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# Antitumor Activity of the Progesterone Antagonists ZK 98.299 and RU 38.486 in the Hormone-dependent MXT Mammary Tumor Model of the Mouse and the DMBA- and the MNU-induced Mammary Tumor Models of the Rat

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**Abstract**—*The antitumor activities of the antiprogesterones ZK* 98.299 *and RU* 38.486 (*RU* 486) *were tested in the hormone-dependent MXT(+) mammary tumor model of the mouse and the DMBA- and MNU-induced mammary tumor models of the rat. In the MXT(+)-tumor model, treatment with the two antiprogesterones* (1–10 mg/kg *daily*) *starting on day* 1 *after tumor implantation led to an almost complete inhibition of tumor growth identical to that accomplished with tamoxifen. Treatment of established MXT(+) tumors with ZK* 98.299 (1, 10 *and* 50 mg/mg) *resulted in a strong, dose-dependent inhibition of tumor growth. At the* 10 *and* 50 mg *doses, the effect of ZK* 98.299 *was superior to that of tamoxifen* (4 mg/kg) *and equal to that of ovariectomy and of RU* 486, *whereas megestrol acetate and medroxyprogesterone acetate had no significant effect. In contrast to the massive induction of cell degeneration and cytolysis in the MXT mammary tumors resulting from ovariectomy, the treatment with the two progesterone antagonists seems rather to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with secretory activity as well as towards the development of spindle-shaped necrobiotic cell populations. The weights of the ovaries were increased after therapy with ZK* 98.299 *and RU* 486. *Due to this inhibition of the negative feedback and an 'unopposed estrogen effect', uterine weight was also significantly increased. In the DMBA-induced mammary carcinoma, ZK* 98.299 (10 mg/kg) *caused strong tumor-inhibiting activity almost comparable to that of ovariectomy. The inhibition was very uniform and in this regard superior to RU* 486. *The MNU-induced mammary carcinoma of the rat was significantly inhibited by ZK* 98.299, *whereas RU* 486 *showed only a weak effect. In the light of these results antiprogesterones can be considered to be a very promising new class of mammary tumor inhibitors.*

## INTRODUCTION

FIRST LINE endocrine therapy of advanced breast cancer in postmenopausal patients is generally performed with tamoxifen [1]. As second line alternatives, high dose progestins like medroxyprogesterone acetate (MPA) and megestrol acetate (Megace) or aminogluthetimide, an inhibitor of steroid biosynthesis and the enzyme aromatase, are used [1]. More specific aromatase inhibitors like 4-hydroxyandrostenedione are currently under development [2].

A new approach in the endocrine therapy of mammary tumors could be treatment with progesterone antagonists [3]. In a preliminary clinical study, a favorable response to RU 486 (Fig. 1) was noted in some patients, who had been pretreated with various modalities [3]. The intention of this trial was based on the antitumor activity of RU 486 in certain experimental mammary tumor models: RU 486 caused growth inhibition of the progesterone receptor positive breast cancer cell lines MCF-7 and T47D *in vitro* via a mode of action different to that of the progestin R 5020 [4]. *In vivo*, the DMBA-induced, hormone-dependent mammary carcinoma of the rat was inhibited by RU 486 to a greater extent than by treatment with progestins, whereas its effect was less than that of ovariectomy [5].

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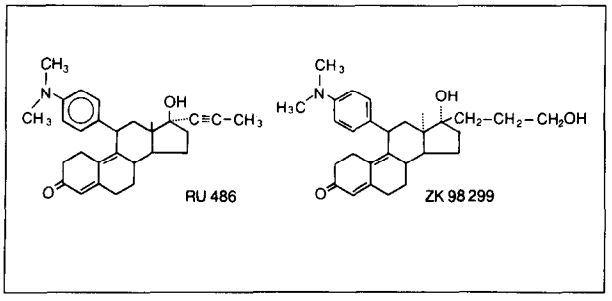


*Fig. 1. Structures of ZK 98.299 and RU 486.*

Whereas in RU 486 the natural configuration of the steroid is preserved, a further group of antiprogesterones with a change from *trans-* to *cis*-fusion between the C and D rings was developed by Neef *et al.* [6]. The leading compound in this group, Schering ZK 98.299 (Fig. 1), has somewhat stronger antiprogestational potency than RU 486 [7, 8].

A particular advantage of ZK 98.299 is the reduced antiglucocorticoid activity *in vitro* and *in vivo* compared to RU 486 [7, 8]. As RU 486 was shown to be a mammary tumor inhibitor, it was of great interest to elucidate the potential of ZK 98.299 in this respect. The aim of this study was therefore to test the antitumor activity of ZK 98.299 in comparison with RU 486 and further standard therapies in relevant mammary tumor models. For this purpose, we used the DMBA-induced, hormone-dependent mammary tumor model of the rat and the transplanted, hormone-dependent MXT mammary tumor model of the mouse which are both inhibited by ovariectomy, high dose estrogens and antiestrogens like tamoxifen, but not or only slightly by progestins [9–12]. Therefore, and because of further similarities to the human disease, these tumors are considered to be excellent models for the evaluation of the antitumor effects of new compounds [9, 11]. In addition, the hormone-dependent MNU-induced mammary tumor model of the rat was used. It has some advantages, such as local tumor invasion and metastasis, over the DMBA model and is therefore complementary to this latter tumor [13]. To give more insight into the endocrinological effects of these compounds during tumor therapy, the histology of the MXT mammary tumors and the weight of the ovaries, uteri and vaginae were determined.

