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SOLICITUD

PATENTE DE INVENCION

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21 EXPEDIENTE N°

64 TÍTULO "Procedimiento para la inducción de apoptosis en una célula".

51 CLASIFICACIÓN INTERNACIONAL A61K 31/57

71 SOLICITANTE Schering Aktiengesellschaft

DOMICILIO Berlín, Alemania

74 APODERADO Gustavo Hollmann Restrepo

22 BOGOTÁ, D. C. 15 de mayo de 2023

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PETITORIO

Industria y Comercio
SUPERINTENDENCIA



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FORMULARIO UNICO DE SOLICITUD DE PATENTE

PCT

ENTRADA EN FASE NACIONAL

①

SOLICITUD DE:



Patente de Invención.



Patente de Modelo de Utilidad.



Capítulo I.



Capítulo II.

②

SOLICITANTE
(71)

Nombre: SCHERING AKTIENGESSELLSCHAFT

Dirección: Müllerstrasse 178
13342 Berlin, Alemania

Nacionalidad o Domicilio: Berlin, Alemania
Lugar de Constitución: Alemania

Teléfono:

Fax:

E-mail:

IDENTIFICACIÓN

C.C.



NIT



C.E.



Otro



Cual

Número

③

REPRESENTANTE
O APODERADO

Nombre: GUSTAVO HOLLMANN RESTREPO

Dirección: Cra. 11A No. 94-76-Of. 308

Teléfono: 6161948

Fax: 6163976

E-mail:

IDENTIFICACIÓN

C.C.



NIT



C.E.



Otro



Cual

Número 2.878.023 TP 5903

④

INVENTOR (ES)
(72)

Nombre: HOFFMANN, Jens

Dirección: Grossstückenfeld 27

Nacionalidad o Domicilio 16567 Mühlenbeck, Alemania

⑤ PCT (86)

Solicitud Internacional No. PCT/EP01/12006

Fecha Octubre 17 de 2001

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Fecha Abril 25 de 2002

⑥

Título (54) "USO DE ANTIPROGESTINAS PARA LA INDUCCION DE APOPTOSIS EN UNA CELULA".

⑦

Clasificación Internacional (51) A61K 31/57

⑧

Prioridad

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Comprobante de pago No. 33,177 y 33,189

Fecha Pago 13, 2003

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

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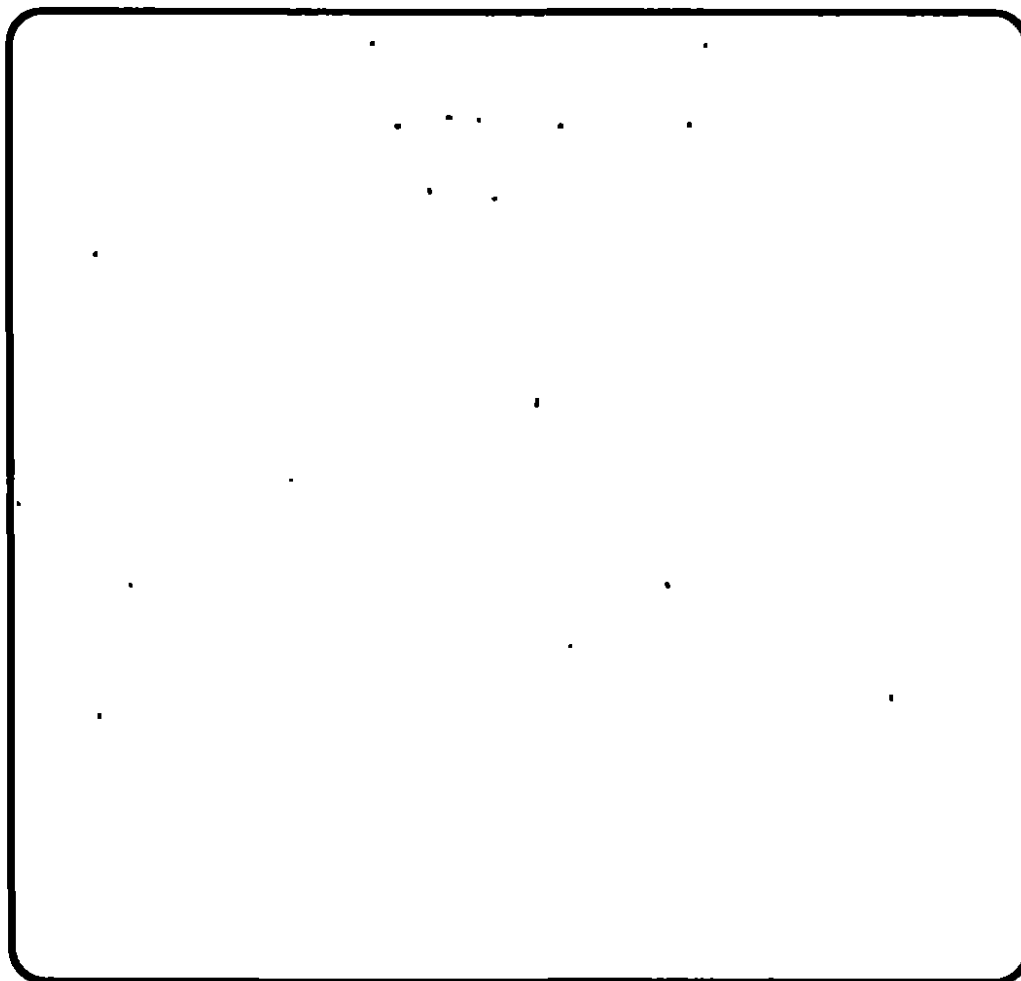
ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☒ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Poderes, si fuere el caso. **Protocolizado bajo el No. 605**
- ☒ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☒ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredita la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico
- ☐ Arte final 12x12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☒ Otros Reporte de Búsqueda Internacional.

Comprobante de pago de la tasa de la Reivindicación de Prioridad

11

FIGURA CARACTERÍSTICA:



12

Solicito la concesión de la patente,

NOMBRE: **GUSTAVO HOLLMANN RESTREPO**

FIRMA:

[Handwritten signature]

C.C. 2.878.023 B/t. T.P. 5903

Instrucciones para la presentación de los anexos, ver reverso de esta página.

**Continuación enumeración del nombre de los Inventores, con
Su correspondiente dirección, código postal y domicilio:**

RECUADRO 4

<u>Nombre</u>	<u>Dirección</u>	<u>Domicilio</u>
LICHTNER, Rosemarie Alemania	Belziger Str. 39,	10823 Berlin,
SIEMEISTER, Gerd	Reimerswalder Steig 26,	13503 Berlin, Alemania
SCHNEIDER, Martin	Schluchseestrasse 6a,	13469 Berlin, Alemania
FUHRMANN, Ulrike	Charlottenburger Ufer 6a,	10587 Berlin, Alemania

Otros anexos que se acompañan a la solicitud.

Copia notificación recepción copia registro (Forma PCT/IB/301)

Copia notificación de registro de un cambio (Forma PCT/IB/306)

Copia notificación entrega o transmisión certificado de prioridad (Forma PCT/IB/304)

Copia de notificación al solicitante sobre la comunicación de la solicitud internacional a las oficinas designadas (Forma PCT/IB/308).

Los dibujos correspondientes.

4

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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

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RECIBO OFICIAL DE CAJA : 03 - 33,177
FECHA : MAYO 13 DE 2003

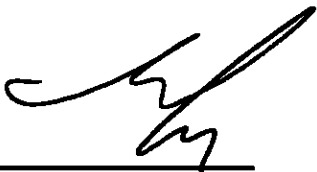
***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
GUSTAVO HOLLMAN	CONSIGNACION	BANCO POPULAR	050-00110-6	11724172	13/05/2003	5,098,000.00

***** C O N C E P T O *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 30005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	400,000.00
	TOTAL :	\$ 400,000.00

SON: CUATROCIENTOS MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

SUPERINDUSTRIA Y COMERCIO
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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

NIT : 900176089-2

RECIBO OFICIAL DE CAJA : 03 - 33,189
FECHA : MAYO 13 DE 2003

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
GUSTAVO HOLLNAN	CONSIGNACION	BANCO POPULAR	050-00110-6	11724172	13/05/2003	5,098,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	02 INVOCACION DE UNA PRIORIDAD EN NU	112,000.00
		TOTAL :	9 112,000.00

SON: CIENTO DOCE MIL PESOS

RESPONSABLE : _____

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- (21) International Application Number: PCT/EP01/12006 (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
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- (71) Applicant (for all designated States except US): SCHERING AKTIENGESELLSCHAFT [DE/DE]; Müllerstr. 178, 13353 Berlin (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): HOFFMANN, Jens [DE/DE]; Grossstückenfeld 27, 16567 Mühlenbeck (DE). LICHTNER, Rosemarie [DE/DE]; Belziger Str. 39, 10823 Berlin (DE). SIEMEISTER, Gerd [DE/DE]; Reimerswalder Steig 26, 13503 Berlin (DE). SCHNEIDER, Martin [DE/DE]; Schluchsestr. 6a, 13469 Berlin (DE). FUHRMANN, Ulrike [DE/DE]; Charlottenburger Ufer 6a, 10587 Berlin (DE).
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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WO 02/32432 A1

(54) Title: USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL

(57) Abstract: The present invention relates to methods and uses for inducing apoptosis in a cell, in particular a breast cancer cell, by the administration of antiprogesterins, in particular the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof. The invention further relates to a treatment of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, said treatment comprising an antiprogesterin, in particular the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof.

USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL

Field of the Invention

The present invention relates to the use of antiprogestins for the induction of apoptosis in a cell. In particular, the invention relates to use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell. The present invention further provides a use of antiprogestins for the preparation of a medicament for the treatment of a type of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

Background of the Invention

Antiprogestins represent a relatively new and promising class of therapeutic agents that could have significant impact on the treatment of hormone-dependent tumors and other diseases. Although antiprogestins were originally created with regard to medicinal non-surgical termination of pregnancy, certain antiprogestins have gained considerable importance, e.g., in the endocrine therapy of those breast cancers which possess receptors for progesterone (T. Maudelonde et al., in: J.G.M. Klijn et al., *Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti) Steroidal Agents*, Raven Press, New York, 1987, pp. 55-59).

This new strategy in endocrine therapy is based on the antitumor activity of antiprogestins in progesterone receptor positive human breast cancer cell lines *in vitro* and in several

hormone-dependent mammary tumors of the mouse and rat *in vivo*. In particular, the antitumor mechanism of the antiprogestins onapristone and mifepristone (RU 486) has already been investigated using the hormone-dependent MXT mammary tumor model of the mouse as well as the DMBA- and the NMU-induced mammary tumor models of the rat (M. R. Schneider et al., *Eur. J. Cancer Clin. Oncol.*, Vol. 25, No. 4, pp. 691-701, 1989; H. Michna et al., *Breast Cancer Research and Treatment* 14:275-288, 1989; H. Michna, *J. Steroid. Biochem.* Vol. 34, Nos 1-6, pp. 447-453, 1989). However, due to low activity and adverse side effects involved with e.g. mifepristone this compound could not be recommended as a single agent in the management of breast cancer (D. Perrault et al., *J. Clin. Oncol.* 1996 Oct, 14(10), pp.2709-2712). Furthermore, mifepristone exhibits strong antigluccorticoid side effects (cf. L.M. Kettel et al., *Fertil. Steril.* 1991 Sep, 56(3), pp. 402-407; X. Bertagna, *Psychoneuroendocrinology* 1997; 22 Suppl. 1, pp. 51-55).

The determination of the percentage of tumor cells in the respective phases of the cell cycle can be performed by the powerful DNA flow cytometry method (cf. G. M. Clark et al., *N. Engl. J. Med.* 320, 1989, March, pp.627-633; L. G. Dressler et al., *Cancer* 61(3), 1988, pp. 420-427 and literature cited therein). It has thus been shown that the stages of the cell cycle of a tumor cell, and specifically, the number of tumor cells in certain stages of the cycle, may be an important clinical predictor of disease progression and success of therapy. The number of cells in the S-phase of the cell cycle are particularly important in this regard.

EP 0 495 825 B1 discloses the use of antiprogestins (competitive progesterone antagonists) for the production of medicaments for the treatment of mammary carcinomas having an increased content of tumor cells in the S-phase of the cell cycle, which is considered to be a high risk factor. This is based on the observation that antiprogestins are capable of blocking the progression of tumor cells in the G₀G₁-phase of the cell cycle resulting in a substantial decrease of tumor cells in the S-phase. This effect was however not observed with the standard breast cancer therapy tamoxifen, estrogen therapy or ovariectomy. The antiprogestins tested in EP 0 495 825 B1 are 11 β -[4-N,N-

dimethylamino)-phenyl]-17 α -hydroxy-17 β -(3-hydroxypropyl)-13 α -methyl-4,9(10)-gonadien-3-one and 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(prop-1-ynyl)-4,9(10)-estradien-3-one.

- 5 17 α -fluoroalkylsteroids having strong antiprogesterin activity as well as methods for producing them are described in WO 98/34947. WO 98/34947 does not discuss or investigate the role that the 17 α -fluoroalkylsteroids disclosed therein may play in cell apoptosis or cell cycle arrest.
- 10 Given the potential value of agents that induce apoptosis in cells, e.g., in the case of tumor cells, by blocking progression in the G₀G₁-phase, it is desirable to identify further agents, e.g., antiprogesterins, having this specific mechanism of action. Such agents would have potential application in treating and preventing certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-
- 15 phase of the cell cycle.

Object of the Invention

- It is thus an object of the present invention to further investigate the mode of action of antiprogesterins in inhibiting hormone-dependent diseases such as breast cancer and to
- 20 provide a method for the targeted induction of apoptosis in cells.

- Surprisingly, the inventors have discovered that the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one (or a pharmaceutically acceptable derivative or analogue thereof) may be used for the induction
- 25 of apoptosis in a cell.

Summary of the Invention

- The present invention is based on the unexpected observation that the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one
- 30 (hereinafter referred to as "antiprogesterin (I)") induces apoptosis and cell death in the

tumor cells of standard breast cancer tumor models. It was found that antiprogesterin (I) is capable of inducing apoptosis in cells via the initiation of terminal differentiation.

Thus, the present invention provides the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the induction of apoptosis in a cell. Preferably, the induction of apoptosis is caused by the initiation of terminal differentiation. The cell is preferably a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of types of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

A further aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell *in vitro*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is a method of inducing apoptosis in a cell by administering an effective amount of antiprogesterin (I) to the cell. This method may be applied *in vitro* or *in vivo*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Due to the ability to induce cell apoptosis the antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be used for the treatment of certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Other types of cancer or hormone-dependent diseases that may be affected and treated by antiprogesterin (I) due to its ability to induce

cell apoptosis may include, e.g., breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e. diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways.

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Brief Description of the Figures

Figure 1 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in a dose-response study in the DMBA-induced mammary carcinoma of the rat, compared with a control, the antiprogesterin onapristone as well as ovariectomy.

10 The study was performed with 0.5, 2.0, 5.0 and 10.0 mg/kg s.c. daily doses of antiprogesterin (I).

Figure 2 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in the NMU-induced mammary carcinoma of the rat, compared with a control and ovariectomy. The study was performed with 0.5 and 1.0 mg/kg s.c. daily doses of antiprogesterin (I).

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Figure 3 shows the induction of apoptosis and thus the tumor growth inhibiting effect of antiprogesterin (I) in a 10 mg/kg s.c. dose on xenotransplanted human T47D tumors in scid mice, compared to a control and ovariectomy.

