J. Steroid Biochem. Molec. Biol. Vol. 37, No. 6, pp. 765–770, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

SENSITIVITY AND INSENSITIVITY OF BREAST CANCER TO TAMOXIFEN

DAVID F. C. GIBSON, MARCO M. GOTTARDIS* and V. CRAIG JORDAN†
Department of Human Oncology, University of Wisconsin Clinical Cancer Center, University of Wisconsin-Madison, Madison, WI 53792, U.S.A.

Summary—Tamoxifen is the endocrine treatment of choice for breast cancer. In several laboratory models *in vivo* tamoxifen is a tumoristatic agent. When MCF-7 breast cancer cells are inoculated into athymic mice, palpable tumors do not grow unless the animals are treated with estrogen, and tamoxifen inhibits estrogen-stimulated growth. If tamoxifen is stopped, tumors regrow. These results suggest that adjuvant tamoxifen therapy should involve long treatment periods (even lifetime) to prevent tumor recurrence. Unfortunately resistance to therapy and patient relapse inevitably occur, and such disease recurrence involving tamoxifen resistance is difficult to treat successfully. A laboratory model of endocrine therapy failure has been developed. When athymic mice with MCF-7 tumors are treated for 6–8 months with tamoxifen, several tumors grew and continued to grow in tamoxifen-treated mice. These estrogen receptor-positive tumors grow with either tamoxifen or estradiol. Tamoxifen-stimulated tumor growth has been observed in human endometrial tumors implanted into athymic animals. Growth of these tamoxifen-stimulated tumors can be inhibited with the pure antiestrogen ICI 164,384 upon withdrawal of tamoxifen. These data are discussed in terms of treatment strategies for tamoxifen-failed patients.

INTRODUCTION

The nonsteroidal antiestrogen, tamoxifen (ICI 46,474; Nolvadex®), developed in 1967 [1], first entered clinical trials at the Christie Hospital in Manchester, England in 1971 [2]. Tamoxifen now represents the endocrine treatment of choice for advanced breast cancer [3, 4], with 50–60% of estrogen receptor-positive patients responding favorably to therapy. Treatment with tamoxifen is safe and effective, and the potency and lack of reported side effects have supported the use of tamoxifen for all types and stages of breast cancer. Clinical trials involving adjuvant tamoxifen treatment of postmenopausal women were initiated towards the end of the 1970s, with the majority of the patients having stage II disease. The reported studies demonstrated an increase in disease-free survival for tamoxifen-treated women [5–7], with a beneficial effect using combination tamoxifen and chemotherapy treatment also reported [8]. Experimental evidence reported during this period, involving *in vivo* laboratory models, demonstrated that tamoxifen acts as a tumoristatic agent [9] and should be administered for extended periods of time to be most effective. Clinically, such long-term therapy (up to 5 yr in duration) produced a significant advantage in node-positive patients [10]. Indeed, a recent overview analysis has confirmed that postmenopausal women have a survival advantage with long-term tamoxifen therapy [11]. A number of these trials [5, 10] involved large numbers of node-negative (stage I) patients. Again, positive results were obtained with tamoxifen treatment. As a result of the favorable data with tamoxifen use from the clinical trials, the NCI (May, 1988) issued an alert to all oncologists, suggesting the use of tamoxifen in the treatment of all stage I estrogen receptor-positive patients. Indeed, tamoxifen now represents a realistic endocrine treatment for all types of breast cancer.

In spite of the clear advantage of tamoxifen treatment, not all estrogen receptor-positive patients respond and therapy that is successful eventually fails. Unfortunately, this failure is invariably fatal. It is essential to understand

Proceedings of the 2nd International EORTC Symposium on "Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti-)Steroidal Agents", Rotterdam, The Netherlands, 9–11 April 1990.

*Present address: Vincent Lombardi Cancer Center, Washington, NW, DC 20007, U.S.A.
†To whom requests for reprints should be addressed: Dr V. Craig Jordan, Department of Human Oncology, University of Wisconsin Clinical Cancer Center, 600 Highland Avenue, Madison, WI 53792, U.S.A.