## MATERIALS AND METHODS

*Chemicals*

ZK 98.299 was synthesized at Schering AG as previously described [6]. RU 38.486 (= RU 486, Roussel-Uclaf [14]) was used as reference compound. Tamoxifen was generously provided by ICI, Macclesfield, U.K. Megestrol acetate (Megace) and Medroxyprogesterone acetate (MPA) were synthesized at Schering AG. 7,12-Dimethylbenz(α)anthracene (DMBA) was purchased from Serva, Heidelberg, F.R.G., and Methylnitrosourea (MNU) from ASH-Stevens, Detroit, U.S.A.

*Hormone-dependent MXT(+) mammary carcinoma of the mouse* [11]

The MXT tumor used was the MXT line M 3.2 generously provided by Dr. A.E. Bogden, EG + G Bogden Laboratories, Worcester, MA, U.S.A. as a frozen sample. After thawing, pieces were implanted subcutaneously in intact, female, 8–10-week-old BDF1 mice (Charles River Wiga, F.R.G.). After the tumor had reached a diameter of about 1 cm, it was further implanted to BDF1 mice as will be described later. Tumors were taken from various transplant generations, frozen and kept in liquid nitrogen. To perform an experiment, tumor pieces from a frozen sample were implanted in three to five mice. At the next passage, the hormone-dependency was tested by implantation in intact and ovariectomized mice [12]. If there was inhibition of tumor growth in the ovariectomized mice of more than 90% after 6 weeks compared to the intact control, these tumors were used for further testing. Two to three tumors from one to two donor animals are taken out, placed in MEM 199 medium and cut into pieces of about 2 mm diameter. These pieces are implanted s.c. in BDF1 mice as above (two tumors/mouse).

(a) *Prophylaxis model.* After tumor implantation, the animals were randomly assigned to groups of 9–10. On the next day, treatment was started. Test compounds were injected daily s.c. as oily solutions (10% benzyl benzoate) or ovariectomy was performed. After 6 weeks of treatment, the animals were killed and weighed. The tumors, ovaries, uteri and vaginae were removed and the wet weights were determined [12]. The tumor used for the experiment shown in Table 1 was frozen in the third passage and was in a third passage during the test.

(b) *Therapy of established tumors.* Twenty days after tumor implantation, the mice were palpated for tumors. Only mice with two palpable tumors were used. These animals were randomly assigned to groups of 9–10. Treatment was started on the following day and continued for 2 or 3 weeks. Test compounds were injected six times a week s.c. The tumor area was determined by caliper measurements once or twice weekly. The tumor area is the product of the longest diameter and its perpendicular. At the end of treatment, the animals were processed as above.

*Table 1. Effect of ZK 98.299, RU 486 and tamoxifen on growth of the MXT(+) mammary tumor (prophylaxis model) and on the weight of ovaries, uterus and vagina**

| | Dose (mg/kg) | Tumor %T/C† | Ovaries %T/C† | Uterus %T/C† | Vagina %T/C† |
|---|---|---|---|---|---|
| Control | — | 100 | 100 | 100 | 100 |
| Tamoxifen | 4.0 | 3.1‡ | 62‡ | 102 | 54‡ |
| ZK 98.299 | 1.0 | 2.9‡ | 138‡ | 118 | 117 |
| | 3.0 | 1.3‡ | 142‡ | 129‡ | 136‡ |
| | 10.0 | 1.4‡ | 155‡ | 97 | 126 |
| RU 486 | 1.0 | 1.4‡ | 133‡ | 117 | 95 |
| | 3.0 | 1.6‡ | 147‡ | 126 | 119 |
| | 10.0 | 1.1‡ | 146‡ | 72 | 92 |

*Compounds were administered daily s.c. starting on *day 1* after tumor implantation (9–10 mice/group; two tumors/mouse). Tumor and organ wet weights were determined after *6* weeks of therapy.
†% T/C = tumor or organ weight of treatment group/control group × 100.
‡Significant ($P < 0.05$); *U*-test.

*DMBA-induced mammary carcinoma of the SD rat* [10, 15]

Female Sprague–Dawley (SD) rats (Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.) were given a single dose of 10 mg DMBA dissolved in 0.5 ml oil by gavage at the age of 50 days. The rats were examined for tumors by palpation once weekly. Animals with at least one tumor with a tumor area of more than 150 $mm^2$ were randomly assigned to experimental groups. The tumor area was determined by caliper measurements of the longest diameter and its perpendicular. Drugs were dissolved in oil (10% benzyl benzoate) and administered daily (0.1 ml/100 g body wt) for 4 weeks. The tumor area was measured weekly. At the start of therapy the total area of all tumors in each animal was termed 100%. Changes in tumor area were calculated in terms of this value. There was an approximately equal distribution of tumors of different latencies, numbers of tumors and total tumor area amongst all the experimental groups.

*MNU-induced mammary carcinoma of the SD rat* [13, 15]

Tumors were induced in 50-day-old female SD rats (Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.) by a single i.v. injection of MNU (50 mg/kg) in the tail vein. Starting 30 days after induction, animals were palpated for tumors. All other experimental details were identical to those described above for the DMBA tumor except that treatment was only performed for 3 weeks with six injections per week. Animals with tumors appearing in weeks 7 to 14 after induction were used.

*Histology of the MXT mammary tumors*

The tumors were excised and rinsed for 1 min with a solution of 0.1 M Na-cacodylate buffer (pH 7.4) with the addition of 1% Novocain® (Hoechst, Frankfurt/Main) at body temperature. The fixation was then performed using a 2.5% glutaraldehyde solution (Merck) chilled to 4°C and the same buffer. For the analysis, blocks of tissue measuring roughly 1 $mm^3$ from the peripheral and central parts of the tumors were obtained. After postfixation and dehydration the samples were embedded in Epon and semi-thin sections were stained with basic fuchsin and methylene blue as described before [16].