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Figure 4 demonstrates the induction of apoptosis and thus the tumor growth inhibiting effect of a 10 mg/kg s.c. dose of antiprogesterin (I) in the MCF-7 human breast cancer model in scid mice, compared to a control and ovariectomy.

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Figures 5A to 5F show histological data relating to the induction of apoptosis in the NMU-induced breast cancer model in rat (cf. Example 5). In particular, figure 5A shows that tumors treated with antiprogesterin (I) display ductal and acinous formations, usually filled with secretory material, compared to the control (figure 5B). Figure 5C shows untreated NMU-induced breast cancer tissue with high PCNA (proliferating cell nuclear antigen) immunoreactivity as compared to NMU-induced breast cancer tissue treated with

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antiprogesterin (I) (figure 5D), which exhibits low PCNA immunoreactivity. Figure 5E shows the appearance of apoptosis in antiprogesterin (I)-treated NMU-induced breast cancer tissue, compared to the control (figure 5E).

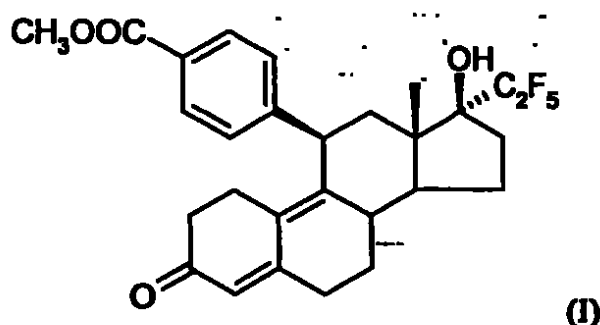
- 5 Figure 6 demonstrates the tumor growth inhibiting effect of antiprogesterin (I) in the T47D breast cancer cell line (stimulated by estradiol) with an effective threshold concentration of 10^{-9} to 10^{-8} mol/l, compared with the antiprogesterin onapristone and the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

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Detailed Description of the Invention

Antiprogesterin (I) — 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one — is represented below by formula (I):

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Antiprogesterin (I) (or a pharmaceutically acceptable derivative or analogue thereof) is a valuable pharmaceutical agent having strong antiprogesterin activity. Antiprogesterin (I) can be used according to the present invention for the induction of apoptosis in cells.

- 25 The term "antiprogesterin" in the context of the present invention is intended to primarily comprise all compounds being capable of competitively inhibiting progesterone receptors.

However, it should also encompass compounds capable of inhibiting the biosynthesis of progestins.

Pharmaceutically acceptable derivatives or analogues of antiprogestin (I) in the context of the present invention may include, for example, any one of the inventive compounds disclosed in WO 98/34947.

The studies performed in the context of the present invention show the potent tumor-inhibiting properties of the antiprogestin (I) in a variety of hormone-dependent tumor models (see Examples 1 to 6). It is further demonstrated that the tumor inhibiting activity of antiprogestin (I) as a result of the induction of apoptosis is stronger than conventional anti-tumor agents, such as, the antiestrogen tamoxifen. The treatment of breast cancer using the antiprogestin (I) according to the present invention is even superior to ovariectomy.

Application of antiprogestin (I) in the various tumor models as demonstrated below in the Examples revealed an accumulation of tumor cells in the G_0G_1 phase of the cell cycle together with a significant and biologically relevant reduction in the number of cells in the S and G_2M phase of the cell cycle. These results indicate an induction of differentiation. Differentiation-specific G_1 arrest has already been proposed earlier for other stem cell systems (see J.J. Wille Jr., *Cancer Res.* 1982, 42(12):5139-46; R.E. Scott, *J. Cell. Biol.* 1982, 94(2):400-405).

The experimental results obtained in the various tumor models revealed that treatment with antiprogestin (I) seems to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with a massive sequestering of secretory products, as well as towards spindle-shaped necrobiotic subpopulations (see Example 5 and in particular figures 5A and 5B). Whereas tumor size, mitotic index and the grade of malignancy decreased distinctly, the volume fraction of glandular structures in the tumors as well as the appearance of apoptosis increased 3-fold compared to the controls (see Example 5, figures 5E and 5F).

Without limitation to any theory, these results indicate that the main mechanism of the antitumor action of antiprogesterin (I) in the tested models is a direct progesterone-receptor-mediated antiproliferative effect at the level of the tumor cells, via the induction of terminal differentiation associated with terminal cell death. In this manner, antiprogesterin (I) appears to be capable of eliminating the intrinsic block in terminal differentiation inherent in malignant tumor cells in progesterone receptor-positive tumors. This antiproliferative effect of antiprogesterin (I) seems to be dissociated from the antihormone (antiprogesterin) activity of antiprogesterin (I).

Agents such as antiprogesterin (I) that induce apoptosis in cells, for example, in the case of tumor cells, by blocking progression in the G₀G₁-phase, have potential applications for treating and preventing numerous conditions. Such agents, including antiprogesterin (I), may be used for treating those cancers where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, such as in breast cancer.

Thus one aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for preparation of a medicament for the induction of apoptosis in a cell. In a preferred embodiment, the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof relates to a medicament for the induction of apoptosis in a tumor cell, preferably a breast tumor cell, in a human. Such medicament could be beneficial in the treatment of hormone-dependent diseases such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

The manufacture of the medicaments may be performed according to methods known in the art. Commonly known and used adjuvants as well as further suitable carriers or diluents may be used. Suitable carriers and adjuvants may be such as recommended for pharmacy, cosmetics and related fields in: *Ullmann's Encyclopedia of Technical Chemistry*, Vol. 4, (1953), pp. 1-39; *Journal of Pharmaceutical Sciences*, Vol. 52 (1963), p. 918ff; H.v.Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete";

Pharm. Ind. 2, 1961, p.72ff; Dr. H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete*, Cantor KG, Aulendorf in Württemberg, 1971.

Antiprogestins suitable for the purposes of the present invention, preferably antiprogestin
5 (I) or a pharmaceutically acceptable derivative or analogue thereof, can be incorporated into pharmaceutical compositions according to known methods of preparing galenics for oral or parenteral, e.g., intraperitoneal, intramuscular, subcutaneous or percutaneous application. They can also be implanted into tissue. Implants can comprise as inert materials e.g. biologically degradable polymers or synthetic silicones such as e.g. silicone
10 rubber.

They can be administered in the form of tablets, pills, dragees, gel capsules, granules, suppositories, implants, injectable sterile aqueous or oily solutions, suspensions or emulsions, ointments, creams, gels or by intravaginal (e.g., vaginal rings) or intrauterine
15 systems (e.g., diaphragms, loops).

For the preparation of a medicament for oral administration, the antiprogestins suitable for the purposes of the present invention as defined above can be admixed with commonly known and used adjuvants and carriers such as for example, gum arabic, talcum, starch,
20 sugars such as, e.g., mannitose, methyl cellulose, lactose, gelatin, surface-active agents, magnesium stearate, aqueous or non-aqueous excipients, paraffin derivatives, cross-linking agents, dispersants, emulsifiers, lubricants, conserving agents and flavoring agents (e.g., ethereal oils). In a pharmaceutical composition, the antiprogestin may be dispersed in a microparticle, e.g. a nanoparticulate, composition.

25 In order to further enhance the bioavailability of the active agent, the antiprogestins suitable for the purposes of the present invention as defined above can also be formulated as cyclodextrin clathrates by reacting them with α -, β - or γ -cyclodextrines or derivatives thereof according to the method as disclosed in PCT/EP95/02656.

30 For parenteral administration the antiprogestins suitable for the purposes of the present invention as defined above can be dissolved or suspended in a physiologically acceptable

diluent, such as, e.g., oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used.

- 5 The amount to be administered (i.e., a "pharmaceutically effective amount") varies within a broad range and depends on the condition to be treated and the mode of administration. It can cover any amount efficient for the intended treatment. Determining a "pharmaceutically effective amount" is within the purview of the person skilled in the art.

- 10 One unit dose may represent about 0.1 to 100 mg active agent(s). For administration to humans, the daily dose of the active agent(s) is about 0.1 to 400 mg, preferably 10 to 100 mg, most preferably 50 mg.

- 15 The medicaments can also be administered via a depot injection or an implant preparation, optionally for sustained delivery of the active agent(s).

The preferred mode of administration is oral administration. The antiprogestins for use according to the invention, and in particular, antiprogestin (I) are particularly suitable for oral administration.

20

- According to all aspects of the present invention it is also possible to combine at least one antiprogestin as defined above, in particular antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof, with at least one antiestrogen, because many hormone-dependent diseases, in particular breast cancer, exhibit not only progesterone
25 receptors, but also estrogen receptors. The antiestrogen may be administered either simultaneously with or sequentially to the antiprogestin, and in particular with/to antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof. The amount of antiprogestin and antiestrogen may be equal or one component may be more predominant than the other, such as in an antiprogestin:antiestrogen ratio of 1:50 to 50:1,
30 preferably 1:30 to 30:1, and most preferably 1:15 to 15:1.

Examples of suitable antiestrogens for use according to the invention are non-steroidal antiestrogens, such as tamoxifen and nafoxidine as well as raloxifen, faslodex and EM800. Examples of steroidal antiestrogens include those disclosed in EP 0 348 341 A and those disclosed in WO 98/07740, in particular, 11 β -flouro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-
5 pentafluoropentylthio-propylamino]-pentyl)}-estra-1,3,5(10)-trien-3,17 β -diol, or those disclosed in WO 99/33855, in particular 11 β -flouro-7 α -{5-[methyl-(7,7,8,8,9,9,10,10,10-nonafluoro-decyl)-amino]-pentyl)}-estra-1,3,5(10)-trien-3,17 β -diol or pharmaceutically acceptable derivatives or analogues thereof. Aromatase inhibitors having an anticestrogen effect, such as those disclosed on pages 7 to 8 of EP 0 495 825 B1 may also be used as
10 antiestrogens.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an
15 increased amount of tumor cells in the S-phase of the cell cycle. The number of tumor cells in the S-phase may be determined by DNA flow cytometry as described in Dressler et.al., "DNA Flow Cytometry and Prognostic Factors in 1331 Frozen Breast Cancer Specimens," *Cancer*, Vol. 61(3), 1988, pp. 420-427; see also McGuire & Dressler, "Emerging Impact of Flow Cytometry in Predicting Recurrence and Survival in Breast
20 Cancer Patients," *JNCI*, Vol. 75(3), 1985, pp. 405-409. A high risk amount of tumor cells in the S-phase indicates a particularly suitable candidate for the use according to the invention. In the case of antiprogesterin (I), the advantage arises from both the potent anti-tumor effect, as evidenced by the standard animal models (see Examples 1 to 4), and the mechanism of action of this agent of inducing apoptosis (see in particular Example 5) and
25 cell cycle arrest.

In an alternative aspect the present invention provides a method for inducing apoptosis in a cell. The cell is preferably a mammalian cell and most preferably a human cell, and the method may be applied *in vitro* or *in vivo*. Preferably, apoptosis is induced via the
30 mechanism of initiating terminal differentiation, for example, by the administration of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof. In the

method, an effective amount of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be applied to the cells in question. For example in the T47D breast cancer cell line, whose growth is stimulated by the administration of estradiol, antiprogesterin (I) induced a complete inhibition of cell growth with an effective threshold concentration of between 10^{-9} and 10^{-8} mol (see Example 6 and figure 6). This is especially surprising as the known antiprogesterin onapristone has no reducing effect on cell growth in this tumor model. Thus, antiprogesterin (I) is superior with regard to potency and efficacy to other antiprogesterins such as onapristone and to antiestrogens such as tamoxifen and even to pure antiestrogens such as 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

The role of antiprogesterin (I) in the induction of apoptosis in the cell indicates that this antiprogesterin (or a pharmaceutically acceptable derivative or analogue thereof) may be useful in a host of conditions, particularly hormone-dependent conditions, where induction of apoptosis is particularly desired. Specifically, it may be used in the treatment of such diseases as breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e., diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways. Antiprogesterins, such as antiprogesterin (I), may thus be further used for the preparation of medicaments for inducing apoptosis or cell death for the treatment of hormone-dependent diseases as already described above.

The invention is further illustrated in the examples. The following examples are not to be understood as a limitation.

Examples

30

Example 1:

Dose-response study in the DMBA-induced tumor model

Materials and Methods:

5 Immature female Sprague-Dawley rats (49 - 51 days old; 10 animals/group) were used in this study. Mammary tumors were induced by a single oral administration of 10 mg 7,12-dimethylbenz[a]anthracene (DMBA, Serva/Heidelberg). Rats with at least one established tumor with a size of more than 150 mm² were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 0,5 mg/kg s.c., 4) antiprogesterin (I), 2 mg/kg s.c., 5) antiprogesterin (I), 5 mg/kg s.c., 6) antiprogesterin (I), 10 mg/kg s.c., and 7) onapristone, 5 mg/kg, s.c., daily. As a parameter for growth inhibition the change of tumor area (in % with respect to initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used. For a further description and evaluation of the DMBA prevention model, see R.G. Mehta, *European Journal of Cancer* 36 (2000), pp. 1275-1282.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a considerable tumor regression in 90% of the animals. Treatment with antiprogesterin (I) at doses of or above 2 mg/kg resulted in a significant induction of apoptosis resulting in inhibition of tumor growth compared with the control (see fig. 2). There was a clear dose-response relationship. Whereas treatment with 0.5 mg/kg antiprogesterin (I) did not significantly prevent the tumor from growing, at 2 mg/kg maximal induction of apoptosis and thus growth inhibition was observed. In this group a complete tumor regression was seen in 50% of the rats. The effect of the highest dose of antiprogesterin (I) tested in this experiment (10 mg/kg), was comparable to that of 2 mg/kg. Onapristone (5 mg/kg, s.c.) was distinctly less effective than antiprogesterin (I) at comparable doses.

30 Conclusion:

In the DMBA-induced mammary tumor model in the rat, antiprogesterin (I) strongly induced apoptosis in the tumor cells and thus completely suppressed the tumor growth in intact animals. It was found that 2 mg/kg antiprogesterin (I) has a maximal apoptotic effect on tumor cells. Antiprogesterin (I) was distinctly superior to onapristone regarding the inhibition of tumor growth.

Example 2:

Tumor growth inhibition in NMU-induced breast cancer model in rat

Materials and Methods:

Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Starting 10 days later, rats with at least one established tumor were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 1.0 mg/kg/day, 4) antiprogesterin (I), 0.5 mg/kg/day and 5) onapristone, 5 mg/kg/day. As a parameter for growth inhibition the change of tumor area (in % of initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor growth inhibition. Treatment with antiprogesterin (I) at doses of 0.5 or 1.0 mg/kg resulted in a significant inhibition of tumor growth due to the induction of apoptosis compared with the control (see fig. 2). Onapristone (5 mg/kg) was distinctly less effective than antiprogesterin (I) at the much lower dose of 0.5 mg/kg.

Conclusions:

In the MNU-induced mammary tumor model in the rat, due to its potent ability to induce apoptosis in tumor cells, antiprogesterin (I) completely suppresses the tumor growth in intact animals. Both doses (1.0 mg/kg as well as 0.5 mg/kg) of antiprogesterin (I) have a significant apoptotic effect on tumor cells.

5

Example 3:**Human T47D breast cancer xenograft in scid mice****10 Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). T47D breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

20

Results:

In the T47D breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 3 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induces apoptosis in the tumor cells. The effect of antiprogesterin (I) is almost comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human T47D breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy) which is considered to be the maximum effective method of inhibiting growth of breast cancer in this model.

