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ZOLADEX® PLUS TAMOXIFEN VERSUS ZOLADEX® ALONE IN PRE- AND PERI-MENOPAUSAL METASTATIC BREAST CANCER

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Summary—Phase II studies examining the endocrinological and clinical efficacy of Zoladex® and Zoladex® plus tamoxifen have been examined in pre- and peri-menopausal women with advanced breast cancer. No adverse endocrinological interaction between the drugs have been observed. Although a higher proportion of static disease was observed with the combination of the drugs, which possibly occurred at the expense of partial remissions, the time to disease progression was extended in women who received Zoladex® plus tamoxifen. Remissions were primarily restricted to patients whose tumours were ER positive. Only occasional responses were seen in ER negative disease. This was especially evident where the ER negative tumours were EGF-R positive and showed high rates of cell proliferation.

INTRODUCTION

In addition to the use of LH-RH agonists as single agents in pre- and peri-menopausal breast cancer patients (reviewed in Ref. [1]), they are also being examined in combination with other endocrine therapies. Emphasis is initially being placed on their actions in combination with the antioestrogen tamoxifen, since although they share a common line of action through their involvement with oestrogens, nevertheless it is envisaged that they have nonoverlapping mechanisms of action. The studies are, therefore, based on the rationale that while LH-RH agonists reduce ovarian activity, they do not interfere with peripheral oestradiol production, a factor which is believed to play a major role in the promotion of hormone sensitive breast cancer growth in post-menopausal women, and that the effects of this may be inhibited by the antioestrogen. Other arguments favouring combined therapies include the possibility that they might: (i) reduce the risk of early tumour flare; (ii) shorten the time required to achieve a full suppression of ovarian activity; (iii) increase time to disease progression in responding tumours; and (iv) have additive antitumour activity and hence increase survival rates.

In light of the above, our group initiated a Phase II study in 1986 to examine the efficacy of combining the LH-RH agonist Zoladex® [goserelin, D-Ser(Bu^t)^{6}Azgly^{10}-LH-RH, ICI 118630] with tamoxifen in pre- and peri-monopausal women with advanced breast cancer [2, 4]. Our intention was to retrospectively compare the results obtained with data derived from an earlier Phase II trial of Zoladex alone [4, 5]. In this way we hoped to provide the impetus for a collaborative randomized trial of Zoladex plus tamoxifen vs Zoladex alone, where patients in the latter group received tamoxifen on progression of their disease. Such a trial began in Europe in 1988 and is now nearing completion.

Against this background the current article summarizes our experience of the Phase II studies. Three endpoints have been examined: endocrine data, clinical data and our ability to select patients for LH-RH agonist therapy.

PATIENTS AND METHODS

Two groups of pre- and peri-menopausal patients with histologically proven advanced breast cancer have been treated with Zoladex. The first group received Zoladex alone either as a daily subcutaneous injection (250–1000 μg/day, $n = 25$) or as a sustained release formulation (3.6 mg depot every 28 days, $n = 50$). On progression of the disease the majority of

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patients were either surgically ovariectomized ($n = 24$) or received second-line tamoxifen therapy (20 mg bd, $n = 20$). The second group of women ($n = 50$) were administered the depots of Zoladex in combination with tamoxifen (20 mg bd). Each patient gave written informed consent and had not received previous endocrine or cytotoxic therapies. The median age of patients on commencing therapy was 44 yr (range 21–55) and 42 yr (range 25–51) for groups 1 and 2, respectively.

Tumour oestrogen receptor (ER) status was known in 60 patients in group 1 and in 38 women in group 2. The assays were performed in the Breast Cancer Unit of the Tenovus Institute using either an in-house ligand binding assay [6] or commercially available ER-enzyme-immunoassay kits [7]. Tumours were considered to be positive when a value greater than 15 fmol/mg cytosol protein was obtained. A proportion of the tumours have also been assayed by immunochemical procedures of ER [8], epidermal growth factor-receptor, EGF-R [9] and Ki67 immunostaining [10] in a multiple antibody study [11]. Hormone assays, although not detailed in this paper, were performed in the Tenovus Institute [2, 4].

Patients were assessed for response according to UICC criteria [12] while adhering to the British Breast Group's recommendation [13] that the minimum duration of remission was 6 months.