## RESULTS

*Antitumor activity in the MXT mammary tumor model of the mouse*

The progesterone antagonists ZK 98.299 and RU 486 were first tested in doses of 1, 3 and 10 mg/kg daily in the hormone-dependent MXT(+) mammary tumor of the BDF1 mouse using a treatment schedule starting on day 1 after tumor implantation (prophylaxis model). The antiestrogen tamoxifen, which was used as a positive control, led to strong inhibition of tumor growth after 6 weeks of therapy, as determined by the tumor weights (Table 1) as was also described previously [12, 15]. Both antiprogesterones caused almost complete tumor inhibition in all doses used (Table 1). Because of these strong effects, no dose-dependency was evaluable.

To gain more insight into the endocrinological effects of these new compounds, the wet weights of the ovaries, uteri and vaginae were determined at the end of therapy (Table 1). In contrast to tamoxifen, which reduced ovarian weight due to its estrogen agonistic activity in the mouse [17], ZK 98.299 and RU 486 significantly increased the weights of

the ovaries at all doses used as can be seen from the elevated % T/C values compared to the intact control. The effects on uteri and vaginae were not as clear as those on ovaries and need further histological evaluation.

As the antitumor effects of the antiprogesterones in the prophylaxis model were so strong, it was necessary to use a tumor model which is more difficult to inhibit so that differences between potencies of compounds become evaluable. This is the case if therapy in the MXT(+) model is not started immediately after tumor implantation, but 3 weeks later, i.e. if established, palpable tumors are treated. The intact control group showed progressive tumor growth, whereas ovariectomy (on day 1 of therapy) caused strong retardation of growth (Fig. 2). In contrast to the prophylaxis experiment (Table 1), tamoxifen exerted only relatively weak tumor inhibition which was just significant according to tumor area and tumor weight after 3 weeks of therapy (Table 2). ZK 98.299 (10 mg/kg) and RU 486 (1 and 10 mg/kg) were, however, superior in their antitumor action to tamoxifen and caused significant inhibition of tumor weight comparable to that of ovariectomy (Table 2, Fig. 2). In the 1 mg/kg dose, RU 486 was more effective than ZK 98.299. However, in order to determine an advantage of one of these antiprogesterones in this model, a comparison using various doses below 10 mg/kg would be necessary. Treatment with the progesterones megestrol acetate (Megace) or medroxyprogesterone acetate (MPA) in doses up to 100 mg/kg did not have any significant effect on the tumor (Table 2, Fig. 2).

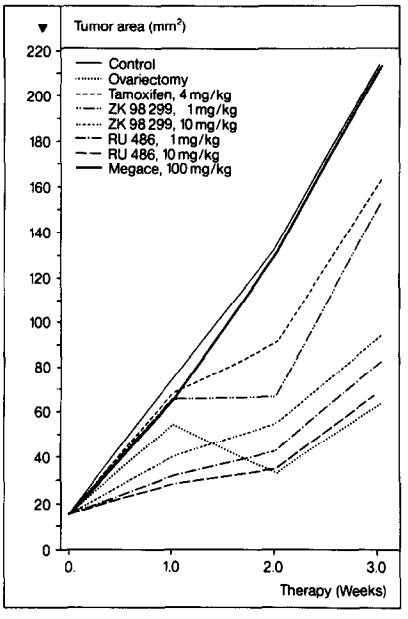


*Fig. 2. Effect of ZK 98.299, RU 486, tamoxifen, megestrol acetate (Megace) and ovariectomy on growth of established MXT(+) mammary tumors of the mouse. Compounds were administered six times weekly s.c. for 3 weeks.*

In a further experiment with established MXT(+) tumors, treatment was only performed for 2 weeks (Fig. 3). The growth curves for tamoxifen (10 mg/kg) and ovariectomy were similar to those shown in Fig. 2, i.e. pronounced inhibition by ovariectomy, but only a weak effect of tamoxifen (Table 3). Administration of the progesterone antagonists ZK 98.299 and RU 486 (50 mg/kg), however, again led to an extremely strong antitumor effect

*Table 2. Effect of ZK 98.99, RU 486, tamoxifen, megestrol acetate (Megace), medroxyprogesterone acetate (MPA) and ovariectomy on growth of the MXT(+) mammary tumor (therapy of established tumors) and on the weight of ovaries and uterus**

| | Dose (mg/kg) | Tumor area ($mm^2$) | Tumor weight (mg) | Ovarian weight (mg) | Uterine weight (mg) |
|---|---|---|---|---|---|
| Control | — | 214 ± 54 | 2561 ± 528 | 10.2 ± 2.7 | 54.4 ± 23.4 |
| Ovariectomy | — | 63 ± 48† | 706 ± 608† | — | 17.0 ± 0.9† |
| Tamoxifen | 4.0 | 161 ± 58† | 1715 ± 660† | 6.4 ± 1.3† | 66.8 ± 21.1 |
| ZK 98.299 | 1.0 | 152 ± 54† | 1453 ± 604† | 11.5 ± 1.3 | 77.0 ± 38.8 |
| | 10.0 | 93 ± 55† | 740 ± 414† | 12.4 ± 2.7 | 76.2 ± 29.0† |
| RU 486 | 1.0 | 82 ± 42† | 956 ± 541† | 11.1 ± 2.0 | 85.1 ± 45.1 |
| | 10.0 | 69 ± 24† | 844 ± 488† | 14.6 ± 2.6† | 79.2 ± 31.2† |
| MPA | 100.0 | 258 ± 116 | 2267 ± 1050 | ND | ND |
| Megace | 25.0 | 173 ± 103 | 2096 ± 1174 | ND | ND |
| | 100.0 | 212 ± 54 | 2160 ± 720 | ND | ND |

*Compounds were administered six times weekly s.c. starting *21 days* after tumor implantation (9–10 mice/group; two tumors/mouse). Tumor and organ wet weights were determined after *3* weeks of therapy.
†Significant ($P < 0.05$); *U*-test.
Ovariectomy was performed on day 21 after tumor implantation.
ND: not determined.