Abbreviations: TAM: tamoxifen; s.c.: subcutaneous; ER: estrogen receptor; ER-EIA: estrogen receptor-enzyme immobilized assay; PR: progesterone receptor.

how such therapeutic failure occurs, so suitable treatment strategies can be developed for patients when tamoxifen resistance occurs. Ultimately, such investigations could aid in the development of novel therapies for the treatment of breast cancer.

Four potential mechanisms of resistance to tamoxifen therapy can be proposed:

1. Metabolic tolerance

Tamoxifen is extensively metabolized in both animals and man [3, 4]. The metabolites observed include 4-hydroxytamoxifen, *N*-desmethyltamoxifen and metabolite Y. Each of these compounds are antiestrogenic in conventional laboratory assays [12], suggesting that the antitumor effects of tamoxifen are a combination of both the parent compound and its metabolites. However, disease recurrence could be caused by the production of estrogenic metabolites during long-term tamoxifen treatment. No such metabolites have been observed. Patient noncompliance during therapy might result in declining tamoxifen levels, with tumor growth occurring during a period of low drug concentration [13]. We are currently conducting a study at the University of Wisconsin to evaluate tamoxifen levels in patients who have successfully completed 10 yr of adjuvant therapy.

2. Hormone-independent growth

On the assumption that tamoxifen operates through the estrogen receptor, it is not surprising that receptor-negative tumors are not controlled by antiestrogen treatment. Breast tumors are known to be a heterogeneous mixture of receptor-positive and negative cells [14], and tamoxifen failure could merely be result of receptor-negative cell outgrowth. While this undoubtedly occurs, up to 13% of receptor-negative tumors exhibit an objective response to tamoxifen. Assuming that the receptor classification is correct, a number of mechanisms of tamoxifen action in these cells have been proposed [15, 16].

3. Paracrine-regulated growth

This mechanism centers upon the stimulation of tamoxifen-inhibited receptor-positive cells by growth factors produced by neighboring cells. These cells could be receptor-negative breast cancer cells or stromal cells. Indeed, it has been shown that EGF can partially reverse the action of tamoxifen on breast cancer cell lines [17]. Clearly, treatments that control or destroy growth factor-producing cells are a priority for future research. A related mechanism involves the stimulation of breast cancer cells by circulating steroids. Tamoxifen is a competitive inhibitor of estrogen action and therefore high concentrations of circulating estrogens may not result in an optimal tumor response. Tamoxifen is known to cause an elevation in estrogen levels in premenopausal women [18]. Elevations in progestin levels have also been observed, and there is evidence that progesterone can have a negative effect upon tamoxifen-controlled breast cancer growth [19, 20]. It is clear that strategies to reduce steroidogenesis (e.g. Zoladex®) should be considered for these patients, to reduce the possibility of tamoxifen failure. Although the role of prolactin in human breast cancer growth is undefined, elevated levels of this hormone may have a stimulatory effect upon cell growth [21]. Again, this would blunt the inhibitory effects of tamoxifen.

4. Tamoxifen-dependent growth

The failure of tamoxifen treatment could involve growth stimulation of tumors by long-term tamoxifen treatment. This paper will focus upon the development of laboratory models of tamoxifen-stimulated hormone-responsive endometrial and breast tumors.

MATERIALS AND METHODS

Breast and endometrial tumors

The breast cancer cell line MCF-7 was obtained from ATCC (Rockville, MD) and shown to be authentic by karyotypic analysis (data not shown). The MCF-7 tumor used in these experiments was originally derived from an inoculation of 10^7 cells into estrogenized athymic mice, as described previously [22]. Ovariectomized BALB/c 4–5-wk-old athymic mice were implanted s.c. in the axillary mammary fat pads with 1-mm^3 pieces of this MCF-7 tumor. This tumor had been passaged previously *in vivo* four times in estrogenized animals. Estrogen stimulation was consistent over these passages and no growth was observed with control or tamoxifen treatment. The tamoxifen-stimulated tumors MCF-7TAM and the endometrial tumor EnCa101 (obtained from Dr Satyaswaroop, Hershey, PA) were maintained by passaging in ovariectomized tamoxifen-treated athymic mice. Tumor implantation was as described previously.