RESULTS

Summary of the endocrine actions of Zoladex

During the first month of treatment of patients with Zoladex alone and in combination

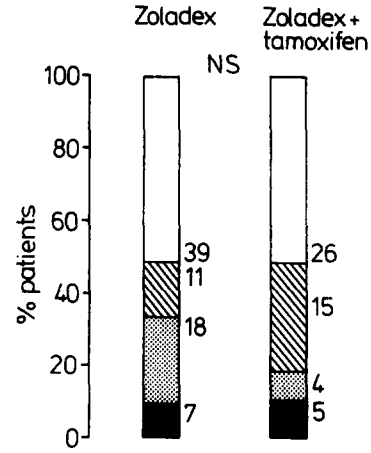

Fig. 1. Relationship between endocrine treatment and response to therapy. Response category: complete, ■; partial ⊡; static, ▨; progressive, □.

with tamoxifen the endocrine actions were identical [14]. In each instance, following an initial rise in circulating gonadotrophin levels (days 1–7), the basal concentrations of LH and FSH fell and were associated with a fall in serum levels of oestradiol and progesterone. In the Zoladex alone group, serum FSH values showed a tendency to rise on long-term therapy, reaching approx. 4 IU/ml after 3–6 months. This was not observed using the combination of drugs. Although this did not markedly influence the already lowered ovarian activity, pooling of the oestradiol data, gathered between 1 and 12 months, showed significantly lower serum oestradiol concentrations in the combination group. This effect was not observed following analysis of the oestradiol data during the first 28 days of therapy, nor was it observed for LH or progesterone. No differences in circulating levels of oestrone, testosterone, androstenedione

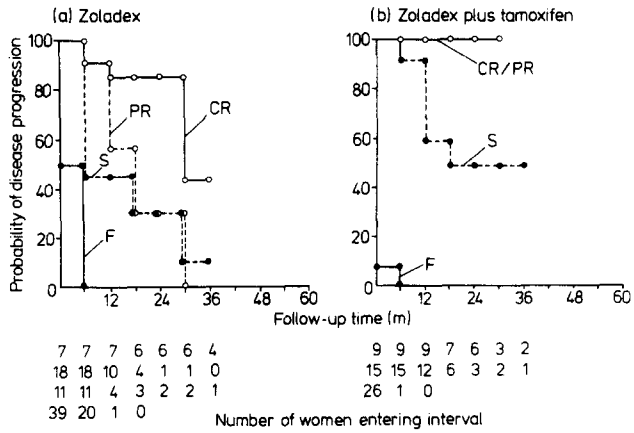

Fig. 2. Time to disease progression in women treated with (a) Zoladex and (b) Zoladex plus tamoxifen. Response category: complete, ○——○; partial, ○- - -○; static, ●- - -●; progressive (failure), ●——●.

and DHAs were observed between the two treatments [15].

The side-effects of Zoladex therapy alone included cessation of menstruation, hot flushes, vaginal dryness and occasional nausea. In patients treated with both Zoladex and tamoxifen similar side-effects were recorded [14].

Clinical actions

Comparison of the proportion of patients responding (CR, PR and SD) to therapy within the two groups of patients (Fig. 1) indicates that the treatments are active against a similar population of pre- and peri-menopausal women (approx. 45%). The use of the combination of drugs, however, appears to be associated with an increased incidence of static disease, which occurs at the expense of partial responses (χ^2, = 7.78, $P < 0.03$).

Using information on the duration of response to the drugs, life table curves have been calculated for the time to disease progression after the initiation of therapy [16 17]. In each instance patients who initially responded to either Zoladex or Zoladex plus tamoxifen have a much more favourable outlook than those women who failed (Fig. 2a,b). Time to progression in responsive patients (CR + PR + S), however, was significantly longer in women treated with the combination of drugs (Fig. 3a). Similarly, examination of the survival curves for these two groups of patients shows a clear benefit to women who respond to Zoladex plus tamoxifen with only one death to date being recorded in this group (Fig. 3b). No significant difference in the time to disease progression or death was observed in women who failed to respond to Zoladex or Zoladex plus tamoxifen (Fig. 3c,d). Subdivision of the responding groups of patients according to the category of response shows that the combination of Zoladex and tamoxifen extends the time to progression in women who experience both disease stabilization and a complete or partial response (Fig. 4a,c). Survival, however, was only significantly extended in women with static disease (Fig. 4b).