5

Example 4:**Human MCF-7 breast cancer xenograft in scid mice****10 Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). MCF7 breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

20

Results:

In the MCF7 breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 4 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induced apoptosis in the tumor cells. The effect of antiprogesterin (I) is comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human MCF7 breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy).

5

Example 5:

NMU-induced breast cancer in rat (histology, proliferation index and TUNEL assay)

Materials and Methods:

10

Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Rats with at least one established tumor with a size of more than 150 mm² were treated for 7 days by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 3 mg/kg s.c., daily. At the end of treatment tumors were excised, fixed in formalin and embedded in paraffin. Histology, proliferation index and apoptosis induction assays were performed on these resected tumors.

15

Histology: For histology tissue slides were stained with haematoxylin and analyzed by microscopy.

20

Proliferation Index: To determine the proliferation index the expression of PCNA was determined. Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear protein associated with the cell cycle. Nuclear PCNA immunoreactivity is found in the proliferative compartment of normal tissues. A monoclonal antibody, that recognizes a fixation and processing resistant epitope has been used to investigate its tissue distribution.

25

TUNEL (Apoptosis Test): The biochemical hallmark of apoptosis is the degradation of the genomic DNA, an irreversible event that results in cell death. This characteristic DNA fragmentation is the result of the activation of nuclear endonucleases, which selectively cleave DNA at sites located between nucleosomal units. These DNA strand breaks were

30

detected by enzymatic labeling of the 3'-OH termini with fluorescein-dUTP using terminal deoxynucleotidyl transferase (TUNEL, Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling, cf. Gavrieli et al., J. Cell. Biol. 119, 493, 1992).

Incorporated fluorescein was detected using the anti-fluorescein antibody alkaline

5 phosphatase conjugate followed by alkaline phosphatase substrate reaction.

Results:

Histology: After treatment with antiprogesterin (I), the tissue sections from the NMU
10 tumors displayed dysplastic ductal and acinous formations, usually filled with secretory material (Figure 5A). Moreover, the volume fraction of glandular structures in the tumors increased compared to controls (Figure 5B). In addition, the mammary tumors of antiprogesterin (I) treated animals showed the morphological features of differentiation.

15 *Proliferation Index:* PCNA immunoreactivity is high in untreated NMU-induced breast cancer tissue (Figure 5C: Untreated control). The number of cells with PCNA immunoreactivity is reduced by induction of differentiation in NMU-induced breast cancer tissue from rats treated with antiprogesterin (I) (Figure 5D). These data demonstrate
20 that in breast cancer, treatment with antiprogesterin reduces the proliferation index by induction of differentiation.

TUNEL (Apoptosis): Figure 5E demonstrates the appearance of apoptosis induced by antiprogesterin (I) in NMU-induced breast cancer tissue in comparison with untreated control (Figure 5F). It is clearly evident that antiprogesterin (I) alone was capable of
25 inducing apoptosis in the NMU-induced breast cancer tissue and thus inhibited the growth of these tumors.

Example 6:

Antiproliferative activity of antiprogesterin (I) *in vitro* in the T47D cell line

Materials and Methods:

T47D cells were grown in charcoal-treated serum supplemented with 0.1 nM E2 (estradiol) plus antiprogesterin (I) for 6 days with one medium change. Following fixation and subsequent staining with crystal violet, the absorbance was recorded and values normalized to the absorbance of untreated-controls as described in R.B. Lichtner, *J. Steroid Biochem. Mol. Biol.* 1999, 71;181-189. The TUNEL assay is performed analogous to above Example 5 with the only difference that instead of tissue sections cells that are cultivated on microscopic slides are used for the assay.

Results:

In this T47D cell line *in vitro*-test, antiprogesterin (I) exhibited potent tumor growth inhibiting activity with an effective threshold concentration as low as 10^{-9} to 10^{-8} mol/l whereas the antiprogesterin onapristone did not show any inhibiting effect. Even the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740) was distinctly less effective than antiprogesterin (I) (see figure 6).

Conclusion:

Antiprogesterin (I) according to the present invention induces complete inhibition of estradiol-stimulated T47D cell growth at very low concentrations and is thus superior regarding potency and efficacy to other antiprogesterins tested such as onapristone and to the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol.

CLAIMS

1. Use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell.
5
2. Use according to claim 1 wherein the induction of apoptosis is caused by the initiation of terminal differentiation.
10
3. Use according to any preceding claim wherein the cell is a mammalian cell.
4. Use according to claim 3 wherein the mammalian cell is a human cell.
5. Use according to any preceding claim wherein the cell is a tumor cell.
15
6. Use according to claim 5 wherein the tumor is breast cancer.
7. Use according to any preceding claims, wherein the medicament further comprises an antiestrogen.
20
8. Use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.
25
9. The use according to claim 8, where the disease is breast cancer.
10. Method of inducing apoptosis in a cell, comprising administering an effective amount of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-
30

pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable analogue or derivative thereof to a cell *in vitro*.

11. The method according to claim 10, wherein the cell is a mammalian tumor cell.

5 12. The method according to claims 10 or 11, wherein the cell is a breast cancer cell.

Fig. 1

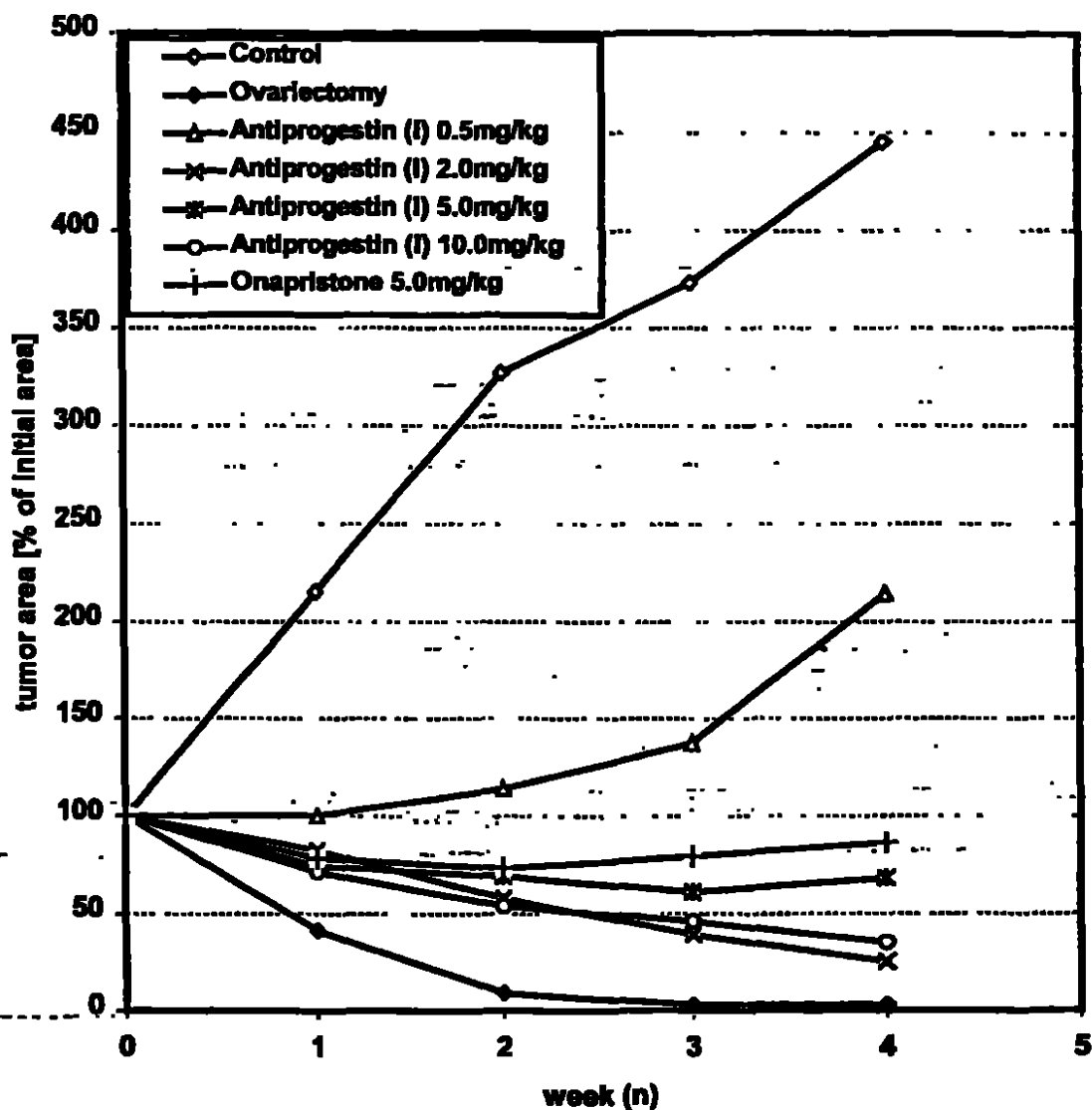


Fig. 2

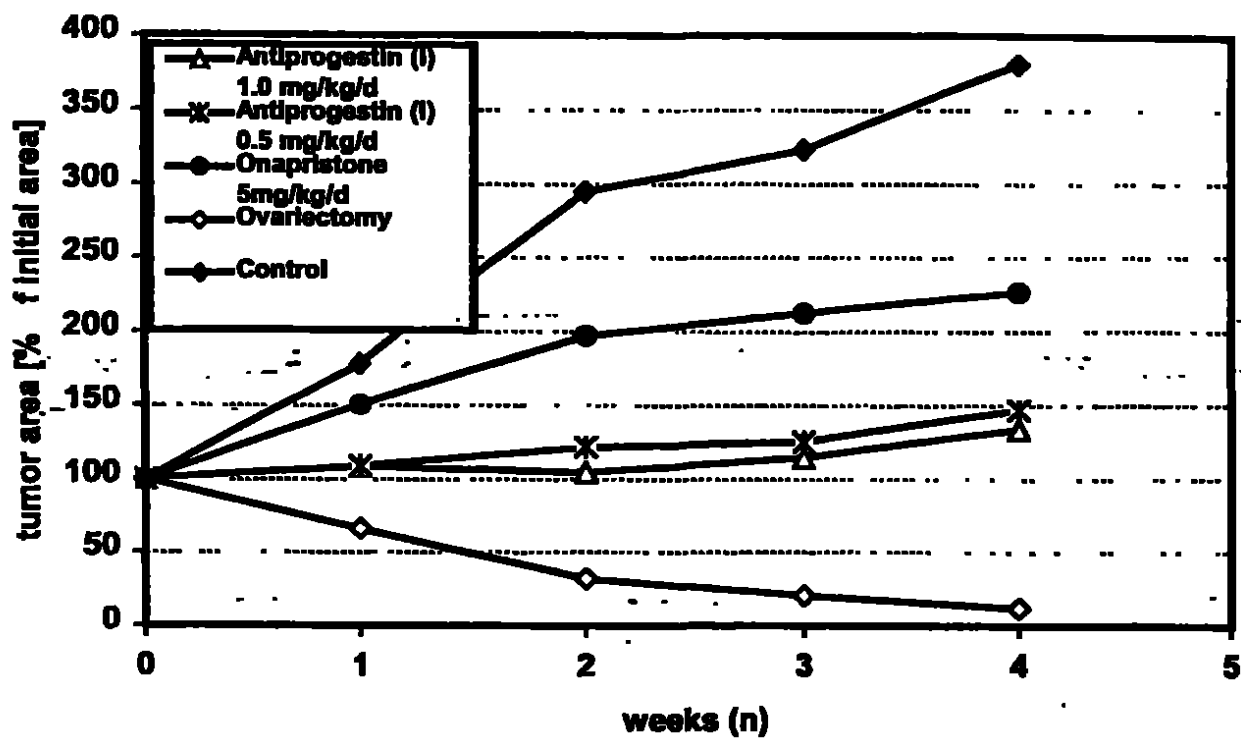
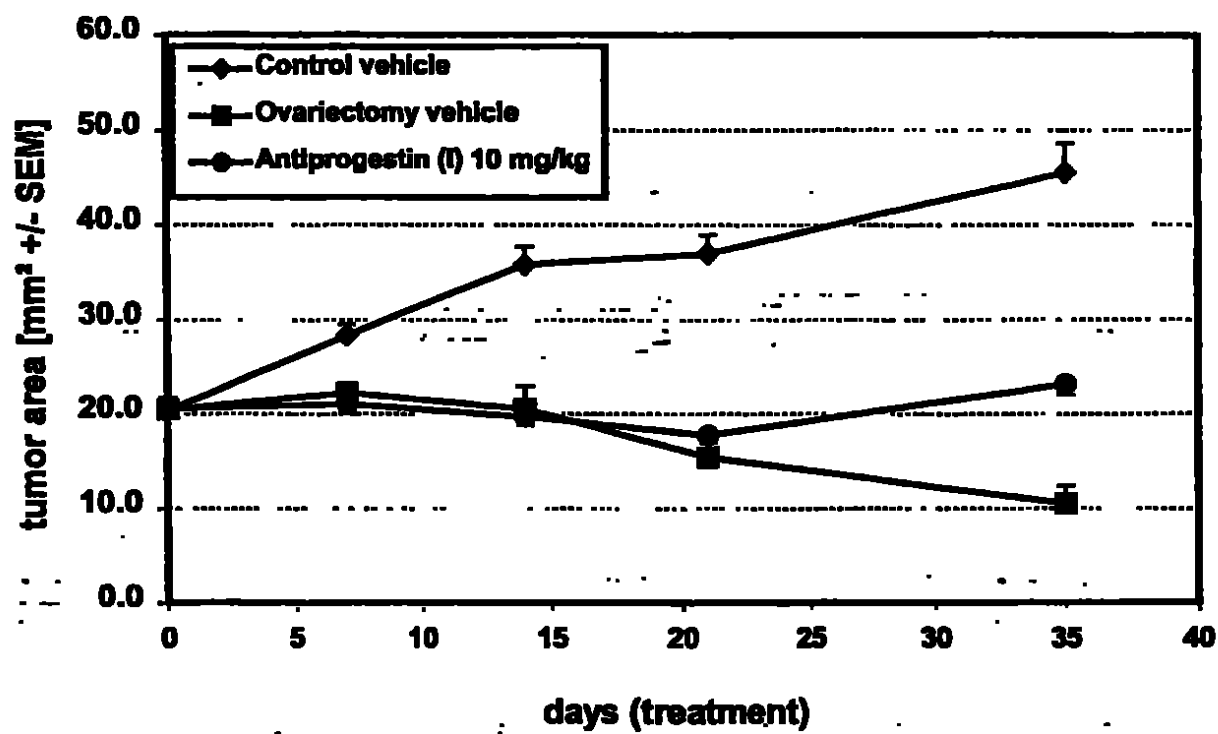