Animals

Ovariectomized athymic mice (BALB/c, 4–5 wk) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and housed under sterile conditions. Tumors were measured weekly and the mean cross-sectional area (cm^2) determined using the formula (length\2) × (width\2) × π.

Hormone treatments

Estradiol (1.7 mg, 8-wk release) and tamoxifen (5 mg, 4-wk release) pellets were custom made (Innovative Research of America, Toledo, OH) and implanted s.c. on the backs of animals using a trocar on the same day as tumor implantation. Tamoxifen was administered where indicated using capsules made from Silastic tubing (Dow Corning, Midland, MI) cut to various lengths and filled with tamoxifen free-base (Stuart Pharmaceuticals, Wilmington, DE). Ends were plugged with Silastic cement (Dow Corning, Midland, MI) and the finished capsules sterilized by γ-irradiation prior to s.c. implantation on the back of the animal. Control capsules, empty and sealed at both ends, were also used. Where indicated, ICI 164,384 (Roussel, Uclaf) was injected s.c. in a 0.1 ml volume. The suspension used was made by dissolving the compound in ethanol, mixing in Tween 80 (Sigma Chemical) and diluting in isotonic saline to precipitate the compound. This approach was necessary because of the poor bioavailability of ICI 164,384.

Tumor-receptor measurements

Tumors were excised from the animals and stored in liquid nitrogen until the assay was performed. ER was measured using a commercially available monoclonal antibody kit (ER-EIA) from Abbott Laboratories (Chicago, IL). Procedures followed recommended protocols [23]. PR levels were determined using specific ligand receptor binding using [^{3}H]17α-methyl-R5020, with free and bound ligand separated using a dextran-coated charcoal separation method. PR levels were quantitated using Scatchard analysis.

Single-point binding analysis of ER was done on some tumors using a similar ligand-binding assay as for PR. Protein values were determined in cytosols using a modified Bradford protein assay [23, 24].

Statistical analysis

Differences in mean tumor area and receptor levels between groups were measured using analysis of variance followed by unpaired Student's t-test.

RESULTS AND DISCUSSION

Tamoxifen-stimulated endometrial tumor growth

Tamoxifen is able to stimulate the growth of a transplantable hormone-responsive endometrial tumor, EnCa101, maintained in athymic animals (Fig. 1) [25]. This model was further developed in collaboration with Dr Satyaswaroop [26]. In this experiment, the endometrial tumor is transplanted on one side of the animal and the breast tumor on the other. As expected, estrogen treatment stimulated the growth of both tumors. However, when the animals were also treated with tamoxifen, the estrogen-stimulated breast tumor growth was inhibited, while the growth of the endometrial tumor was actually further stimulated (Fig. 2). In summary, tamoxifen is able to encourage the growth of a hormone-responsive tumor, EnCa101. Is this a mechanism by which breast tumor growth recurs during tamoxifen therapy?

Tamoxifen-stimulated breast tumor growth

Investigations in this laboratory were conducted to mimic the long-term treatment of women with tamoxifen. MCF-7 breast tumors were grown in athymic animals under the influence of estrogen, and following removal of this

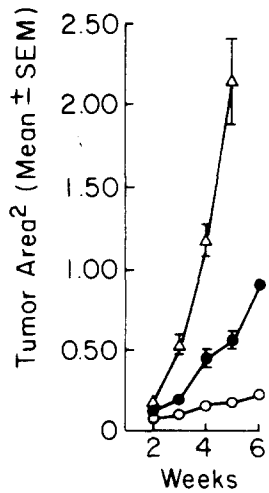

Fig. 1. Growth rates of EnCa101 tumor (n = 12) transplants in athymic mice treated by sustained release pellets containing 17β-estradiol (1.7 mg, 8-wk release; △); TAM (5 mg, 4-wk release replaced at wk 4; ●); no therapy (○). Adapted from Ref. [26].