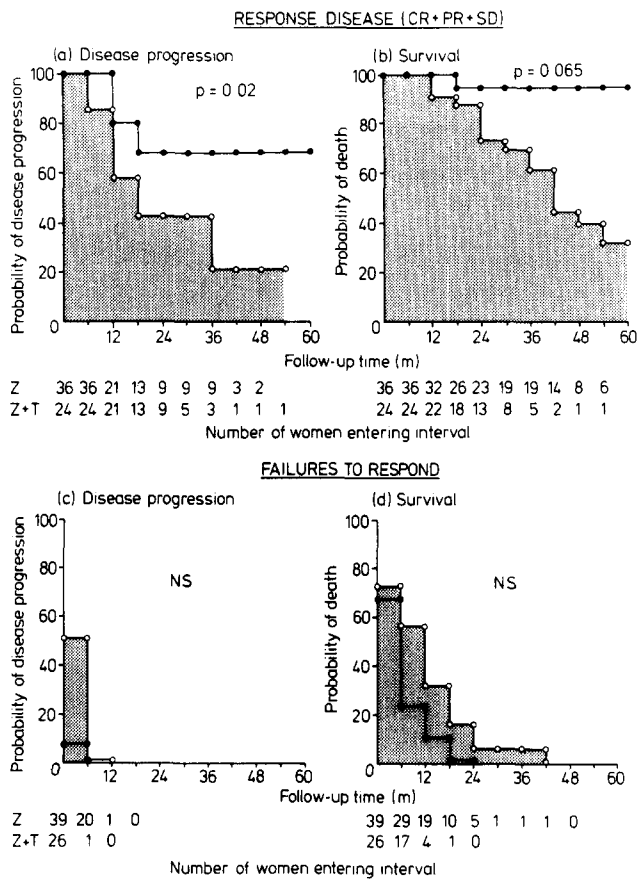

Fig. 3. Time to disease progression and death in women treated with Zoladex (○) and Zoladex plus tamoxifen (●): influence of response category. (a) and (b), Responsive disease; (c) and (d), progressive disease.

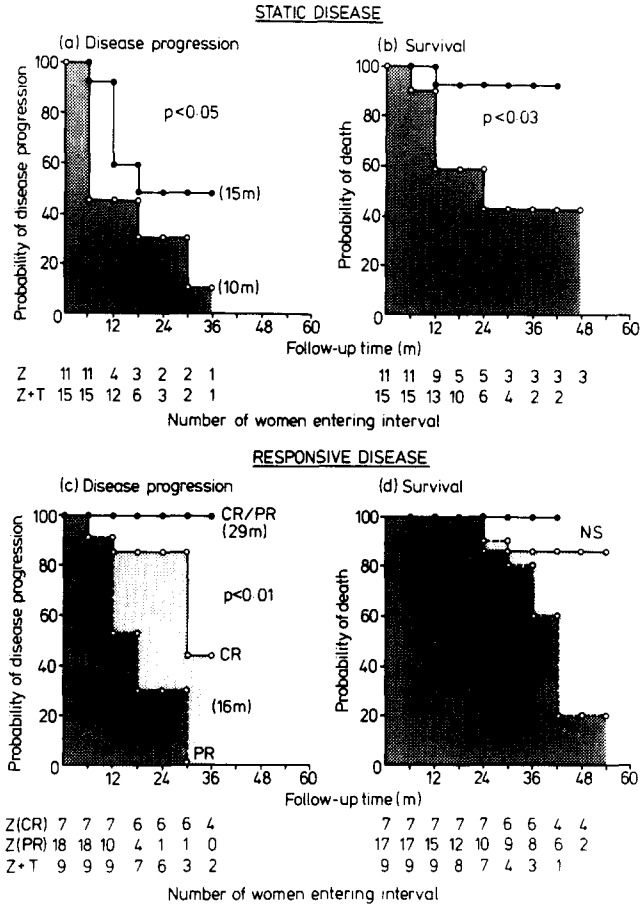

Fig. 4. Time to disease progression and death in women treated with Zoladex (○) and Zoladex plus tamoxifen (●): influence of response category. (a) and (b), Static disease, (c) and (d), responsive disease. Figures in parentheses represent the median time to disease progression in individual response categories.