Fig. 3



4/11

Fig. 4

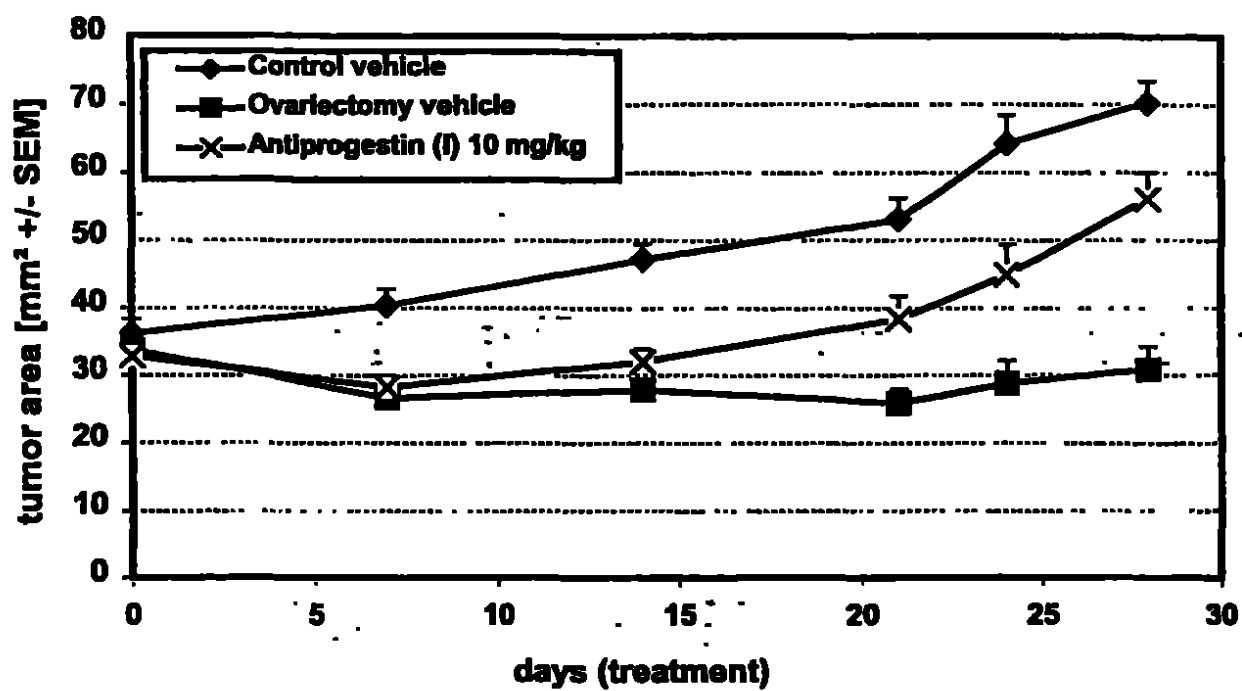


Fig. 5A

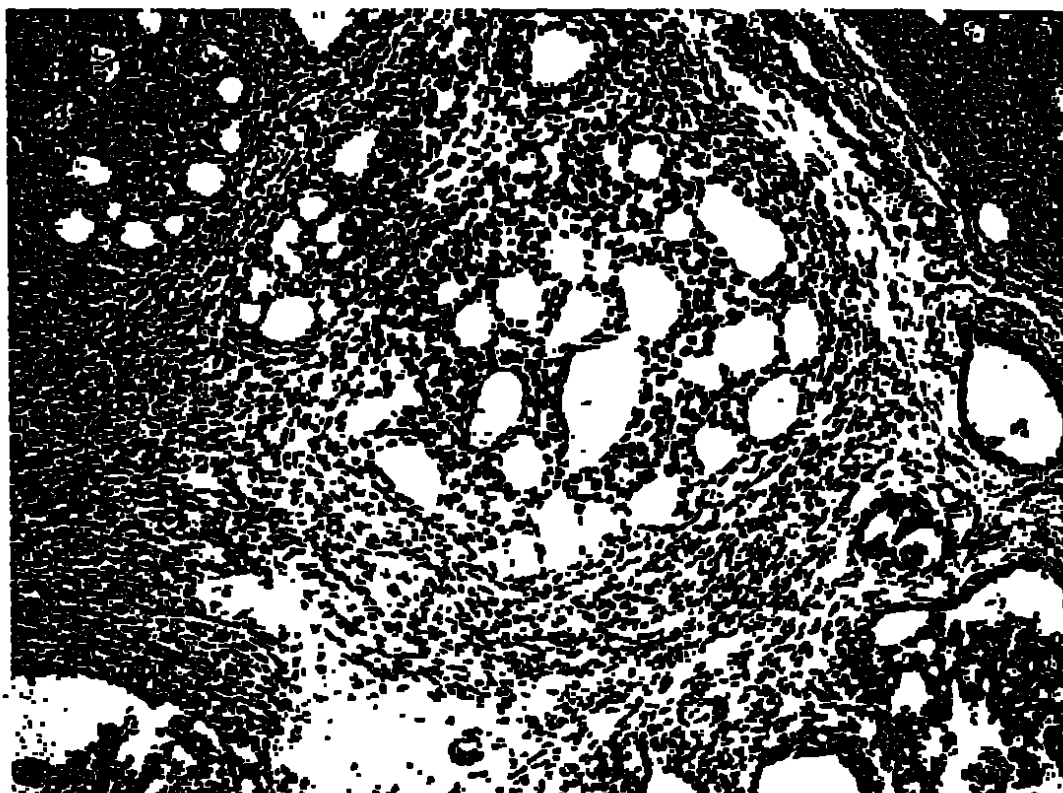


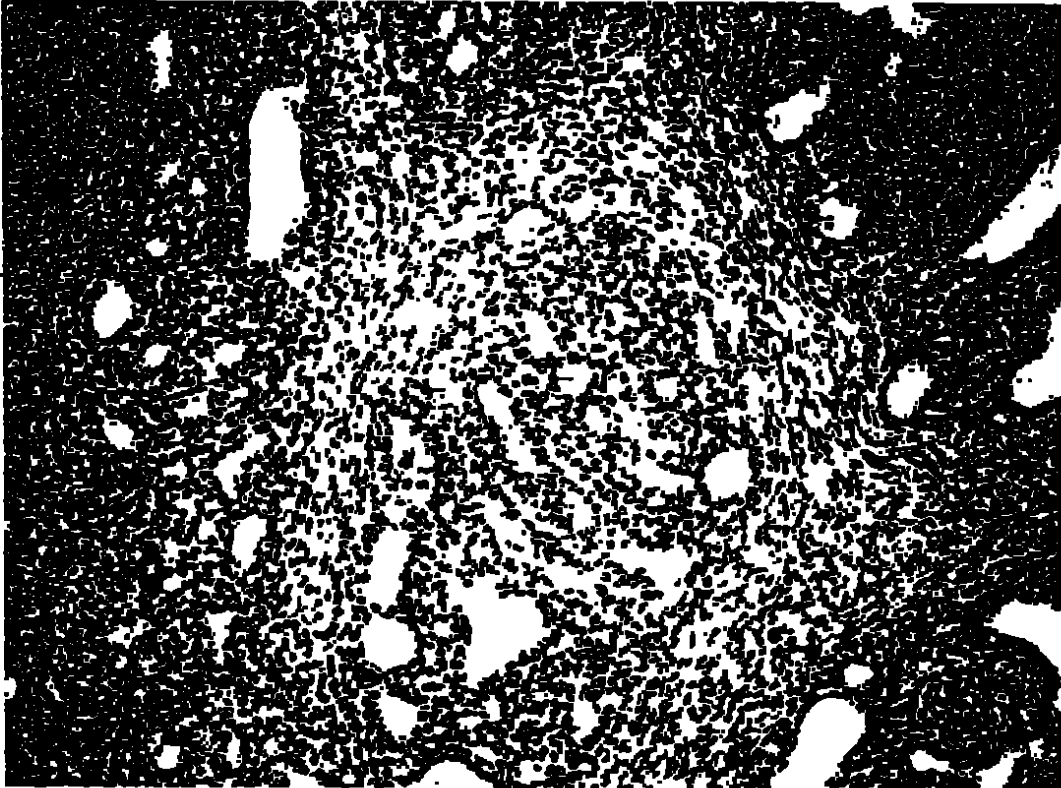
Fig. 5B

Fig. 5C

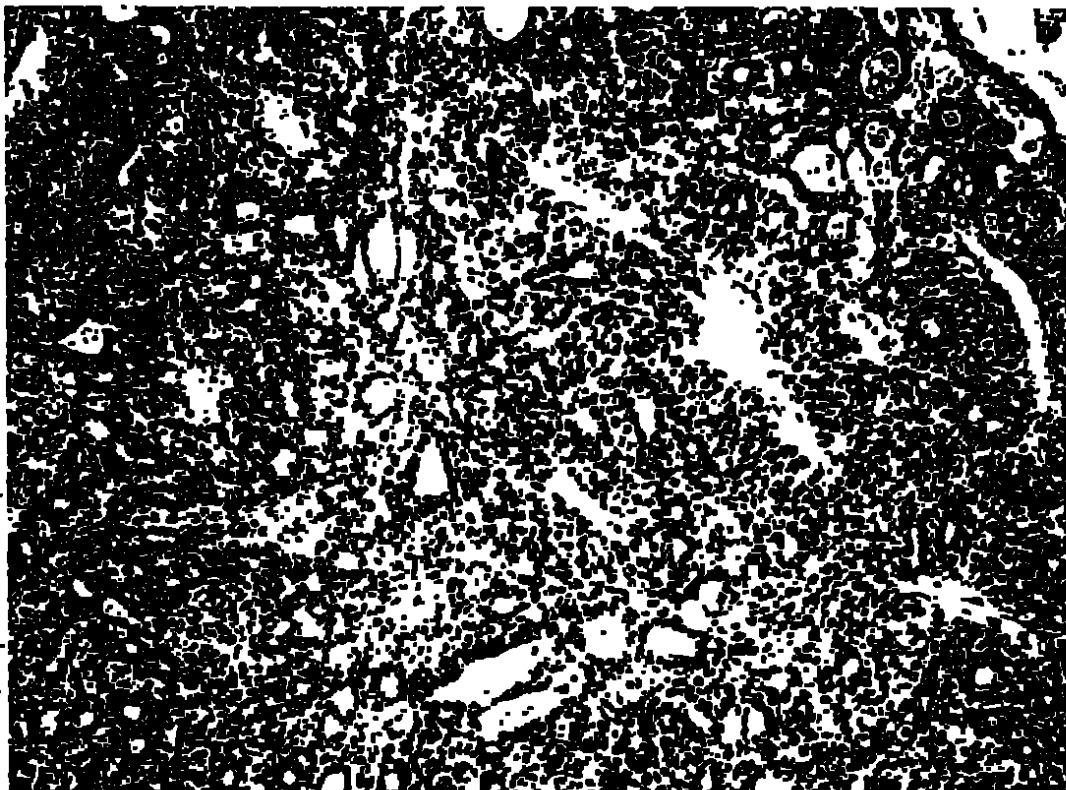


Fig. 5D

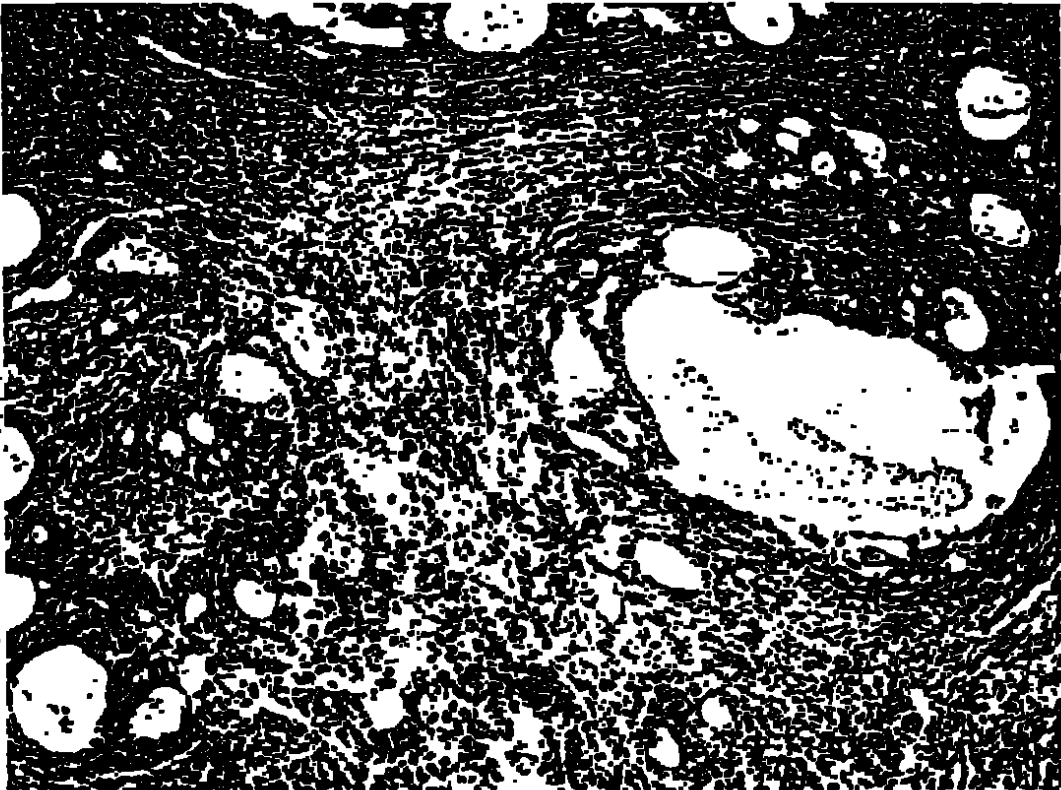


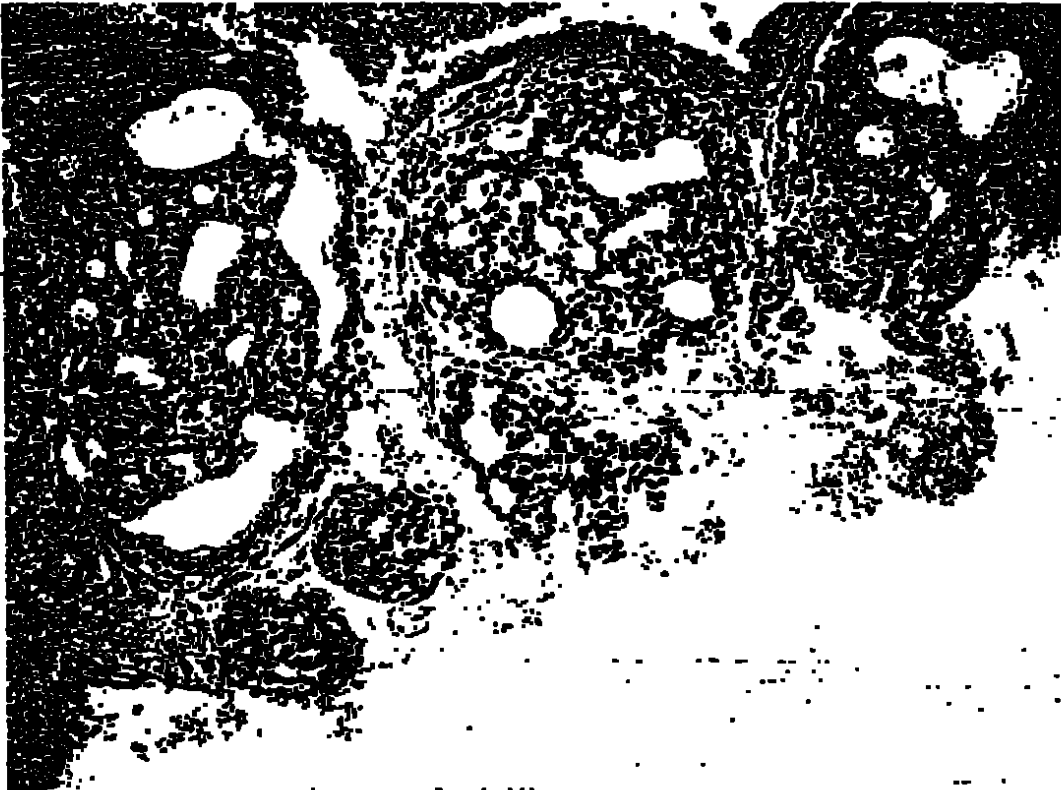
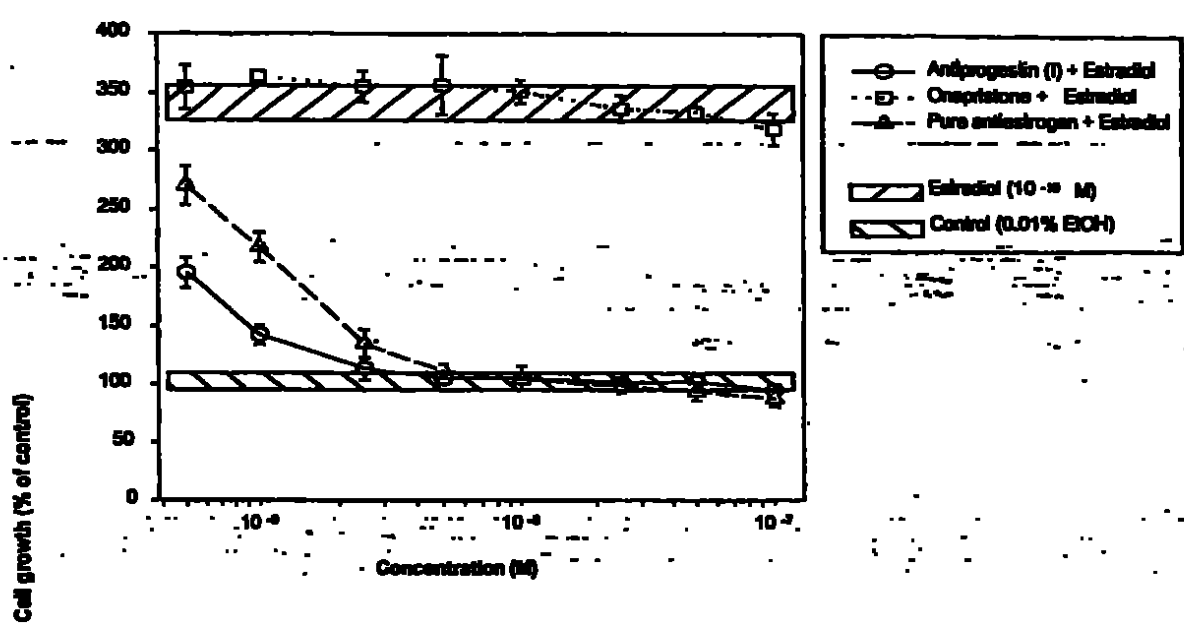
Fig. 5E