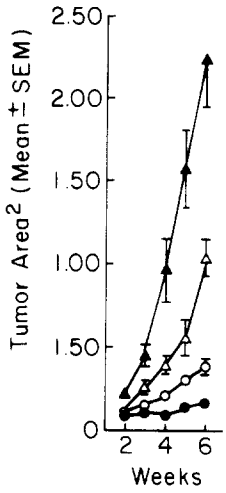

Fig. 2. Growth responses of EnCa101 (triangles) and MCF-7 (circles) tumors ($n = 6$) transplanted on opposite sides of the same athymic mouse and treated by sustained release pellets containing 17β-estradiol (1.7 mg, 8-wk release; open symbols) or 17β-estradiol (1.7 mg, release) plus TAM (5 mg, 4-wk release replaced at week 4; solid symbols). Adapted from Ref. [26].

stimulus, subject to long-term tamoxifen treatment [23]. After the estrogen is removed, there is a period of tumor regression. However, after approx. 6 months of tamoxifen treatment, tumor regrowth was observed (Fig. 3). Tamoxifen-stimulated tumors were removed and transplanted into new tamoxifen-treated animals; in this way, a transplantable, tamoxifen-stimulated breast cancer was attained (termed MCF-7TAM). Whilst tamoxifen is stimulating tumor growth in these animals, it is acting as an antiestrogen in another hormone-responsive tissue, the uterus, as seen by the lack of growth over control levels (Table 1).

The MCF-7TAM tumors contain significantly higher ER levels than estrogen-stimulated MCF-7 tumors (Table 1) and

Table 1. Uterine wet weights and steroid hormone receptor values of MCF-7 tumors (estradiol and tamoxifen stimulated) under various treatments (adapted from Refs [23] and [32])

Treatment group	Uterine wet weight[a] (mg)	Steroid receptor values[b] (fmol/mg cytosol protein) ER[c]	PR[d]
MCF-7 wild + E_2	105 ± 4.1[e]	173 ± 14	132 ± 31
MCF-7TAM + TAM	18.4 ± 1.8	390 ± 37[f]	55 ± 27
MCF-7TAM + CON	20.2 ± 1.3	ND[g]	ND

[a]Animals subject to 7 months of tamoxifen or control treatments; E_2 treatment was 2 months in duration.
[b]Tamoxifen treatment of 5.5 months in duration; E_2 treatment of 2 months in duration.
[c]Measurement by ER-EIA.
[d]Measurement by Scatchard.
[e]Mean ± SE.
[f]Significantly different from ER-EIA level in other group.
[g]ND, not done.

measurable levels of PR, although PR levels in MCF-7TAM tumors were not stimulated by tamoxifen treatment (Table 1). These data suggest that if tamoxifen is acting through the estrogen receptor in the MCF-7TAM tumor, the ER/PR pathway is altered in some way. A detailed investigation into the regulation of the expression of steroid hormone receptors in this tumor variant is currently underway. Tamoxifen was able to stimulate tumor growth in a dose-dependent fashion, with increasing serum tamoxifen levels resulting in increased tumor growth (Fig. 4) [23]. However, when the tamoxifen was removed, tumor growth was arrested [23]. Therefore, this growth appears to be stimulated by tamoxifen in a dose-dependent fashion.