Examination of known prognostic markers for survival after the initiation of endocrine therapy [18], including sites of disease, histological grade of malignancy and ER status, failed to show an uneven distribution of the parameters between the treatment groups [16, 17].

SELECTION OF PATIENTS FOR LH-RH AGONIST THERAPY

ER on solubilized preparations of breast tumours using an EIA

ER assays were carried out on approx. 60% of the tumours and are related to response to therapy in Fig. 5. Tumour remissions to either Zoladex alone or Zoladex plus tamoxifen stem primarily from patients with ER positive disease. Examination of the time to disease progression curves for these women shows a more favourable outlook for patients with ER positive tumours (cf. Fig. 6a,b with Fig. 6c,d). This is especially pronounced in the Zoladex plus tamoxifen group for time to disease progression (Fig. 6a).

Immunohistochemical assays on frozen sections of breast tumours

Three antibodies have proved particularly useful in assessing the hormone insensitivity of breast cancer [11]: (i) Ki67, an antibody which

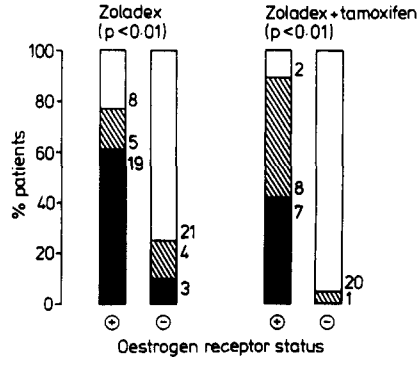

Fig. 5. Relationship between endocrine treatment, response to therapy and ER status. Response category: complete and partial, ■; static, ▨; progressive, □.

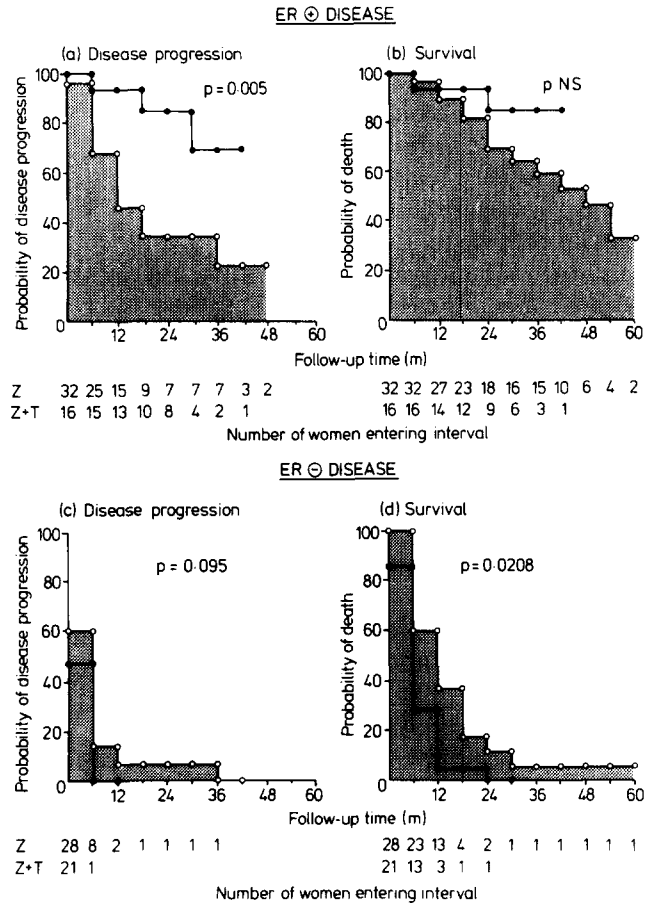

Fig. 6. Time to disease progression and death in women treated with Zoladex (○) and Zoladex plus tamoxifen (●): influence of ER status. (a) and (b), ER-positive disease; (c) and (d), ER-negative disease.

detects a protein expressed in proliferating cells [19]; (ii) R1, an antibody to the external domain of the EGF-R [20]; and (iii) H222, an antibody to ER [21]. Approximately one-third of patients have tumours which are ER-negative, EGF-R-positive and show appreciable levels of Ki67 immunostaining (>30% cells Ki67 positive). As may be seen from Fig. 7, these patients do not respond to endocrine measures and have an extremely poor outlook after the initiation of therapy for their advanced disease.