Fig. 5F



Fig. 6



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A. CLASSIFICATION OF SUBJECT MATTER

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, CHEM ABS Data, BIOSIS, MEDLINE, CANCERLIT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	DE 197 06 061 A (SCHERING AG) 13 August 1998 (1998-08-13) cited in the application page 3, line 30 page 4, line 56,57; claim 1	1-12

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex

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Name and mailing address of the ISA

European Patent Office, P. B. 5818 Pisonville 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx 31 051 epo nl,
Fax (+31-70) 340-3018

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 (71) Solicitante: (para todos los países designados excepto EEUU):
 SCHERING AKTIENGESELLSCHAFT [DE/DE], Müllerstrasse 178,
 13342 Berlin (DE).
 (72) Inventor y
 (75) Inventores en la solicitud (para EEUU solamente): HOFFMANN, Jens [DE/DE]; Gross
 stückenfeld 27, 16567 Möhlenbeck (DE). LICHTNER, Rosemarie [DE/DE]; Belziger Str. 39,
 10823 Berlin (DE). SIEMEISTER, Gerd [DE/DE]; Reimerswalder Steig 26, 13503 Berlin (DE).
 SCHNEIDER, Martin [DE/DE]; Schluchsestr. 6a, 13469 Berlin (DE). FUHRMANN; Ulrike
 [DE/DE]; Charlottenburger Ufer 6a, 10587 Berlin (DE).
 (74) Representada comunmente por: SCHERING AKTIENGESELLSCHAFT; Müllerstr. 178,
 13353 Berlin (DE).
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(54) Título: Uso de antiprogestinas para la inducción de apoptosis en una célula.

(57) Resumen: La presente invención se relaciona con métodos y usos para inducir apoptosis en una célula, en particular una célula de cáncer de mama, mediante la administración de antiprogestinas, en particular la antiprogestina 11β-(4-acetilfenil)-17β-hidroxi-17α-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona o un derivado o análogo de la misma aceptable para uso farmacéutico. La invención se relaciona además con un tratamiento para el cáncer, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular, tratamiento que comprende una antiprogestina, en particular la antiprogestina 11β-(4-acetilfenil)-17β-hidroxi-17α-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona o un derivado o análogo de la misma aceptable para uso farmacéutico.

USO DE ANTIPROGESTINAS PARA LA INDUCCIÓN DE APOPTOSIS EN UNA CÉLULA

Campo de la Invención

La presente invención se relaciona con el uso de anti-progestinas para la inducción de apoptosis en una célula. En particular, la invención se relaciona con el uso de la anti-progestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona, o un derivado o análogo de la misma aceptable para uso farmacéutico, para la inducción de apoptosis en una célula. La presente invención provee además un uso de las antiprogestinas para la preparación de un medicamento para el tratamiento de un tipo de cáncer, tal como cáncer de mama, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular.

Antecedentes de la Invención

Las antiprogestinas representan una clase relativamente nueva y prometedora de agentes terapéuticos que podrían tener un impacto significativo en el tratamiento de tumores dependientes de hormonas y otras enfermedades. Aunque las antiprogestinas fueron creadas originalmente para la terminación medicinal no quirúrgica del embarazo, algunas antiprogestinas han ganado una importancia considerable, por ejemplo, en la terapia en aquellos cánceres de mama que poseen receptores para progesterona (T. Maudelonde et al., en: J.G.M. Klijn et al., *Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti) Steroidal Agents*, Raven Press, Nueva York, 1987, páginas 55-59).

Esta nueva estrategia en terapia endócrina se basa en

la actividad antitumoral de las antiprogestinas en las líneas de células humanas de cáncer de mama positivas para receptores *in vitro* y en varios tumores mamarios dependientes de hormonas de ratón y rata *in vivo*. En particular, el mecanismo antitumoral de las antiprogestinas onapristona y mifepristona (RU 486) ya ha sido investigado usando el modelo de tumor mamario MXT dependiente de hormonas, así como los modelos de tumores mamarios inducidos por DMBA y NMU en rata (M. R. Schneider et al., *Eur. J. Cancer Clin. Oncol.*, Volumen 25, N° 4, páginas 691-701, 1989; H. Michna et al., *Brest Cancer Research and Treatment* 14:275-288, 1989; H. Michna, *J. Steroid. Biochem.* Volumen 34, N° 1-6, páginas 447-453, 1989). Sin embargo, debido a la baja actividad y a los efectos colaterales adversos relacionados con, por ejemplo, la mifepristona, no pudo recomendarse este compuesto como agente individual en el manejo del cáncer de mama (D. Perrault et al., *J. Clin. Oncol.* 1996 Oct, 14(10), páginas 2709-2712). Además, la mifepristona muestra fuertes efectos colaterales antiglucocorticoides (véanse L.M. Kettel et al., *Fertil. Steril.* 1991 Sep, 56(3), páginas 402-407; X. Bertagna, *Psychoneuroendocrinology* 1997; 22 Suplemento 1, páginas 51-55).

La determinación del porcentaje de células tumorales en las fases respectivas del ciclo celular puede realizarse por el método poderoso de citometría de flujo de ADN (véanse G. M. Clark et al., *N. Engl. J. Med.* 320, 1989, March, páginas 627-633; L. G. Dressler et al., *Cancer* 61(3), 1988, páginas 420-427 y la literatura citada en ellos). Se ha demostrado así que las etapas del ciclo celular en una célula tumoral,

y específicamente, la cantidad de células tumorales en algunas etapas del ciclo, puede ser un elemento clínico importante de la progresión de la enfermedad y el éxito de la terapia. La cantidad de células en la fase S del ciclo celular es particularmente importante en este aspecto.

La EP 0 495 825 B1 describe el uso de antiprogestinas (antagonistas competitivos de la progesterona) para la producción de medicamentos para el tratamiento de carcinomas mamarios que tienen un contenido incrementado de células tumorales en la fase S del ciclo celular, que se considera como un alto factor de riesgo. Esto se basa en la observación de que las antiprogestinas son capaces de bloquear la progresión de células tumorales en la fase G_0G_1 del ciclo celular, lo que resulta en una disminución sustancial en las células tumorales en fase S. Sin embargo, no se observó este efecto con la terapia común de cáncer tamoxifen, terapia de estrógeno u ovariectomía. Las antiprogestinas evaluadas en EP 0 495 825 B1 son 11β -[4-N,N-dimetilamino)-fenil]- 17α -hidroxi- 17β -(3-hidroxipropil)- 13α -metil-4,9(10)-gonadien-3-ona y 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(prop-1-enil)-4,9(10)-estradien-3-ona.

Los 17α -fluoroalquilesteroides que tienen una fuerte actividad antiprogestina, así como los métodos para producirlos, se describen en WO 98/34947. WO 98/34947 no describe o investiga la función que pueden tener los 17α -fluoroalquilesteroides que se describen allí en la apoptosis o la detención del ciclo celular.

Dado el valor potencial de los agentes que inducen

apoptosis en las células, por ejemplo, en el caso de las células tumorales, mediante el bloqueo de la progresión en la fase G_0G_1 , es deseable para identificar otros agentes, por ejemplo, antiprogestinas, que tienen este mecanismo de acción específico. Tales agentes tendrían una aplicación potencial en el tratamiento y la prevención de algunos tipos de cáncer, tales como cáncer de mama, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular.

Objeto de la Invención

Es así un objeto de la presente invención investigar adicionalmente el modo de acción de las antiprogestinas en la inhibición de las enfermedades dependientes de hormonas tales como el cáncer de mama y proveer un método para la inducción dirigida de apoptosis en las células.

Sorprendentemente, los inventores han descubierto que la antiprogestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona (o un derivado o análogo de la misma aceptable para uso farmacéutico) puede usarse para la inducción de apoptosis en una célula.

Resumen de la Invención

La presente invención se basa en la observación inesperada de que la antiprogestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona (conocida de aquí en adelante como "antiprogestina (I)") induce apoptosis y muerte celular en las células tumorales de modelos comunes de tumores de cáncer de mama. Se halló que la antiprogestina (I) es capaz de inducir apoptosis en células

a través del inicio de diferenciación terminal.

Así, la presente invención provee el uso de la antiprogestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, para la preparación de un medicamento para la inducción de apoptosis en una célula. Preferiblemente la inducción de apoptosis es causada por la iniciación de diferenciación terminal. La célula es preferiblemente una célula de mamífero, más preferiblemente una célula humana y más preferiblemente una célula tumoral, donde el tumor es preferiblemente cáncer de mama.

Otro aspecto de la presente invención es el uso de antiprogestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, para la preparación de un medicamento para el tratamiento de tipos de cáncer donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular.

Otro aspecto de la presente invención es el uso de antiprogestina (I) o un derivado o análogo de la misma aceptable para uso farmacéutico para la inducción de apoptosis en una célula *in vitro*. Preferiblemente, la célula es una célula de mamífero, más preferiblemente una célula humana y más preferiblemente una célula tumoral, donde el tumor es preferiblemente cáncer de mama.

Otro aspecto de la presente invención es un método para inducir apoptosis en una célula por medio de la administración de una cantidad efectiva de antiprogestina (I) a la célula. Este método puede aplicarse *in vitro* o *in vivo*. Preferiblemente, la célula es una célula de mamífero, más prefe-

blemente una célula humana y más preferiblemente una célula tumoral, donde el tumor es preferiblemente cáncer de mama.

Debido a la capacidad de inducir apoptosis celular, puede usarse la antiprogestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, para el tratamiento de algunos tipos de cáncer, tal como cáncer de mama, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular. Otros tipos de cáncer o enfermedades dependientes de hormonas que pueden ser afectados o tratados con antiprogestina (I) debido a su capacidad de inducir apoptosis celular pueden incluir, por ejemplo, cáncer de mama, cáncer de ovario, cáncer de endometrio, mieloma, infertilidad anovulatoria, meningioma, es decir, enfermedades que se originan o están influenciadas por la presencia de receptores de hormonas y/o vías dependientes de hormonas.

Breve Descripción de las Figuras

La figura 1 muestra el efecto inhibidor del crecimiento de los tumores como resultado de la inducción de apoptosis por antiprogestina (I) en un estudio de respuesta a la dosis en el carcinoma mamario inducido por DMBA en rata, en comparación con un control, la antiprogestina onapristona, así como una ovariectomía. El estudio se realizó con dosis diarias de 0,5, 2,0, 5,0 y 10,0 mg/kg s.c. de antiprogestina (I).

La figura 2 muestra el efecto inhibidor del crecimiento tumoral como resultado de la inducción de apoptosis por antiprogestina (I) en el carcinoma mamario inducido por NMU de la rata, comparado con un control y ovariectomía. El estudio

se realizó con dosis diarias de 0,5 y 1,0 mg/kg s.c. de antiprogestina (I).

La figura 3 muestra la inducción de apoptosis y, así, el efecto inhibidor de tumores de la antiprogestina (I) con una dosis de 10 mg/kg s.c. sobre tumores xenotransplantados T47D en ratones SCID, en comparación con un control y ovariectomía.

La figura 4 demuestra la inducción de apoptosis y el efecto inhibidor de tumores de una dosis de 10 mg/kg s.c. de antiprogestina (I) en el modelo de cáncer de mama humano MCF-7 en ratones SCID, en comparación con un control y ovariectomía.

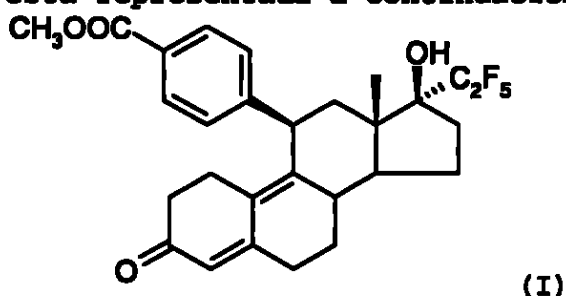
Las figuras 5A a 5F muestran datos histológicos relacionados con la inducción de apoptosis en el cáncer inducido por NMU, sobre un modelo de cáncer de mama en rata (véase el ejemplo 5). En particular, la figura 5A muestra que los tumores tratados con antiprogestina (I) muestran formaciones ductales y acinosas, comúnmente rellenas con material secretorio, en comparación con el control (figura 5B). La figura 5C muestra un tejido de cáncer de mama sin tratar inducido por NMU con inmunorreactividad elevada contra PCNA (antígeno celular nuclear proliferante), en comparación con tejido de cáncer de mama inducido por NMU tratado con antiprogestina (I) (figura 5D), que muestra una baja inmunorreactividad contra PCNA. La figura 5E muestra la aparición de la apoptosis en tejido de cáncer de mama tratado con antiprogestina (I) inducido por NMU, en comparación con el control (fig. 5E).