*Table 3. Effect of ZK 98.299, RU 486, tamoxifen and ovariectomy on growth of the MXT(+) mammary tumor (therapy of established tumors) and on the weight of ovaries and uterus**

| | Dose (mg/kg) | Tumor area ($mm^2$) | Tumor weight (mg) | Ovarian weight (mg) | Uterine weight (mg) |
|---|---|---|---|---|---|
| Control | — | 143 ± 29 | 1548 ± 484 | 7.2 ± 2.3 | 39.5 ± 15.6 |
| Ovariectomy‡ | — | 65 ± 30† | 527 ± 279† | — | 18.1 ± 1.6† |
| Tamoxifen | 10.0 | 102 ± 47 | 1145 ± 563 | 4.9 ± 1.5† | 68.8 ± 17.7† |
| ZK 98.299 | 50.0 | 50 ± 24† | 501 ± 189† | 9.6 ± 1.9† | 81.7 ± 24.4† |
| RU 486 | 50.0 | 44 ± 35† | 503 ± 291† | 9.8 ± 1.6† | 53.2 ± 40.0 |

*Compounds were administered six times weekly s.c. starting *21 days* after tumor implantation (9–10 mice/group; two tumors/mouse). Tumor and organ wet weights were determined after *2* weeks of therapy.
†Significant ($P < 0.05$); *U*-test.
‡Ovariectomy was performed on day 21 after tumor implantation.

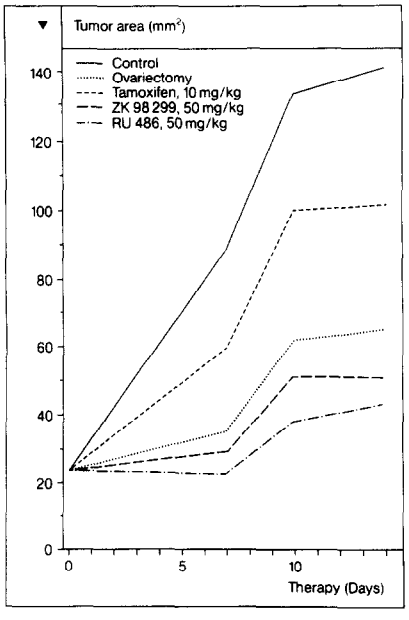


*Fig. 3. Effect of ZK 98.299, RU 486, tamoxifen and ovariectomy on growth of established MXT(+) mammary tumors of the mouse. Compounds were administered six times weekly s.c. for 2 weeks.*

that even surpassed, though not significantly, that of ovariectomy (Fig. 3, Table 3). When the results of both experiments with established MXT(+) tumors are taken together, dose-dependent antitumor activity, especially of ZK 98.299, is obvious. In this tumor model, both antiprogesterones are vastly superior to progesterones and to tamoxifen, and are at least as effective as ovariectomy.

Histological analysis of the MXT tumors showed differentiated adenocarcinomas in the control groups with acini and glandular structures varying in size (Fig. 4). The tumor cells were mostly large. Their nuclei contained one to three prominent nucleoli. Mitotic figures were common. Thus, morphology is in line with the observations of Danguy *et al.* [18]. After treatment with progesterone antagonists, many epithelial ducts were detected (Fig. 4). Each of these ducts and acinar structures contained signs of secretory activity. Mitoses were rare. Epithelial ducts and acinar structures were surrounded by spindle-shaped tumor cells. By way of contrast, only a few epithelial ducts and acinar structures were observed in the ovariectomy group. Necrobiotic cells and signs of cytolysis were numerous [19, 20].

In both experiments with established tumors, the weights of the ovaries and uteri were determined after 3 (Table 2) or 2 (Table 3) weeks of treatment. There was again an increase in the ovarian weights after therapy with ZK 98.299 and RU 486, though this was only significant at the higher doses due to the shorter treatment period compared to the prophylaxis model. Tamoxifen again significantly decreased the weights of the ovaries. In the experiment shown in Table 2, there was also an increase in uterine weight induced by the antiprogesterones in these mature, intact mice which was significant in the 10 mg dose. The same also holds true for ZK 98.299, 50 mg (Table 3), whereas the effect of RU 486 was not significant due to the high standard deviation. It was striking in all groups treated with the antiprogesterones that there was a huge variation in the uterine weights within one and the same group. In the 50 mg dose of ZK 98.299 and RU 486 about half of the uteri showed remarkable water imbibition, whereas others did not. Histological studies are in progress.

*Antitumor activity in the DMBA- and MNU-induced mammary tumor models of the rat*

For the characterization of the antitumor effect of new compounds the use of several tumor models including transplantable as well as induced carcinomas is highly recommended [21]. For this reason, ZK 98.299 and RU 486 in a dose of 10 mg/kg were tested in the DMBA- as well as the MNU-induced mammary carcinoma of the SD rat. In the DMBA model, ovariectomy on day 1 of therapy of established tumors led to almost complete inhibition of tumor growth as is shown by the individual growth curves, whereas tumors in the intact controls all

showed progressive growth (Fig. 5, note scale in the $y$-axis of the control). Treatment with ZK 98.299 caused strong and very uniform inhibition of tumor growth (Fig. 5). As can be seen from the individual growth curves, not one animal had progressing tumors. In contrast to ZK 98.299, RU 486 led to a very variable response in tumor growth (Fig. 5). Whereas some animals had progressing tumors, others showed complete remissions. This difference in tumor response between ZK 98.299 and RU 486 is also demonstrated in a boxplot of this experiment (Fig. 6). While the median response to both antiprogesterones is similar and almost comparable to ovariectomy, a certain advantage of ZK 98.299 could be its uniform tumor inhibition.