A central question remains, what is the mechanism by which tamoxifen stimulates growth in the MCF-7TAM tumor? A logical explanation involves the known estrogenic properties of tamoxifen [3]. Interestingly, both *in vitro* [27] and *in vivo* [28], wild-type MCF-7 cells can be stimulated by tamoxifen under the appropriate

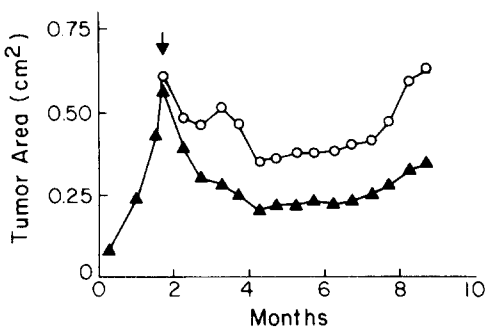

Fig. 3. Mean MCF-7 tumor growth in animals treated with estradiol pellets for 7 wk. Animals had estrogen removed (arrow) and were randomized into groups treated with placebo pellets ($n = 10$) (▲) or TAM pellets ($n = 12$) (○) for 7 months. Adapted from Ref. [23].

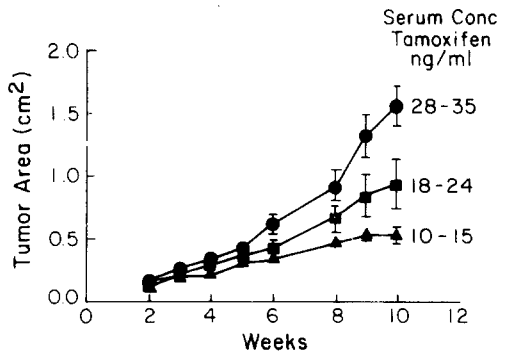

Fig. 4. MCF-7 TAM tumor growth in athymic mice ($n = 6$) treated with TAM silastic capsules of varying sizes (2 cm, ●; 1 cm, ■; 0.25 cm, ▲). Points = mean; bars = SE. Adapted from Ref. [32].

conditions. In addition, two clinical observations support the idea of tumor growth stimulated by the estrogenic properties of tamoxifen. Some breast cancer patients experience "tumor flare" at the onset of antiestrogen treatment [29]. Secondly, some success has been observed with second-line endocrine therapies (e.g. aminoglutethimide) following tamoxifen failure [30]. These data suggest that tamoxifen can indeed stimulate breast tumor growth, and that this stimulation is a result of the estrogenicity of this compound. Indeed, estrogen is able to stimulate the growth of MCF-7TAM tumors to an extent equivalent to that attained with tamoxifen treatment [32]. Collectively, these concepts suggest that merely stopping the tamoxifen in failed patients will not be sufficient to arrest tumor growth, as circulating estrogens will be able to maintain tumor cell proliferation.

The response of MCF-7TAM tumors to pure antiestrogens would aid in the dissection of this tamoxifen-stimulated growth. These compounds [31] are steroidal in nature, and in standard assays *in vitro* and *in vivo* show no estrogenic effects. A member of this family of pure antiestrogens, ICI 164,384, was used in the MCF-7TAM animal model [32]. ICI 164,384 was able to inhibit tamoxifen-stimulated MCF-7TAM tumor growth (Fig. 5) and EnCa101 tumor growth [33]. Importantly, this steroidal antiestrogen did not stimulate growth above control levels in either model, when administered alone. These results support the concept that tamoxifen-stimulated growth of these two tumors is a result of the estrogenic properties of tamoxifen.

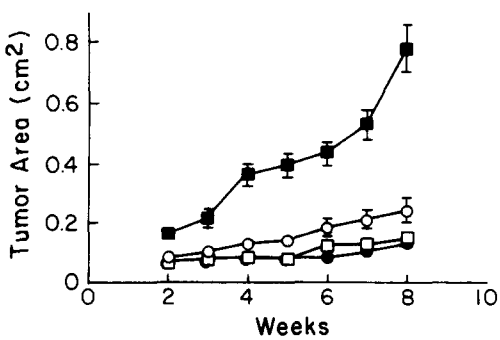

Fig. 5. Inhibition of TAM-stimulated MCF-7 TAM tumor growth ($n = 16$). Mice were treated continuously with 0.5 cm TAM silastic capsules alone (■), TAM capsules and 1 mg of ICI 164,384 injected s.c. every other day (○), 1 mg of ICI 164,384 injected s.c. alone every other day (□) or with an empty 0.5 cm placebo capsule (●). Points = mean; bars = SE. Adapted from Ref. [32].