La figura 6 demuestra el efecto inhibidor de tumores de

la antiproggestina (I) en la línea de células de tumores T47D de cáncer de mama (estimulados por estradiol) con una concentración umbral efectiva de 10^{-9} a 10^{-8} mol/l, comparado con la antiproggestina onapristona y el antiestrógeno puro 11β -fluoro- 7α -(5-[N-metil-N-3-(4,4,5,5,5-pentafluoropentil-tio)-propilamino]-pentil)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

Descripción Detallada de la Invención

La antiproggestina (I) - 11β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona - está representada a continuación por la fórmula (I):



La antiproggestina (I) (o un derivado o análogo de la misma aceptable para uso farmacéutico) es un agente farmacéutico valioso que tiene una fuerte actividad antiproggestina. La antiproggestina (I) puede usarse de acuerdo con la presente invención para la inducción de apoptosis en células.

El término "antiproggestina" en el contexto de la presente invención pretende comprender primariamente todos los compuestos que son capaces de inhibir competitivamente los receptores de progesterona. Sin embargo, debería abarcar también compuestos capaces de inhibir la biosíntesis de progesterinas.

Los derivados o análogos aceptables para el uso farma-

céutico de la antiprogestina (I) en el contexto de la presente invención pueden incluir, por ejemplo, cualquiera de los compuestos de la invención que se describen en WO 98/34947.

Los estudios realizados en el contexto de la presente invención muestran las potentes propiedades inhibitoras de tumores de la antiprogestina (I) en una variedad de modelos de tumores dependientes de hormonas (véanse los Ejemplos 1 a 6). Se demuestra además que la actividad inhibidora de tumores de la antiprogestina (I) como resultado de la apoptosis es más fuerte que los agentes antitumorales convencionales tales como el antiestrógeno tamoxifen. El tratamiento de cáncer de mama usando la antiprogestina (I) de acuerdo con la presente invención es aún superior a la ovariectomía.

La aplicación de antiprogestina (I) en los distintos modelos de tumores, como se demuestra a continuación en los Ejemplos, reveló una acumulación de tumorales en la fase G_0G_1 del ciclo celular, junto con una reducción significativa y relevante desde el punto de vista biológico en la cantidad de células en las fases S y G_2M del ciclo celular. Estos resultados indican una inducción de la diferenciación. La detención específica para la diferenciación en G_1 se ha propuesto anteriormente para otros sistemas de células madre (véanse J.J. Wille Jr., *Cancer Res.* 1982, 42(12):5139-46; R.E. Scott, *J. Cell. Biol.* 1982, 94(2):400-405).

Los resultados experimentales hallados en los distintos modelos de tumores revelaron que el tratamiento con antiprogestina (I) parece provocar a las células tumorales poligo-

nales mitóticamente activas para que generen estructuras glandulares y acinos con un secuestro masivo de productos secretorios, así como subpoblaciones necrobióticas con forma de huso (véase el ejemplo 5 y en particular las figuras 5A y 5B). Si bien el tamaño del tumor, el índice mitótico y el grado actividad maligna disminuyeron marcadamente, la fracción de volumen de las estructuras glandulares en los tumores, así como la apariencia de la apoptosis aumentaron 3 veces en comparación con los controles (véase el Ejemplo 5 y las figuras 5E y 5F).

Sin limitarse a teoría alguna, estos resultados indican que el mecanismo principal de acción antitumoral de la anti-progestina (I) en los modelos evaluados es un efecto antiproliferativo directo mediado por receptores de progesterona en el ámbito de las células tumorales, mediante la inducción de diferenciación terminal asociada con la muerte terminal de las células. De esta manera, la antiprogestina (I) parece ser capaz de eliminar el bloqueo intrínseco en la diferenciación terminal inherente en las células tumorales malignas en tumores positivos para receptores de progesterona. Este efecto antiproliferativo de la antiprogestina (I) parece estar dissociado de la actividad antihormonal (antiprogestional) de la antiprogestina (I).

Los agentes tales como la antiprogestina (I), que inducen apoptosis en células, por ejemplo, en el caso de las células tumorales, bloqueando la progresión en la fase G_0G_1 , tienen aplicaciones potenciales para el tratamiento y la prevención de numerosas condiciones. Tales agentes, inclu-

yendo la antiproggestina (I), pueden usarse para tratar aquellos cánceres donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular, tal como en cáncer de mama.

Así, un aspecto de la presente invención es el uso de antiproggestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, para la preparación de un medicamento para la inducción de apoptosis en una célula. En una realización preferida, el uso de antiproggestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, se relaciona con un medicamento para la inducción de apoptosis en una célula tumoral, preferiblemente una célula tumoral de mama, en un humano. Tal medicamento sería beneficioso en el tratamiento de enfermedades dependientes de hormonas tales como el cáncer de mama, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular.

La fabricación de medicamentos puede realizarse de acuerdo con métodos conocidos en la técnica. Pueden usarse coadyuvantes, así como otros vehículos o diluyentes adecuados. Los vehículos o coadyuvantes adecuados pueden ser como se los recomienda para campos farmacéuticos, cosméticos y relacionados en: *Ullmann's Encyclopedia of Technical Chemistry*, Volumen 4, (1953), páginas 1-39; *Journal of Pharmaceutical Sciences*, Volumen 52 (1963), pág. 918ff; H.v. Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete"; *Pharm. Ind.* 2, 1961, p.72ff; Dr. H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende*

Gebiete, Cantor KG, Aulendorf in Württemberg, 1971.

Las antiprogestinas adecuadas para los propósitos de la presente invención, preferiblemente antiprogestina (I) o un derivado o análogo de la misma aceptable para uso farmacéutico, pueden incorporarse en composiciones farmacéuticas de acuerdo con métodos conocidos para preparar composiciones medicinales para aplicación oral o parenteral, por ejemplo, intraperitoneal, intramuscular, subcutánea o percutánea. Pueden implantarse también en el tejido. Los implantes pueden comprender materiales inertes, por ejemplo, polímeros biológicamente degradables o siliconas sintéticas tales como, por ejemplo, goma de silicona.

Pueden administrarse bajo la forma de tabletas, píldoras, grageas, cápsulas de gel, gránulos, supositorios, implantes, soluciones, suspensiones o emulsiones acuosas u oleosas estériles inyectables, ungüentos, cremas, geles o mediante sistemas intravaginales (por ejemplo, anillos vaginales) o intrauterinos (por ejemplo, diafragmas, rizos).

Para la preparación de un medicamento para administración oral, las antiprogestinas adecuadas para los propósitos de la presente invención que se definieron previamente pueden mezclarse con coadyuvantes o vehículos comúnmente conocidos tales como por ejemplo, goma arábiga, talco, almidón, azúcares tales como, por ejemplo, manitosa, metil celulosa, lactosa, gelatina, agentes activos en superficie, estearato de magnesio, excipientes acuosos o no acuosos, derivados de parafina, agentes reticulantes, dispersantes, emulsificadores, lubricantes, agentes conservantes y agentes saborizan-

tes (por ejemplo, aceites etéreos). En una composición farmacéutica, la antiprogestina puede dispersarse en una nanopartícula, por ejemplo, una composición nanoparticulada.

Con el fin de mejorar la biodisponibilidad del agente activo, las antiprogestinas adecuadas para los propósitos de la presente invención que se definieron previamente pueden formularse también como clatratos de ciclodextrinas haciéndolas reaccionar con α -, β - o γ -ciclodextrinas o derivados de las mismas de acuerdo con el método que se describe en PCT/EP95/02656.

Para administración parenteral, las antiprogestinas adecuadas para los propósitos de la presente invención que se definieron previamente pueden disolverse o suspenderse en un diluyente aceptable para uso fisiológico tal como, por ejemplo, aceites con o sin solubilizadores, agentes activos en superficie, dispersantes o emulsificadores. Como aceites, por ejemplo y sin limitación, puede usarse aceite de oliva, aceite de maní, aceite de semilla de algodón, aceite de soja, aceite de castor y aceite de sésamo.

La cantidad a administrar (es decir, una "cantidad efectiva para el uso farmacéutico") varía dentro de un amplio rango y depende de la condición a tratar y del modo de administración. Puede cubrir cualquier cantidad suficiente para el tratamiento que se pretende. La determinación de una "cantidad efectiva para el uso farmacéutico" está dentro del conocimiento de aquél entrenado en la técnica.

Una dosis unitaria puede representar entre aproximadamente 0,1 y 100 mg de agentes activos. Para una administra-

ción a humanos, la dosis diaria de agente activo es entre aproximadamente 0,1 y 400 mg, preferiblemente entre 10 y 100 mg, más preferiblemente de 50 mg.

Los medicamentos pueden también administrarse vía una inyección de depósito o una preparación de implante, opcionalmente para la administración sostenida de los agentes activos.

El modo preferido de administración es administración oral. Las antiprogestinas que se usan de acuerdo con la invención, y en particular, la antiprogestina (I), son particularmente adecuadas para administración oral.

De acuerdo con todos los aspectos de la presente invención, es también posible combinar al menos una antiprogestina como se definió previamente, en particular antiprogestina (I) o un derivado o análogo de la misma aceptable para uso farmacéutico, con al menos un antiestrógeno, ya que muchas enfermedades dependientes de hormonas, en particular cáncer de mama, muestran no solamente receptores de progesterona sino también receptores de estrógeno. El antiestrógeno puede administrarse simultáneamente con/a la antiprogestina (I) o un derivado o análogo de la misma aceptable para uso farmacéutico. La cantidad de antiprogestina y antiestrógeno puede ser igual o un componente puede ser más predominante que el otro, tal como en una relación antiprogestina: antiestrógeno ratio de 1:50 a 50:1, preferiblemente de 1:30 a 30:1, y más preferiblemente de 1:15 a 15:1.

Los ejemplos de antiestrógenos adecuados para un uso de acuerdo con la invención son antiestrógenos no esteroideos

tales como tamoxifen y nafoxidina, así como raloxifen, faslodex y EM800. Los ejemplos de antiestrógenos esteroidales incluyen aquellos descritos en EP 0 348 341 A y aquellos descritos en WO 98/07740, en particular, 11β -flouro- 7α -(5-[N-metil-N-3-(4,4,5,5,5-pentafluoropentiltio-propilamino)-pentil]-estra-1,3,5(10)-trien-3,17 β -diol, o aquellos descritos en WO 99/33855, en particular 11β -flouro- 7α -(5-[metil-(7,7,8,8,9,9,10,10,10-nonafluoro-decil)-amino]-pentil)-estra-1,3,5(10)-trien-3,17 β -diol, o los derivados o análogos de los mismos aceptables para el uso farmacéutico. Pueden usarse también como antiestrógenos inhibidores de aromatasas que tienen un efecto antiestrogénico, tales como aquellos que se describen en las pág. 7 a 8 de EP 0 495 825 B1.

Otro aspecto de la presente invención es el uso de antiprogestina (I), o un derivado o análogo de la misma aceptable para uso farmacéutico, para la preparación de un medicamento para el tratamiento de un tipo de cáncer donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular. La cantidad de células tumorales en la fase S puede determinarse por citometría de flujo de ADN, como se describe en Dressler et al., "DNA Flow Cytometry and Prognostic Factors in 1331 Frozen Breast Cancer Specimens," *Cancer*, Volumen 61(3), 1988, páginas 420-427; véase también McGuire & Dressler, "Emerging Impact of Flow Cytometry in Predicting Recurrence and Survival in Breast Cancer Patients," *JNCI*, Volumen 75(3), 1985, páginas 405-409. Una gran cantidad de riesgo de células tumorales en la fase S indica un candidato particularmente

adecuado para el uso de acuerdo con la invención. En el caso de la antiprogestina (I), la ventaja surge del potente efecto antitumoral, como lo evidencian los modelos animales comunes (véanse los Ejemplos 1 a 4), y el mecanismo de acción de este agente, incluyendo la apoptosis (véase en particular el ejemplo 5) y el arresto del ciclo celular.

En una realización alternativa, la presente invención provee un método para inducir apoptosis en una célula. La célula es preferiblemente una célula de mamífero y más preferiblemente una célula humana, y el método puede aplicarse *in vitro* o *in vivo*. Preferiblemente, la apoptosis se induce mediante el mecanismo de inducción de diferenciación terminal, p.ej., a través de la administración de antiprogestina (I) o un derivado o análogo de la misma aceptable para uso farmacéutico. En este método, puede aplicarse una cantidad efectiva de antiprogestina (I), o de un derivado o análogo de la misma aceptable para uso farmacéutico, a las células en cuestión. Por ejemplo, en la línea T47D de células de cáncer de mama, cuyo crecimiento es estimulado por la administración de estradiol, la antiprogestina (I) indujo una inhibición completa del crecimiento celular, con una concentración umbral efectiva de entre 10^{-9} y 10^{-8} mol (véase el ejemplo 6 y la fig. 6). Esto es especialmente sorprendente, ya que la antiprogestina conocida onapristona no tiene efectos reductores sobre el crecimiento de las células en este modelo tumoral. Así, la antiprogestina (I) es superior respecto de la potencia y la eficacia a otras antiprogestinas tales como la onapristona, y a antiestrógenos tales como el tamoxifen, y aún

a antiestrógenos puros tales como 11 β -fluoro-7 α -{5-[N-metil-N-3-(4,4,5,5,5-pentafluoropentiltio)-propilamino]-pentil}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

La función de la antiprogestina (I) en la inducción de la apoptosis en la célula indica que esta antiprogestina (o un derivado o análogo de la misma aceptable para uso farmacéutico) puede ser útil en un conjunto de condiciones, particularmente condiciones dependientes de hormonas, donde se desea particularmente la inducción de la apoptosis. Específicamente, puede usarse en el tratamiento de enfermedades tales como el cáncer de mama, cáncer de ovario, cáncer de endometrio, mieloma, infertilidad anovulatoria, meningioma, es decir, enfermedades que se originan o están influidas sustancialmente por la presencia de receptores de hormonas y/o vías dependientes de hormonas. Las antiprogestinas, tales como la antiprogestina (I), pueden así usarse adicionalmente para la preparación de medicamentos para inducir apoptosis o muerte celular para el tratamiento de enfermedades dependientes de hormonas, como ya se ha descrito previamente.

La invención se ilustra adicionalmente en los ejemplos. Los siguientes ejemplos no deben interpretarse como una limitación.

Ejemplos

Ejemplo 1:

Estudio de respuesta a la dosis en el modelo de tumor inducido por DMBA

Materiales y Métodos:

Se usaron ratas hembras inmaduras Sprague-Dawley (49 -

51 días de edad; 10 animales/grupo) en este estudio. Se indujeron tumores mamarios a través de una única administración oral de 10 mg de 7,12-dimetilbenz[a]antraceno (DMBA, Serva/Heidelberg). Las ratas con al menos un tumor establecido con un tamaño de más de 150 mm² se trataron por 4 semanas con: 1) solvente control, 2) ovariectomía al inicio del tratamiento, 3) antiprogestina (I), 0,5 mg/kg s.c., 4) antiprogestina(I), 2 mg/kg s.c., 5) antiprogestina(I), 5 mg/kg s.c., 6) antiprogestina(I), 10 mg/kg s.c., y 7) onapristona, 5 mg/kg, s.c., diariamente. Como parámetro de inhibición de crecimiento, se usó el cambio en el área tumoral (en % respecto del tamaño inicial del tumor) determinado por mediciones semanales de calibre. Para el análisis estadístico de las diferencias entre grupos de valores medios, se usó la prueba de Kruskal-Wallis. Para una descripción y una evaluación adicional del modelo de prevención DMBA, véase R.G. Metha, *European Journal of Cancer* 36 (2000), pág. 1275-1282.