In the MNU model, established mammary tumors induced by a single dose of MNU were treated for 3 or 6 weeks (Fig. 7). The intact control revealed progressive tumor growth as was determined by the percentage change of tumor area. In sharp contrast, ovariectomy performed at day 1 of treatment, caused almost complete and highly significant ($P < 0.0001$) inhibition of tumor growth. Seven out of 10 animals were without palpable tumors.

Both antiprogesterones, ZK 98.299 and RU 486 were administered in a 10 mg/kg dose. RU 486 only caused retardation of tumor growth which was not statistically significant ($P > 0.05$) compared to the intact control after 3 weeks (Figs. 7, 8). Because of the big tumors in the intact control and in the group treated with RU 486, animals of these groups were sacrificed after 3 weeks, ZK 98.299 caused strong and highly significant ($P < 0.001$) tumor inhibition after 3 weeks (Figs. 7, 8), though its effect was not as pronounced as that of ovariectomy. ZK 98.299 treatment was, therefore, continued for a further 3 weeks. During the whole treatment period, ZK 98.299 was able to hold tumor growth at the initial value (Fig. 7). In terms of this experiment, a certain advantage of ZK 98.299 over RU 486 is obvious, though the difference between the effects of the two antiprogesterones after 3 weeks is not significant ($P = 0.06$).

Evaluation of the endocrine effects of ZK 98.299 and RU 486 on the ovaries, uterus and vagina (weight, histology) as well as the study of the morphology of the mammary tumors in these rat models will be published elsewhere.

## DISCUSSION

Taking the results in these various mammary tumor models together, antiprogesterones can be considered to be a very potent new class of mammary tumor inhibitors. In the prophylaxis model of the hormone-dependent MXT(+) tumor of the mouse, both ZK 98.299 and RU 486 achieved almost complete inhibition of tumor growth even at doses as low as 1 mg/kg. This antitumor effect is identical to that of tamoxifen (Table 1), ovariectomy of or high-dose estrogens [12]. Treatment of established MXT (+) tumors with the antiprogesterones led to an effect comparable to that of ovariectomy and superior to that of tamoxifen and of high-dose progesterones (Tables 2, 3).

ZK 98.299 and RU 486 inhibited the DMBA-induced mammary carcinoma strongly and almost comparably to ovariectomy (Fig. 6), whereas the MNU-induced mammary carcinoma was significantly inhibited only by ZK 98.299. RU 486 has already been tested in the DMBA model and found to be superior to progestins, but inferior to ovariectomy [5]. Compared to published data on the effect of tamoxifen in this model [22, 23], the inhibition accomplished by these antiprogesterones is at least as good or even better.

In the MXT(+) tumor of the mouse, the antiprogesterones increased the weights of the ovaries and in some cases consequently those of the uteri too (Tables 1–3). Similar results were reported in rats bearing the DMBA tumor after treatment with RU 486 [5]. In this study, besides significantly elevated ovarian and uterine weights, greatly increased plasma levels of LH, estradiol and progesterone were noted. Bearing this in mind, the potent tumor-inhibiting effects of the antiprogesterones in intact animals in spite of the inhibition of the negative feedback mechanism and the thereby elevated pituitary and ovarian hormones, is striking. The hypersecretion of the ovarian steroids and the blockade of the peripheral progesterone action by the competitive progesterone antagonists lead to an 'unopposed' estrogenic effect at different target organs such as the uterus and vagina.

On the basis of the above-mentioned results, an antigonadotrophic effect of the antiprogesterones in these models (chemical castration/pituitary and ovarian blockade) can be excluded. As corticosterone levels as well as adrenal weights were unchanged by RU 486 [5], participation of the antiglucocorticoid effects in the antitumor activity is unlikely [5]. Based on these results and on *in vitro* data [4], a 'direct antiprogestational effect at the level of the mammary tumor cells via the progesterone receptor' [5] seems to be the main mechanism of the antitumor action of progesterone antagonists.

Moreover, *in vitro* studies with mammary carcinoma cell lines by Bardon *et al.* [24] suggest a mechanism of antitumor action of RU 486 via a progesterone receptor-mediated, antiproliferative effect which is dissociated from its antihormone activity. This is in agreement with our *in vivo* studies in the MXT(+) model, as low doses of only 1 mg/kg, i.e. 0.02 mg/mouse, were sufficient to induce strong tumor inhibition, whereas higher doses of more than 0.1 mg/mouse are necessary to achieve a