In summary, the variant tumor, MCF-7TAM, whose growth is stimulated by both tamoxifen and estrogen, represents a useful model of tamoxifen-treatment failure. Investigations of the kind described here will provide useful information, important in the development of strategies to combat antiestrogen resistance. The fact that estrogen can stimulate the MCF-7TAM tumor suggests that treatment withdrawal will be unproductive, as estrogens will be sufficient to maintain growth. To this end, novel pure antiestrogens represent an effective treatment for tamoxifen-failed patients.

Acknowledgement—We wish to thank Dr P. G. Satyaswaroop for the generous gift of human tumor EnCa101.

REFERENCES

1. Harper M. J. K. and Walpole A. L.: A new derivative of triphenylethylene: effect on implantation and mode of action in rats. *J. Reprod. Fert.* **13** (1967) 101–119.
2. Cole M. P., Jones C. T. A. and Dodd I. D. H.: A new antiestrogenic agent in late breast cancer. *Br. J. Cancer* **25** (1971) 270–275.
3. Furr B. J. A. and Jordan V. V.: The pharmacology and clinical uses of tamoxifen. *Pharmac. Ther.* **35** (1984) 127–205.
4. Jordan V. C. (Ed.): *Estrogen/Antiestrogen Action and Breast Cancer Therapy*. University of Wisconsin Press, Madison, WI (1986).
5. Baum M. and other members of the Nolvadex Adjuvant Trial Organization: controlled trial of tamoxifen adjuvant agent in management of early breast cancer. *Lancet* **1** (1983) 257–261.
6. Baum M. and other members of the Nolvadex Adjuvant Trial Organization: controlled trial of tamoxifen as a single adjuvant agent in the management of early breast cancer. *Lancet* **1** (1985) 836–840.
7. Ribeiro G. and Palmer M. K.: Adjuvant tamoxifen for operable carcinoma of the breast; report of a clinical trial by the Christie Hospital and Holt Radium Institute. *Br. Med. J.* **286** (1983) 827–830.
8. Fisher B. and other NSABP investigators: Adjuvant chemotherapy with and without tamoxifen in the treatment of primary breast cancer: five-year results from the National Surgical Adjuvant Breast and Bowel Project Trial. *J. Clin. Oncol.* **4** (1986) 459–471.
9. Jordan V. C.: Long-term tamoxifen therapy for breast cancer. In *Important Advances in Oncology* (Edited by V. T. DeVita Jr, S. Hellman and S. A. Rosenberg). Lippincott, Philadelphia (1989) pp. 179–192.
10. Breast Cancer Trials Committee, Scottish Cancer Trials Office: Adjuvant tamoxifen in the management of operable breast cancer. The Scottish Trial. *Lancet* **2** (1987) 171–175.
11. EBCTCC: Effects of adjuvant tamoxifen and of cytotoxic therapy on mortality in early breast cancer. *N. Engl. J. Med.* **319** (1988) 1681–1691.
12. Kemp J. V., Adam H. K., Wakeling A. E. and Slater R.: Identification and biological activity of tamoxifen metabolites in human serum. *Biochem Pharmac.* **32** (1983) 2045–2052.
13. Jordan V. C., Fritz N. F. and Tormey D. C.: Endocrine effects of adjuvant chemotherapy and long-term tamoxifen administration on node-positive patients with breast cancer. *Cancer Res.* **47** (1987) 624–630.