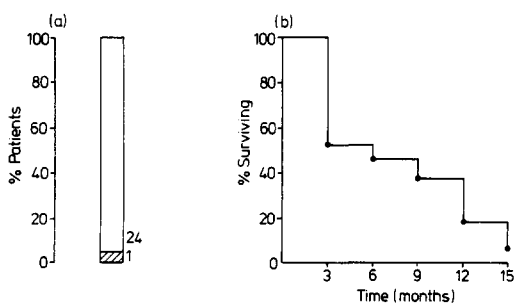

Fig. 7. Influence of the ER-negative, EGF-R-positive and Ki67 positive phenotype on (a) response to therapy and (b) survival after initiation of therapy in 25 advanced breast cancer patients. Response category: static, ▨; progressive, □.

CONCLUSIONS AND DISCUSSION

From the data presented it is clear that there are no adverse endocrinological or clinical interactions between the LH-RH agonist Zoladex and the antioestrogen tamoxifen which would preclude their use in combination therapy. Indeed, in both instances advantages appear to accrue from the combination of drugs. Combination therapy results in a more effective suppression of circulating concentrations of FSH and a further small, but significant, decline in serum oestradiol concentrations [14]. The effective suppressive effect of Zoladex and tamoxifen on serum concentrations of FSH may result from the partial oestrogen agonist properties of tamoxifen [22] which has been shown to partially reduce gonadotrophin levels in postmenopausal women [23, 24]. Although the combination of drugs did not promote a higher

response rate than that observed with Zoladex alone, and a higher proportion of static disease was observed in the combination group, nevertheless the time to disease progression was extended in women who receive Zoladex plus tamoxifen. This result might be anticipated, since a response to surgical oophorectomy in premenopausal women is predictive for a subsequent response to tamoxifen. The survival of patients receiving the combination of drugs also appears to be extended in comparison with Zoladex alone. This stems primarily from patients with static disease who have an extended period of disease stabilization and may represent a developing response to tamoxifen, possibly mediated through the cytostatic effects of the antioestrogenic drug [22]. The above beneficial effects of the combination of drugs do not appear to be due to an uneven distribution of known prognostic variables [18], including ER, tumour grade of malignancy and site of metastases [16, 17].

Finally, it is apparent from our study that when the UICC criteria are used to assess response to therapy in combination with the British Breast Group recommendation of a minimum duration of response of 6 months, that very few tumour remissions are seen in patients with ER negative tumours. This is particularly evident when these tumours are also EGF-R positive and show high rates of cell proliferation. In view of the expense of LH-RH agonist treatment and the unfavourable outlook for advanced breast cancer patients who fail to respond to LH-RH agonist therapy, their use in this subgroup of women does not seem justified. Projection of these results to primary breast cancer and adjuvant LH-RH agonist therapy argues for patient selection based on the above parameters with a subsequent saving in drug costs.

In summary, the clinical and endocrine data generated in our Phase II studies provide considerable encouragement for the combined use of LH-RH agonists and antioestrogens in selected groups of primary and advanced breast cancer patients. Their superiority to single agent therapy requires to be confirmed in prospective randomized trials. Such a trial is nearing completion for Zoladex and tamoxifen.