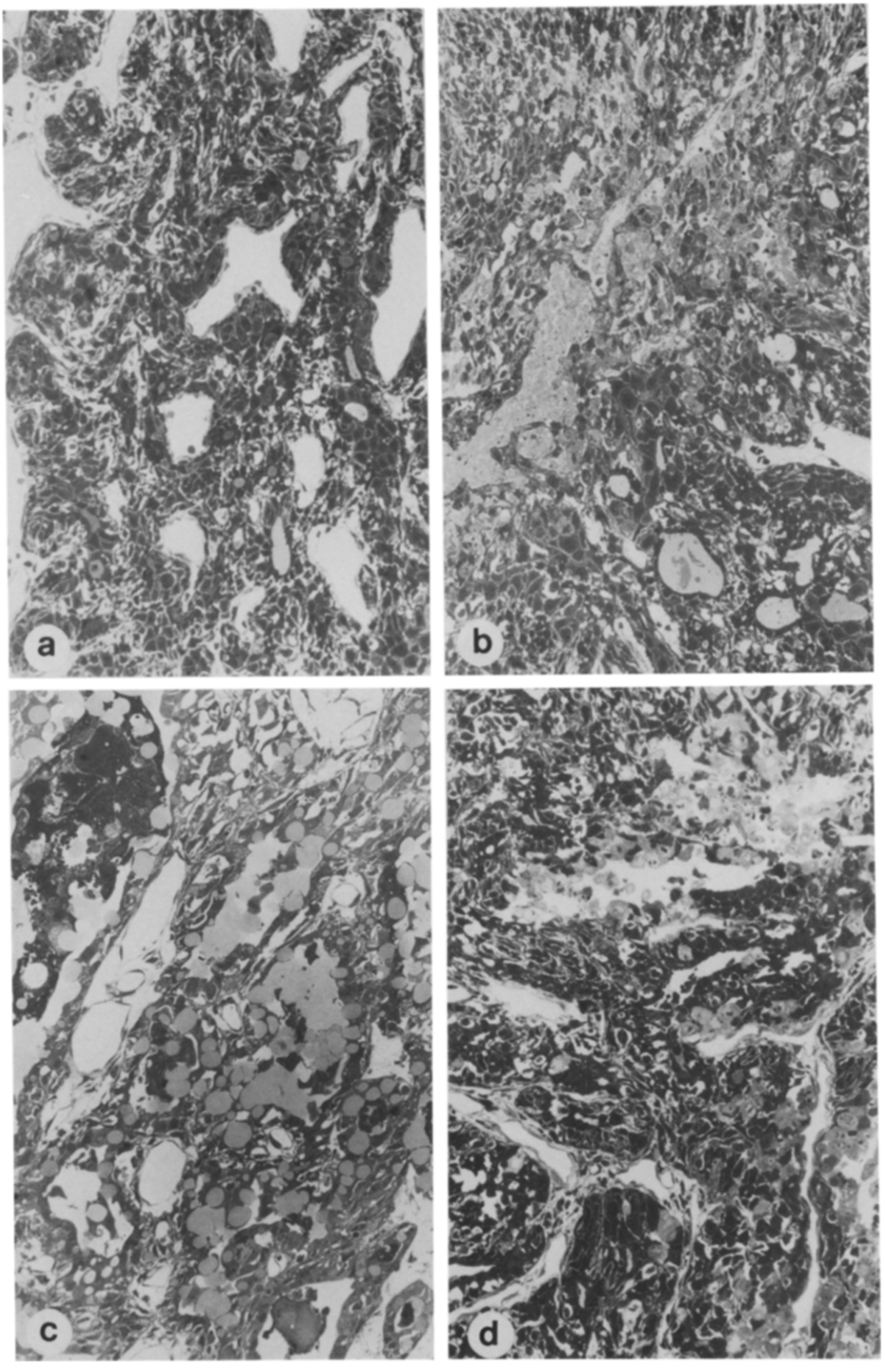


*Fig. 4. Typical histological patterns of the MXT(+) mammary tumor (experiment shown in Fig. 2). (a) Intact control: a well-differentiated carcinoma displaying acini with different sizes and shapes; stroma is scarce and a well-developed capillary network is present. (b) Ovariectomy: necrobiotic epithelial tumor cells with a mostly spindle-shaped cell body are induced; note loss of epithelial acini, and appearance of thick collagen fibers. The progesterone antagonists induced massive secretory activity and an enhanced number of acini.* 238 ×. *(c) ZK* 98.299, 10 mg/kg. *(d) RU* 486, 10 mg/kg.