14. Jordan V. C.: The control of hormone-dependent breast cancer growth—are we talking about estrogen alone? *Eur. J. Cancer Clin. Oncol.* **24** (1988) 1245–1248.
15. Jordan V. C.: Biochemical pharmacology of antiestrogen action. *Pharmac. Rev.* **36** (1984) 245–276.
16. Berry J., Green B. J. and Matheson D. S.: Modulation of natural killer cell activity by tamoxifen in stage I postmenopausal breast cancer. *Eur. J. Cancer Clin. Oncol.* **23** (1987) 517–520.
17. Koga M. and Sutherland R. L.: Epidermal growth factor partially reverses the inhibitory effects of antiestrogens on T47D human breast cancer cell growth. *Biochem. Biophys. Res. Commun.* **147** (1987) 739–745.
18. Ravdin P. M., Fritz N. F., Tormey D. C. and Jordan V. C.: Endocrine status of premenopausal node-positive breast cancer patients following adjuvant chemotherapy and long-term tamoxifen. *Cancer Res.* **48** (1988) 1026–1029.
19. Mouridsen H. T., Ellemann K., Mattsson W., Palshoff T., Daehnfeldt J. L. and Rose C.: Therapeutic effect of tamoxifen versus tamoxifen combined with medroxyprogesterone acetate in advanced breast cancer in postmenopausal women. *Cancer Treat. Rep.* **63** (1979) 171–175.
20. Robinson S. P. and Jordan V. C.: Reversal of the antitumor effects of tamoxifen by progesterone in the 7,12-dimethylbenzanthracene-induced rat mammary carcinoma model. *Cancer Res.* **47** (1987) 5386–5390.
21. Biswas R. and Vonderhaar B. K.: Role of serum in the prolactin responsiveness of MCF-7 human breast cancer cells in long-term tissue culture. *Cancer Res.* **47** (1987) 3509–3514.
22. Gottardis M. M., Robinson S. P. and Jordan V. C.: Estradiol-stimulated growth of MCF-7 tumors implanted in athymic mice: a model to study the tumoristatic action of tamoxifen. *J. Steroid Biochem.* **30** (1988) 311–314.
23. Gottardis M. M. and Jordan V. C.: Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen administration. *Cancer Res.* **48** (1988) 5183–5187.
24. Bradford M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.* **72** (1976) 248–254.
25. Satyaswaroop P. G., Zaino R. J. and Mortel R.: Estrogen-like effects of tamoxifen on human endometrial carcinoma transplanted into nude mice. *Cancer Res.* **44** (1984) 4006–4010.
26. Gottardis M. M., Robinson S. P., Satyaswaroop P. G. and Jordan V. C.: Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res.* **48** (1988) 812–815.
27. Berthois Y., Katzenellenbogen J. A. and Katzenellenbogen B. S.: Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. Natl. Acad. Sci. U.S.A.* **83** (1986) 2496–2500.
28. Osbourne C. K., Coronado E. B. and Robinson J. P.: Human breast cancer in the athymic nude mouse: cytostatic effects of long-term antiestrogen therapy. *Eur. J. Cancer Clin. Oncol.* **23** (1987) 1189–1196.
29. Plotkin D., Lechner J. J., Jung W. E. and Rosen P. J.: Tamoxifen flare in advanced breast cancer. *JAMA* **240** (1978) 2644–2646.
30. Smith I. E., Harris A. L., Morgan M., Ford H. T., Gazet J. T., Harmer C. L., White H., Parsons C. A., Villardo A., Walsh G. and McKinna J. A.: Tamoxifen versus amino-glutethimide in advanced breast carcinoma: a randomized cross-over trial. *Br. Med. J.* **283** (1981) 1432–1434.
31. Wakeling A. E. and Bowler J.: Steroidal pure antiestrogens. *J. Endocrinol.* **112** (1987) R7–R10.
32. Gottardis M. M., Jiang S. Y., Jeng M. H. and Jordan V. C.: Inhibition of tamoxifen-stimulated growth of an MCF-7 tumor variant in athymic mice by novel steroidal antiestrogens. *Cancer Res.* **49** (1989) 4090–4093.
33. Lerner L. J. and Jordan V. C.: Development of antiestrogens and their use in breast cancer. *Cancer Res.* **50** (1990) 4177–4189.