REFERENCES

1. Nicholson R. I. and Walker K. J.: Use of LH-RH agonists in the treatment of breast disease. *Proc. Soc. R. Soc. Edin.* **95B** (1989) 271–281.
2. Nicholson R. I., Walker K. J., Bouzubar N., Wills R. J., Gee J. M. W., Rushmere N. R. and Davies P.: Estrogen deprivation in breast cancer. *Ann. N.Y. Acad. Sci.* **595** (1990) 316–327.
3. Robertson J. F. R., Walker K. J., Nicholson R. I. and Blamey R. W.: Combined endocrine effects of LH-RH agonist (Zoladex) and tamoxifen (Nolvadex) therapy in premenopausal women with breast cancer. *Br. J. Cancer* **76** (1989) 1262–1265.
4. Nicholson R. I. and Turkes A. *et al.*: The British experience with the LH-RH agonist Zoladex (ICI 118630) in the treatment of breast cancer. In *Hormonal Manipulation of Cancer* (Edited by J. G. M. Klijn, R. Paridaens and J. A. Foekens). Raven Press, New York (1987) pp. 331–341.
5. Williams M. R., Walker K. J., Turkes A., Elston C. W., Blamey R. W. and Nicholson R. I.: The use of an LH-RH agonist (ICI 118630, Zoladex) in advanced premenopausal breast cancer. *Br. J. Cancer* (1986) 629–636.
6. Nicholson R. I., Campbell F. C., Blamey R. W., Elston C. W., George D. and Griffiths K.: Steroid receptors in early breast cancer: value in prognosis. *J. Steroid Biochem.* **15** (1981) 193–199.
7. Nicholson R. I. and Colin P. *et al.*: Evaluation of an enzymeimmunoassay for estrogen receptors in human breast cancers. *Cancer Res.* **46** (1986) 4299–4302.
8. Walker K. J. and Bouzubar N. *et al.*: Immunocytochemical localisation of estrogen receptor in human breast tissue. *Cancer Res.* **48** (1988) 6517–6522.
9. Lewis F., Ellis C. W., Blamey R. W. and Nicholson R. I.: EGF-R expression in human breast carcinoma. *J. Clin. Pathol.* **43** (1990) 385–389.
10. Bouzubar N., Walker K. J., Griffiths K., Ellis I. O., Elston C. W., Robertson J. F. R., Blamey R. W. and Nicholson R. I.: Ki67 immunostaining in primary breast cancer, pathological and clinical associations. *Br. J. Cancer* **59** (1989) 943–994.
11. McClelland R. A. and Nicholson R. I.: (submitted for publication).
12. Hayward J. L., Carbonne P. P., Heuson J. C., Kumaoka S., Segalof A. and Rubens R.: Assessment of response to therapy in advanced breast cancer. *Cancer* **39** (1977) 1289–1293.
13. British Breast Group: Assessment of response to treatment in advanced breast cancer. *Lancet* **ii** (1974) 38–39.
14. Walker K. J., Walker R. F., Turkes A., Robertson J. F. R., Blamey R. W., Griffiths K. and Nicholson R. I.: Endocrine effects of combination antioestrogen and LH-RH agonist therapy in premenopausal patients with advanced breast cancer. *Eur. J. Cancer Clin. Oncol.* **25** (1989) 651–654.
15. Nicholson R. I., Walker K. J., Walker R. F., Read G. F., Finlay E., Robertson J. F. R., Blamey R. W. and Griffiths K.: Oestrogen deprivation in breast cancer using LH-RH agonists and antioestrogens. In *Progress in Cancer Research and Therapy, Vol. 35. Hormones and Cancer 3* (Edited by F. Bresciani, R. J. B. King, M. E. Lippman and J. P. Raynaud). Raven Press, New York (1988) pp. 359–364.
16. Dixon A. R., Robertson J. F. R., Nicholson R. I., Walker K. J. and Blamey R. W.: Goserelin (Zoladex) in premenopausal advanced breast cancer: duration of response and survival. *Br. J. Cancer* (1990).
17. Dixon A. R., Robertson J. F. R., Nicholson R. I., Walker K. J. and Blamey R. W.: Zoladex plus tamoxifen in premenopausal advanced breast cancer: duration of response and survival (1990) (submitted for publication).
18. Williams M. R., Todd J. H., Nicholson R. I., Elston C. W., Blamey R. W. and Griffiths K.: Survival patterns in hormone treated advanced breast cancer. *Br. J. Surg.* **73** (1986) 752–755.

19. Gerdes J. and Lelle R. J. *et al.* Growth fractions in breast cancers determined *in situ* with monoclonal antibody, Ki67. *J. Clin. Path.* **39** (1986) 977–984.
20. Mayes E. L. V. and Waterfield M. D.: *EMBO J.* **3** (1984) 231–537.
21. Greene G. I. and Jensen E. V.: Monoclonal antibodies as probes for estrogen receptor detection and characterization. *J. Steroid Biochem.* **16** (1982) 353–359.
22. Nicholson R. I.: Antioestrogens and breast cancer therapy. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Bailliere Tindall, London (1987) pp. 60–86.
23. Furr B. J. A. and Jordan J. C.: The pharmacological and clinical uses of tamoxifen. *Pharmac. Ther.* **25** (1984) 127–205.
24. Sutherland R. L., Reddel R. R. and Green M. D.: Effects of oestrogens on cell proliferation and cell cycle kinetics. A hypothesis on cell cycle effects of antioestrogens. *Eur. J. Cancer Clin. Oncol.* **19** (1983) 307–318.