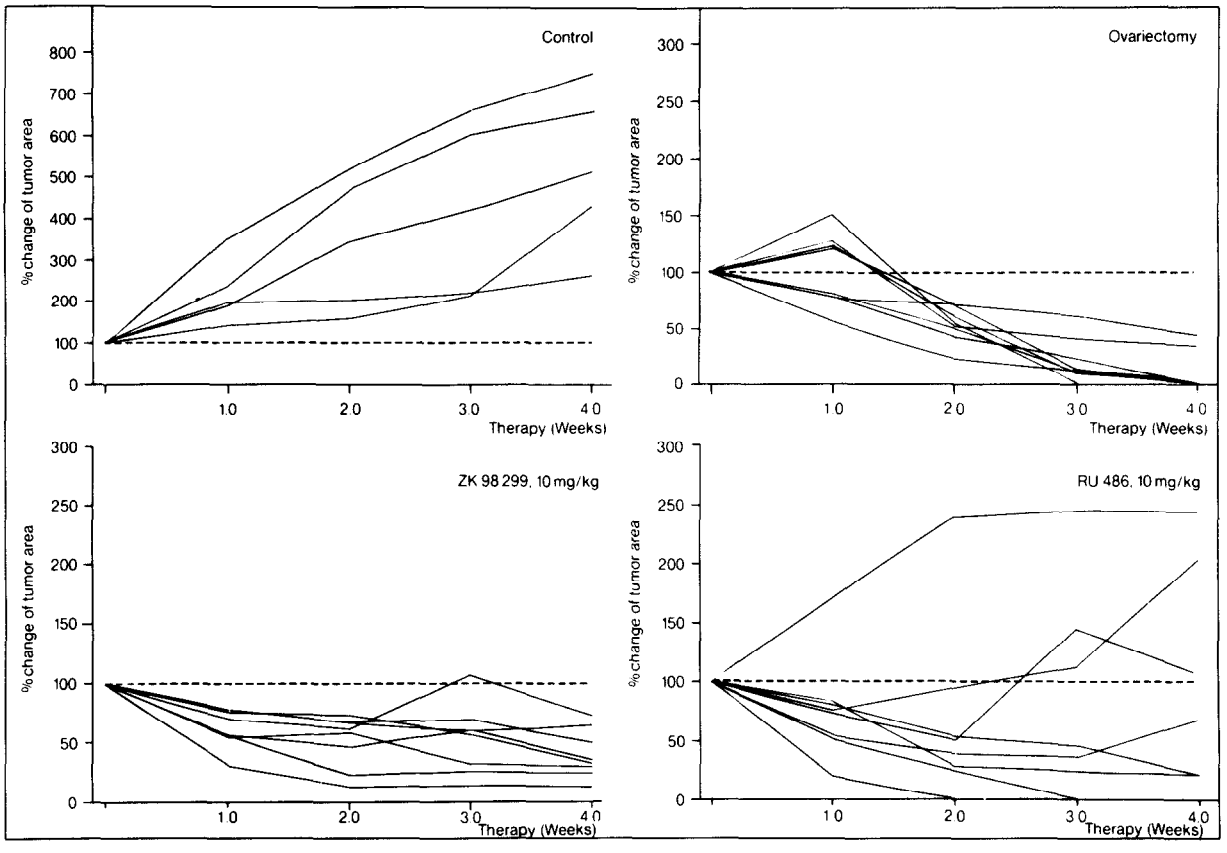


*Fig. 5. Effect of ZK 98.299, RU 486 and ovariectomy on growth of established, DMBA-induced mammary tumors of the rat. Each line represents tumor growth of one animal. Tumor area was termed 100 at start of therapy. Compounds were administered daily s.c.*

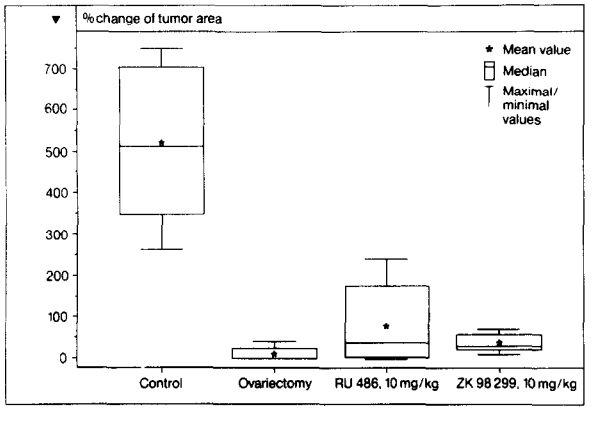


*Fig. 6. Percentage change of tumor area of DMBA-induced mammary tumor after 4 weeks of therapy: from Fig. 5 shown as box plots.*

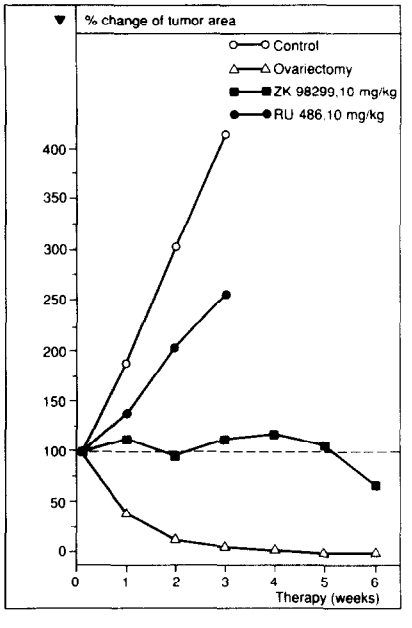


*Fig. 7. Effect of ZK 98.299, RU 486 and ovariectomy on mean percentage change of tumor area of established MNU-induced mammary tumors of the rat (10 animals/group). Compounds were administered six times weekly s.c.*

100% abortion rate in pregnant mice (S. Beier, unpublished data).

This possible 'receptor-mediated antiproliferative effect' would explain the enormous antitumor effect of antiprogesterones, as this mechanism would not mean a mere competition with the tumor growth

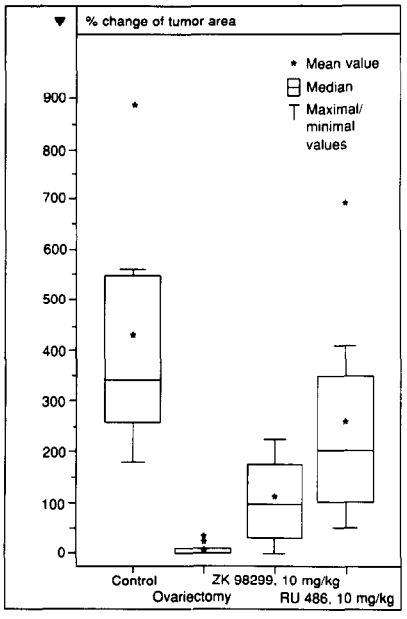


*Fig. 8. Percentage change of tumor area of MNU-induced mammary tumors after 3 weeks of therapy: from Fig. 7 shown as box plots.*

stimulating activity of progesterone, which is considered to be low [9]. Moreover, the histological findings of the MXT mammary tumors show that the treatment with both progesterone antagonists seems rather to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with secretory activity as well as towards the development of spindle-shaped necrobiotic cell populations. This is in contrast to the massive induction of cell degeneration and cytolysis in the MXT mammary tumors as a result of ovariectomy. Therefore antiprogesterones are a very interesting class of new mammary tumor inhibitors, not only because of their excellent antitumor effects in various tumor models, but also because of their possibly innovative mechanism of antitumor action.