Resultados:

En un grupo control intacto de animales, se observó un crecimiento progresivo de los tumores, mientras que la ovariectomía causó una regresión considerable de los tumores en 90% de los animales. El tratamiento con antiprogestina (I) a dosis de 2 mg/kg o superiores resultó en una inducción significativa de apoptosis, que dio como consecuencia una inhibición del crecimiento del tumor en comparación con el control (véase la fig. 2). Existió una relación clara de dosis-respuesta. Mientras que el tratamiento con 0,5 mg/kg de antiprogestina (I) no evitó significativamente el crecimien-

to del tumor, a 2 mg/kg se observó una inducción máxima de apoptosis y, por consecuencia, una inhibición del crecimiento. En este grupo, se observó una regresión completa del tumor en 50% de las ratas. El efecto de la mayor dosis de antiprogestina (I) evaluada en este experimento (10 mg/kg), fue comparable a aquél de 2 mg/kg. La onapristona (5 mg/kg, s.c.) fue notablemente menos efectiva que la antiprogestina (I) a dosis comparables.

Conclusión:

En el modelo de tumor mamario inducido por DMBA en rata, la antiprogestina (I) indujo fuertemente apoptosis en las células tumorales y suprimió así completamente el crecimiento tumoral en animales intactos. Se halló que 2 mg/kg de antiprogestina (I) presentan un efecto apoptótico máximo en células tumorales. La antiprogestina (I) fue distintivamente superior a la onapristona en lo que respecta a la inhibición del crecimiento tumoral.

Ejemplo 2:

Inhibición del crecimiento tumoral en un modelo de cáncer de mama inducido por NMU en rata

Materiales y Métodos:

Se indujeron tumores mediante una única inyección intravenosa de NMU (nitrosometilurea, 50 mg/kg) en ratas hembra Sprague-Dawley (obtenidas de Tierzucht Schönwalde, edad: 50-55 días). Comenzando 10 después, las ratas con al menos un tumor establecido se trataron por 4 semanas con: 1) solvente control, 2) ovariectomía al inicio del tratamiento, 3) antiprogestina (I), 1,0 mg/kg/día, 4) antiprogestina (I),

0,5 mg/kg/día y 5) onapristona, 5 mg/kg/día. Como parámetro de inhibición del crecimiento, se usó el cambio en el área tumoral (en % del tamaño inicial del tumor) determinado a través de mediciones semanales con calibre. Para el análisis estadístico de las diferencias entre grupos de los valores medios, se usó la prueba de Kruskal-Wallis.

Resultados:

En los animales control intactos, se observó un crecimiento progresivo de los tumores, mientras que la ovariectomía causó una inhibición completa de los tumores. El tratamiento con antiprogesterina (I) a dosis de 0,5 ó 1,0 mg/kg resultó en una inhibición significativa del crecimiento del tumor debido a la inducción de apoptosis, en comparación con el control (véase la fig. 2). La onapristona (5 mg/kg) fue notablemente menos efectiva que la antiprogesterina (I) a la dosis mucho menor de 0,5 mg/kg.

Conclusiones:

En el modelo de tumor mamario inducido por MNU en rata, debido a su potente capacidad de inducir apoptosis en células tumorales, la antiprogesterina (I) suprime completamente el crecimiento tumoral en animales intactos. Ambas dosis (1,0 mg/kg, así como 0,5 mg/kg) de antiprogesterina (I) tienen un efecto o significativo sobre las células tumorales.

Ejemplo 3:

Xenoinjerto cáncer de mama humano T47D en ratones SCID

Materiales y Métodos:

Se suplieron ratones hembra Fox Chase SCID (M&B) con pellets de estradiol (Innovative Research of America). Las

células T47D de cáncer de mama, obtenidas de cultivos celulares y suspendidas en matrigel, se implantaron por vía s.c. en la región inguinal del ratón. El tratamiento comenzó cuando los tumores tenían aproximadamente 25 mm³ de tamaño. Se continuó el tratamiento hasta que progresaran los tumores. Los grupos experimentales fueron: 1) control (vehículo), 2) ovariectomía, 3) antiprogesterina (I), 10 mg/kg s.c. Se determinó el área tumoral por mediciones de calibre. Para el análisis estadístico de las diferencias entre grupos de los valores medios, se usó la prueba de Kruskal-Wallis.

Resultados:

En el modelo T47D de cáncer de mama, la ovariectomía resultó en una inhibición considerable del crecimiento de los tumores, en comparación con el crecimiento rápido del control. La figura 3 muestra claramente que la aplicación s.c. de 10 mg/kg de antiprogesterina (I) induce apoptosis en las células tumorales. El efecto de la antiprogesterina (I) es casi comparable con el efecto de la terapia convencional de privación de estrógeno (ovariectomía).

Conclusión:

El efecto de la antiprogesterina (I) en la inducción de la apoptosis y, así, en la inhibición del crecimiento del cáncer de mama T47D xenoinjertado en ratones Fox Chase SCID es comparable al efecto de la terapia común de privación de estrógeno (ovariectomía), que se considera como el método más efectivo para inhibir el crecimiento de cáncer de mama en este modelo.

Ejemplo 4:

Xenoinjerto de cáncer de mama humano MCF-7 en ratones SCID

Materiales y Métodos:

Se suplieron ratones hembra Fox Chase SCID (M&B) con pellets de estradiol (Innovative Research of America). Las células MCF7 de cáncer de mama, obtenidas de cultivos celulares y suspendidas en matrigel, se implantaron por vía s.c. en la región inguinal del ratón. El tratamiento comenzó cuando los tumores tenían aproximadamente 25 mm³ de tamaño. Se continuó el tratamiento hasta que progresaran los tumores. Los grupos experimentales fueron: 1) control (vehículo), 2) ovariectomía, 3) antiprogestina (I), 10 mg/kg s.c. Se determinó el área tumoral por mediciones de calibre. Para el análisis estadístico de las diferencias entre grupos de los valores medios, se usó la prueba de Kruskal-Wallis.

Resultados:

En el modelo MCF7 de cáncer de mama, la ovariectomía resultó en una inhibición considerable del crecimiento de los tumores, en comparación con el crecimiento rápido del control. La figura 4 muestra claramente que la aplicación s.c. de 10 mg/kg de antiprogestina (I) induce apoptosis en las células tumorales. El efecto de la antiprogestina (I) es casi comparable con el efecto de la terapia convencional de privación de estrógeno (ovariectomía).

Conclusión:

El efecto de la antiprogestina (I) en la inducción de la apoptosis y, así, en la inhibición del crecimiento del cáncer de mama MCF7 xenoinjertado en ratones Fox Chase SCID es comparable al efecto de la terapia común de privación de

estrógeno (ovariectomía).

Ejemplo 5:

Cáncer de mama inducido por NMU en rata (histología, índice de proliferación y ensayo TUNEL)

Materiales y Métodos:

Se indujeron tumores mediante una única inyección intravenosa de NMU (nitrosometilurea, 50 mg/kg) en ratas hembra Sprague-Dawley (obtenidas de Tierzucht Schönwalde, edad: 50-55 días). Las ratas con al menos un tumor establecido con un tamaño de más de 150 mm² se trataron por 7 días con: 1) solvente control, 2) ovariectomía al inicio del tratamiento, 3) antiprogestina (I), 3 mg/kg s.c., diariamente. Al final del tratamiento, los tumores se separaron, se fijaron en formalina y se embebieron en parafina. La histología, el índice de proliferación y los ensayos de inducción de apoptosis se realizaron en estos tumores separados.

Histología: para la histología, se colorearon láminas de tejido con hematoxilina y eosina, y se las analizó por microscopía.

Índice de proliferación: para determinar el índice de proliferación, se determinó la expresión de PCNA. El antígeno celular nuclear proliferante (PCNA) es una proteína nuclear de 36 kD asociada con el ciclo celular. La inmunorreactividad nuclear contra PCNA se halla en el compartimiento proliferativo de los tejidos normales. Se usó un anticuerpo monoclonal que reconoce la fijación y el procesamiento de un epítopo resistente para investigar su distribución en los tejidos.

TUNEL (prueba de apoptosis): la marca bioquímica de la apop-

tosís es la degradación del ADN genómico, un suceso irreversible que resulta en la muerte celular. Esta fragmentación característica del ADN es el resultado de la activación de endonucleasas nucleares, que cortan selectivamente el ADN en sitios localizados entre unidades nucleosomales. Se detectaron estos quiebres en las cadenas de ADN mediante la marca enzimática del extremo 3'-OH con fluoresceína-dUTP usando desoxinucleotidil transferasa terminal (TUNEL, Terminal Deo-xinucleotidyl Transferase-Mediated dUTP Nick End Labeling, véase Gavrieli et al., J. Cell. Biol. 119, 493, 1992). Se detectó la fluoresceína incorporada usando un anticuerpo anti-fluoresceína conjugado con fosfatasa alcalina, seguido por una reacción con sustrato de fosfatasa alcalina.

Resultados:

Histología: después del tratamiento con antiprogestina (I), las secciones de tejido de los tumores NMU mostraron formaciones ductales y acinosas, comúnmente rellenas con material secretorio (Figura 5A). Más aún, la fracción de volumen de las estructuras glandulares en los tumores aumentó en comparación con los controles (Fig. 5B). además, los tumores mamarios de los animales tratados con antiprogestina (I) mostraron las características morfológicas de la diferenciación.

Índice de proliferación: la inmunorreactividad contra PCNA es alta en el tejido de cáncer de mama inducido por NMU sin tratar (Figura 5C: control sin tratar). La cantidad de células con inmunorreactividad contra PCNA es reducida por la inducción de la diferenciación en tejido de cáncer de mama inducido por NMU de ratas tratadas con antiprogestina (I)

(Figura 5D). Estos datos demuestran que en el cáncer de mama, el tratamiento con antiprogestina reduce el índice de proliferación mediante la inducción de la diferenciación.

TUNEL (Apoptosis): la figura 5E demuestra la aparición de la apoptosis inducida por antiprogestina (I) en tejido de cáncer de mama inducido por NMU en comparación con el control sin tratar (Figura 5F). Es claramente evidente que la antiprogestina (I) sola fue capaz de inducir apoptosis en el tejido de cáncer de mama inducido por NMU e inhibió así el crecimiento de estos tumores.

Ejemplo 6:

Actividad antiproliferativa de la antiprogestina (I) in vitro en la línea de células T47D

Materiales y Métodos:

Se cultivaron células T47D en suero tratado con carbón suplid con E2 0,1 nM (estradiol) más antiprogestina (I) por 6 días con un cambio de medio. Después de la fijación y coloración subsiguiente con violeta cristal, se registró la absorbancia y se normalizaron los valores con la absorbancia de los controles sin tratar como se describe en R.B. Lichtner, *J. Steroid Biochem.Mol. Biol.* 1999, 71;181-189. Se realizó el ensayo TUNEL de una forma análoga al ejemplo 5 anterior, con la única diferencia de que, en lugar de secciones de tejidos, se usaron células que se cultivaron en láminas microscópicas para el ensayo.

Resultados:

En esta prueba sobre la línea de células T47D in vitro, la antiprogestina (I) mostró una potente actividad inhibido-

ra del crecimiento tumoral con una concentración umbral tan baja como entre 10^{-9} y 10^{-8} mol/l, mientras que la antiprogestina onapristona no mostró efecto inhibitorio alguno. Aún el antiestrógeno puro 11β -fluoro- 7α -{5-[N-metil-N-3-(4,4,5,5,5-pentafluoropentiltio)-propilamino]-pentil}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740) fue notablemente menos efectivo que la antiprogestina (I) (véase la figura 6).

Conclusión:

La antiprogestina (I) de acuerdo con la presente invención induce una inhibición completa del crecimiento estimulado por estradiol de células T47D a concentraciones muy bajas, y es así superior en lo que respecta a potencia y eficacia a otras antiprogestinas evaluadas, tal como onapristona, y al antiestrógeno puro 11β -fluoro- 7α -{5-[N-metil-N-3-(4,4,5,5,5-pentafluoropentiltio)-propilamino]-pentil}-estra-1,3,5(10)-trien-3,17 β -diol.

REIVINDICACIONES

1. Uso de la antiprogestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona, o un derivado o análogo de la misma aceptable para uso farmacéutico, que es para la inducción de apoptosis en una célula.

2. El uso de acuerdo con la reivindicación 1 en donde la inducción de apoptosis es causada por la iniciación de diferenciación terminal.

3. El uso de acuerdo con cualquier reivindicación precedente en donde la célula es una célula de mamífero.

4. El uso de acuerdo con la reivindicación 3 en donde la célula de mamífero es una célula humana.

5. El uso de acuerdo con cualquier reivindicación precedente en donde la célula es una célula tumoral.

6. El uso de acuerdo con la reivindicación 5 en donde el tumor es cáncer de mama.

7. El uso de acuerdo con cualquier reivindicación precedente en donde el medicamento comprende además un anti-estrógeno.

8. El uso de la antiprogestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona, o un derivado o análogo de la misma aceptable para uso farmacéutico, que es para la preparación de un medicamento para el tratamiento de un tipo de cáncer, donde un indicador de alto riesgo es una cantidad incrementada de células tumorales en la fase S del ciclo celular.

9. El uso de acuerdo con la reivindicación 8 en donde

la enfermedad es cáncer de mama.

10. Un método para inducir apoptosis en una célula que comprende administrar una célula efectiva de la antiprogestina 11β -(4-acetilfenil)- 17β -hidroxi- 17α -(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona, o un análogo o derivado de la misma aceptable para uso farmacéutico, a una célula *in vitro*.

11. El método de acuerdo con la reivindicación 10 en donde la célula es una célula tumoral de mamífero.

12. El método de acuerdo con las reivindicaciones 10 u 11, en donde la célula es una célula de cáncer de mama.

5 USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL

Field of the Invention

10 The present invention relates to the use of antiprogestins for the induction of apoptosis in a cell. In particular, the invention relates to use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell. The present invention further provides a use of antiprogestins for the preparation of a
15 medicament for the treatment of a type of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

20 Background of the Invention

Antiprogestins represent a relatively new and promising class of therapeutic agents that could have significant impact on the treatment of hormone-dependent tumors and other diseases. Although antiprogestins were originally created with regard to medicinal non-surgical termination of pregnancy, certain antiprogestins have gained considerable importance, e.g., in the endocrine therapy of those breast cancers which possess receptors for progesterone (T. Maudelonde et al., in: J.G.M. Klijn et al., *Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti) Steroidal Agents*, Raven Press, New York, 1987, pp. 55-59).

30 This new strategy in endocrine therapy is based on the antitumor activity of antiprogestins in progesterone receptor positive human breast cancer cell lines *in vitro* and in several

hormone-dependent mammary tumors of the mouse and rat *in vivo*. In particular, the antitumor mechanism of the antiprogestins onapristone and mifepristone (RU 486) has already been investigated using the hormone-dependent MXT mammary tumor model of the mouse as well as the DMBA- and the NMU-induced mammary tumor models of the rat (M. R. Schneider et al., *Eur. J. Cancer Clin. Oncol.*, Vol. 25, No. 4, pp. 691-701, 1989; H. Michna et al., *Breast Cancer Research and Treatment* 14:275-288, 1989; H. Michna, *J. Steroid. Biochem.* Vol. 34, Nos 1-6, pp. 447-453, 1989). However, due to low activity and adverse side effects involved with e.g. mifepristone this compound could not be recommended as a single agent in the management of breast cancer (D. Perrault et al., *J. Clin. Oncol.* 1996 Oct, 14(10), pp.2709-2712). Furthermore, mifepristone exhibits strong antiglucocorticoid side effects (cf. L.M. Kettel et al., *Fertil. Steril.* 1991 Sep, 56(3), pp. 402-407; X. Bertagna, *Psychoneuroendocrinology* 1997; 22 Suppl. 1, pp. 51-55).