## REFERENCES


1. Mouridsen HT, Paridaens R. Advanced breast cancer—new approaches to treatment: workshop report. *Eur J Cancer Clin Oncol* 1988, **24**, 99–105.
2. Powles TJ. Advanced breast cancer—new approaches to treatment: a review. *Eur J Cancer Clin Oncol* 1988, **24**, 95–98.
3. Maudelonde T, Romieu G, Ulmann A *et al.* First clinical trial on the use of antiprogestin RU 486 in advanced breast cancer. In: Klijn JGM *et al.*, eds. *Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti)Steroidal Agents.* New York, Raven Press, 1987, 55–59.
4. Gill PG, Vignon F, Bardon S, Derocq D, Rochefort H. Difference between R 5020 and the antiprogestin RU 486 in antiproliferative effects on human breast cancer cells. *Breast Cancer Res Treat* 1987, **10**, 37–45.
5. Bakker GH, Setyono-Han B, De Jong FH, Klijn JGM. Mifepristone in treatment of experimental breast cancer in rats. In: Klijn JGM *et al.*, eds. *Hormonal Manipulation of Cancer: Peptides, Growth Factors, and New (Anti)Steroidal Agents.* New York, Raven Press, 1987, 39–45.
6. Neef G, Beier S, Elger W, Henderson D, Wiechert R. New steroids with antiprogestational and antiglucocorticoid activities. *Steroids* 1984, **44**, 349–372.
7. Henderson D. Antiprogestational and antiglucocorticoid activities of some novel 11β-aryl substitute steroids. In: Furr BJA, Wakeling AE, eds. *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action.* London, Bailliere Tindall, 1987, 184–211.
8. Elger W, Beier S, Chwalisz K *et al.* Studies on the mechanism of action of progesterone antagonists. *J Steroid Biochem* 1986, **25**, 835–845.
9. Welsch CW. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res* 1985, **45**, 3415–3443.
10. Schneider MR, Kranzfelder G, von Angerer E *et al.* The tumor-inhibiting effect of diethylstilbestrol-3,4-oxide. *J Cancer Res Clin Oncol* 1981, **100**, 247–254.
11. Watson C, Medina D, Clark JH. Estrogen receptor characterization in a transplantable mouse mammary tumor. *Cancer Res* 1977, **37**, 3344–3348.
12. Schneider MR, Ball H, Schönenberger H. Acetoxy-substituted 1,1,2-triphenylbut-1-enes with antiestrogenic and mammary tumor inhibiting properties. *J Med Chem* 1985, **28**, 1880–1885.
13. McCormick DL, Adamowski CB, Fiks A, Moon RC. Lifetime dose–response relationship for mammary tumor induction by a single administration of *N*-methyl-*N*-nitrosourea. *Cancer Res* 1981, **41**, 1690–1694.
14. Herrman W, Wyss R, Riondel A *et al.* Effet d'un stéroïde anti-progestérone chez la femme: interruption du cycle menstruel et de la grossesse au début. *CR Acad Sci (Paris)* 1982, **294**, 933–938.
15. Von Angerer E, Prekajac J, Schneider MR, Berger MR. The inhibitory effect of 4-chloro-2-(2,6-dichloro-4-hydroxy-phenyl)-1-ethyl-6-hydroxyindole (D15413) on estrogen-dependent mammary tumors. *J Cancer Res Clin Oncol* 1985, **110**, 216–220.

16. Huber JD, Packer F, Odland GF. A basic fuchsin and alkalinized methylene blue rapid stain for epoxy-embedded tissue. *Stain Technol* 1968, **43**, 85–87.
17. Schneider MR. Acetoxy substituted 1,1,2-triphenylbut-1-enes: estrogenic, antiestrogenic and mammary tumor inhibiting activity. *J Cancer Res Clin Oncol* 1986, **112**, 119–124.
18. Danguy A, Kiss R, Leclercq G, Heuson JC, Pasteels JL . Morphology of MXT mouse mammary tumors. Correlation with growth characteristics and hormone sensitivity. *Eur J Cancer Clin Oncol* 1986, **22**, 69–75.
19. Leclercq G, Danguy A, Heuson JC. MXT mouse mammary tumor: effect of ovariectomy on estrogen receptors and histopathology. *IRCS Med Sci* 1982, **10**, 71–72.
20. Paridaens RJ, Danguy AJ, Leclercq G, Kiss R, Heuson JC. Effect of castration and 17-beta-estradiol pulse on cell proliferation in the uterus and the MXT mouse mammary tumor. *J Natl Cancer Inst* 1985, **74**, 1239–1246.
21. Bogden AE. Therapy in experimental breast cancer models. In: McGuire WL, ed. *Breast Cancer*. New York, Plenum Press, 1978, Vol. 2, 283–336.
22. Wakeling AE, Bowler J. Biology and mode of action of pure antiestrogens. *J Steroid Biochem* 1988, **30**, 141–147.
23. Schieweck K, Bhatnagar AS, Matter A. CGS 16949 A, a new nonsteroidal aromatase inhibitor: effects of hormone-dependent and -independent tumors *in vivo*. *Cancer Res* 1988, **48**, 834–838.
24. Bardon S, Vignon F, Montcourrier P, Rochefort H. Steroid receptor-mediated cytotoxicity of an antiestrogen and an antiprogestin in breast cancer cells. *Cancer Res* 1987, **47**, 1441–1448.