The determination of the percentage of tumor cells in the respective phases of the cell cycle can be performed by the powerful DNA flow cytometry method (cf. G. M. Clark et al., *N. Engl. J. Med.* 320, 1989, March, pp.627-633; L. G. Dressler et al., *Cancer* 61(3), 1988, pp. 420-427 and literature cited therein). It has thus been shown that the stages of the cell cycle of a tumor cell, and specifically, the number of tumor cells in certain stages of the cycle, may be an important clinical predictor of disease progression and success of therapy. The number of cells in the S-phase of the cell cycle are particularly important in this regard.

EP 0 495 825 B1 discloses the use of antiprogestins (competitive progesterone antagonists) for the production of medicaments for the treatment of mammary carcinomas having an increased content of tumor cells in the S-phase of the cell cycle, which is considered to be a high risk factor. This is based on the observation that antiprogestins are capable of blocking the progression of tumor cells in the G₀G₁-phase of the cell cycle resulting in a substantial decrease of tumor cells in the S-phase. This effect was however not observed with the standard breast cancer therapy tamoxifen, estrogen therapy or ovariectomy. The antiprogestins tested in EP 0 495 825 B1 are 11 β -[4-N,N-

dimethylamino)-phenyl]-17 α -hydroxy-17 β -(3-hydroxypropyl)-13 α -methyl-4,9(10)-gonadien-3-one and 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(prop-1-ynyl)-4,9(10)-estradien-3-one.

5 17 α -fluoroalkylsteroids having strong antiprogesterin activity as well as methods for producing them are described in WO 98/34947. WO 98/34947 does not discuss or investigate the role that the 17 α -fluoroalkylsteroids disclosed therein may play in cell apoptosis or cell cycle arrest.

10 Given the potential value of agents that induce apoptosis in cells, e.g., in the case of tumor cells, by blocking progression in the G₀G₁-phase, it is desirable to identify further agents, e.g., antiprogesterins, having this specific mechanism of action. Such agents would have potential application in treating and preventing certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-
15 phase of the cell cycle.

Object of the Invention

It is thus an object of the present invention to further investigate the mode of action of antiprogesterins in inhibiting hormone-dependent diseases such as breast cancer and to
20 provide a method for the targeted induction of apoptosis in cells.

Surprisingly, the inventors have discovered that the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one (or a pharmaceutically acceptable derivative or analogue thereof) may be used for the induction
25 of apoptosis in a cell.

Summary of the Invention

The present invention is based on the unexpected observation that the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one
30 (hereinafter referred to as "antiprogesterin (I)") induces apoptosis and cell death in the

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tumor cells of standard breast cancer tumor models. It was found that antiprogesterin (I) is capable of inducing apoptosis in cells via the initiation of terminal differentiation.

Thus, the present invention provides the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the induction of apoptosis in a cell. Preferably, the induction of apoptosis is caused by the initiation of terminal differentiation. The cell is preferably a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of types of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

A further aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell *in vitro*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is a method of inducing apoptosis in a cell by administering an effective amount of antiprogesterin (I) to the cell. This method may be applied *in vitro* or *in vivo*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Due to the ability to induce cell apoptosis the antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be used for the treatment of certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Other types of cancer or hormone-dependent diseases that may be affected and treated by antiprogesterin (I) due to its ability to induce

cell apoptosis may include, e.g., breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e. diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways.

5

Brief Description of the Figures

Figure-1 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in a dose-response study in the DMBA-induced mammary carcinoma of the rat, compared with a control, the antiprogesterin onapristone as well as ovariectomy. The study was performed with 0.5, 2.0, 5.0 and 10.0 mg/kg s.c. daily doses of antiprogesterin (I).

Figure 2 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in the NMU-induced mammary carcinoma of the rat, compared with a control and ovariectomy. The study was performed with 0.5 and 1.0 mg/kg s.c. daily doses of antiprogesterin (I).

Figure-3 shows the induction of apoptosis and thus the tumor growth inhibiting effect of antiprogesterin (I) in a 10 mg/kg s.c. dose on xenotransplanted human T47D tumors in scid mice, compared to a control and ovariectomy.

Figure 4 demonstrates the induction of apoptosis and thus the tumor growth inhibiting effect of a 10 mg/kg s.c. dose of antiprogesterin (I) in the MCF-7 human breast cancer model in scid mice, compared to a control and ovariectomy.

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Figures 5A to 5F show histological data relating to the induction of apoptosis in the NMU-induced breast cancer model in rat (cf. Example 5). In particular, figure 5A shows that tumors treated with antiprogesterin (I) display ductal and acinous formations, usually filled with secretory material, compared to the control (figure 5B). Figure 5C shows untreated NMU-induced breast cancer tissue with high PCNA (proliferating cell nuclear antigen) immunoreactivity as compared to NMU-induced breast cancer tissue treated with

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antiprogesterin (I) (figure 5D), which exhibits low PCNA immunoreactivity. Figure 5E shows the appearance of apoptosis in antiprogesterin (I)-treated NMU-induced breast cancer tissue, compared to the control (figure 5E).

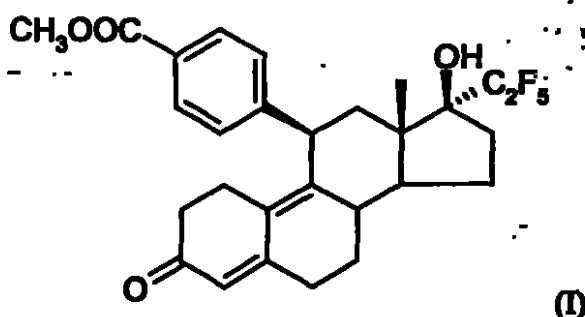
- 5 Figure 6 demonstrates the tumor growth inhibiting effect of antiprogesterin (I) in the T47D breast cancer cell line (stimulated by estradiol) with an effective threshold concentration of 10^{-9} to 10^{-8} mol/L, compared with the antiprogesterin-onapristone and the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

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Detailed Description of the Invention

Antiprogesterin (I) — 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one — is represented below by formula (I):

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Antiprogesterin (I) (or a pharmaceutically acceptable derivative or analogue thereof) is a valuable pharmaceutical agent having strong antiprogesterin activity. Antiprogesterin (I) can be used according to the present invention for the induction of apoptosis in cells.

- 25 The term "antiprogesterin" in the context of the present invention is intended to primarily comprise all compounds being capable of competitively inhibiting progesterone receptors.

However, it should also encompass compounds capable of inhibiting the biosynthesis of progestins.

Pharmaceutically acceptable derivatives or analogues of antiprogesterin (I) in the context of the present invention may include, for example, any one of the inventive compounds disclosed in WO 98/34947.

The studies performed in the context of the present invention show the potent tumor-inhibiting properties of the antiprogesterin (I) in a variety of hormone-dependent tumor models (see Examples 1 to 6). It is further demonstrated that the tumor inhibiting activity of antiprogesterin (I) as a result of the induction of apoptosis is stronger than conventional anti-tumor agents, such as, the antiestrogen tamoxifen. The treatment of breast cancer using the antiprogesterin (I) according to the present invention is even superior to ovariectomy.

Application of antiprogesterin (I) in the various tumor models as demonstrated below in the Examples revealed an accumulation of tumor cells in the G₀G₁ phase of the cell cycle together with a significant and biologically relevant reduction in the number of cells in the S and G₂M phase of the cell cycle. These results indicate an induction of differentiation. Differentiation-specific G₁ arrest has already been proposed earlier for other stem cell systems (see J.J. Wille Jr., *Cancer Res.* 1982, 42(12):5139-46; R.E. Scott, *J. Cell. Biol.* 1982, 94(2):400-405).

The experimental results obtained in the various tumor models revealed that treatment with antiprogesterin (I) seems to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with a massive sequestering of secretory products, as well as towards spindle-shaped necrobiotic subpopulations (see Example 5 and in particular figures 5A and 5B). Whereas tumor size, mitotic index and the grade of malignancy decreased distinctly, the volume fraction of glandular structures in the tumors as well as the appearance of apoptosis increased 3-fold compared to the controls (see Example 5, figures 5E and 5F).

Without limitation to any theory, these results indicate that the main mechanism of the antitumor action of antiprogesterin (I) in the tested models is a direct progesterone-receptor-mediated antiproliferative effect at the level of the tumor cells, via the induction of terminal differentiation associated with terminal cell death. In this manner, antiprogesterin (I) appears to be capable of eliminating the intrinsic block in terminal differentiation inherent in malignant tumor cells in progesterone-receptor-positive tumors. This antiproliferative effect of antiprogesterin (I) seems to be dissociated from the antihormone (antiprogesterin) activity of antiprogesterin (I).

Agents such as antiprogesterin (I) that induce apoptosis in cells, for example, in the case of tumor cells, by blocking progression in the G₀G₁-phase, have potential applications for treating and preventing numerous conditions. Such agents, including antiprogesterin (I), may be used for treating those cancers where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, such as in breast cancer.

Thus one aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for preparation of a medicament for the induction of apoptosis in a cell. In a preferred embodiment, the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof relates to a medicament for the induction of apoptosis in a tumor cell, preferably a breast tumor cell, in a human. Such medicament could be beneficial in the treatment of hormone-dependent diseases such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

The manufacture of the medicaments may be performed according to methods known in the art. Commonly known and used adjuvants as well as further suitable carriers or diluents may be used. Suitable carriers and adjuvants may be such as recommended for pharmacy, cosmetics and related fields in: *Ullmann's Encyclopedia of Technical Chemistry*, Vol. 4, (1953), pp. 1-39; *Journal of Pharmaceutical Sciences*, Vol. 52 (1963), p. 918ff; H.v.Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete";

Pharm. Ind. 2, 1961, p.72ff; Dr. H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete*, Cantor KG, Aulendorf in Württemberg, 1971.

Antiprogestins suitable for the purposes of the present invention, preferably antiprogestin
5 (I) or a pharmaceutically acceptable derivative or analogue thereof, can be incorporated
into pharmaceutical compositions according to known methods of preparing galenics for
oral or parenteral, e.g., intraperitoneal, intramuscular, subcutaneous or percutaneous
application. They can also be implanted into tissue. Implants can comprise as inert
materials e.g. biologically degradable polymers or synthetic silicones such as e.g. silicone
10 rubber.

They can be administered in the form of tablets, pills, dragees, gel capsules, granules,
suppositories, implants, injectable sterile aqueous or oily solutions, suspensions or
emulsions, ointments, creams, gels or by intravaginal (e.g., vaginal rings) or intrauterine
15 systems (e.g., diaphragma, loops).

For the preparation of a medicament for oral administration, the antiprogestins suitable for
the purposes of the present invention as defined above can be admixed with commonly
known and used adjuvants and carriers such as for example, gum arabic, talcum, starch,
20 sugars such as, e.g., mannitose, methyl cellulose, lactose, gelatin, surface-active agents,
magnesium stearate, aqueous or non-aqueous excipients, paraffin derivatives, cross-
linking agents, dispersants, emulsifiers, lubricants, conserving agents and flavoring agents
(e.g., ethereal oils). In a pharmaceutical composition, the antiprogestin may be dispersed
in a microparticle, e.g. a nanoparticulate, composition.

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In order to further enhance the bioavailability of the active agent, the antiprogestins
suitable for the purposes of the present invention as defined above can also be formulated
as cyclodextrin clathrates by reacting them with α -, β - or γ -cyclodextrines or derivatives
thereof according to the method as disclosed in PCT/EP95/02656.

30 For parenteral administration the antiprogestins suitable for the purposes of the present
invention as defined above can be dissolved or suspended in a physiologically acceptable

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diluent, such as, e.g., oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used.

- 5 The amount to be administered (i.e., a "pharmaceutically effective amount") varies within a broad range and depends on the condition to be treated and the mode of administration.

----- It can cover any amount efficient for the intended treatment. Determining a . . .
"pharmaceutically effective amount" is within the purview of the person skilled in the art.

- 10 One unit dose may represent about 0.1 to 100 mg active agent(s). For administration to humans, the daily dose of the active agent(s) is about 0.1 to 400 mg, preferably 10 to 100 mg, most preferably 50 mg.

- The medicaments can also be administered via a depot injection or an implant preparation,
15 optionally for sustained delivery of the active agent(s).

The preferred mode of administration is oral administration. The antiprogesterins for use, according to the invention, and in particular, antiprogesterin (I) are particularly suitable for oral administration.

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- According to all aspects of the present invention it is also possible to combine at least one antiprogesterin as defined above, in particular antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof, with at least one antiestrogen, because many hormone-dependent diseases, in particular breast cancer, exhibit not only progesterone
25 receptors, but also estrogen receptors. The antiestrogen may be administered either simultaneously with or sequentially to the antiprogesterin, and in particular with/to antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof. The amount of antiprogesterin and antiestrogen may be equal or one component may be more predominant than the other, such as in an antiprogesterin:antiestrogen ratio of 1:50 to 50:1,
30 preferably 1:30 to 30:1, and most preferably 1:15 to 15:1.

Examples of suitable antiestrogens for use according to the invention are non-steroidal antiestrogens, such as tamoxifen and nafoxidine as well as raloxifen, faslodex and EM800. Examples of steroidal antiestrogens include those disclosed in EP 0 348 341 A and those disclosed in WO 98/07740, in particular, 11 β -flouro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio-propylamino)-pentyl]-estra-1,3,5(10)-trien-3,17 β -diol, or those
5 disclosed in WO 99/33855, in particular 11 β -flouro-7 α -(5-[methyl-(7,7,8,8,9,9,10,10,10-nonfluoro-decyl)-amino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol or pharmaceutically acceptable derivatives or analogues thereof. Aromatase inhibitors having an antiestrogen effect, such as those disclosed on pages 7 to 8 of EP 0 495 825-B1 may also be used as
10 antiestrogens.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an
15 increased amount of tumor cells in the S-phase of the cell cycle. The number of tumor cells in the S-phase may be determined by DNA flow cytometry as described in Dressler et al., "DNA Flow Cytometry and Prognostic Factors in 1331 Frozen Breast Cancer Specimens," *Cancer*, Vol. 61(3), 1988, pp. 420-427; see also McGuire & Dressler, "Emerging Impact of Flow Cytometry in Predicting Recurrence and Survival in Breast
20 Cancer Patients," *JNCI*, Vol. 75(3), 1985, pp. 405-409. A high risk amount of tumor cells in the S-phase indicates a particularly suitable candidate for the use according to the invention. In the case of antiprogesterin (I), the advantage arises from both the potent anti-tumor effect, as evidenced by the standard animal models (see Examples 1 to 4), and the mechanism of action of this agent of inducing apoptosis (see in particular Example 5) and
25 cell cycle arrest.

In an alternative aspect the present invention provides a method for inducing apoptosis in a cell. The cell is preferably a mammalian cell and most preferably a human cell, and the method may be applied *in vitro* or *in vivo*. Preferably, apoptosis is induced via the
30 mechanism of initiating terminal differentiation, for example, by the administration of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof. In the

method, an effective amount of antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof may be applied to the cells in question. For example in the T47D breast cancer cell line, whose growth is stimulated by the administration of estradiol, antiprogestin (I) induced a complete inhibition of cell growth with an effective threshold concentration of between 10^{-9} and 10^{-8} mol (see Example 6 and figure 6). This is especially surprising as the known antiprogestin onapristone has no reducing effect on cell growth in this tumor model. Thus, antiprogestin (I) is superior with regard to potency and efficacy to other antiprogesterins such as onapristone and to antiestrogens such as tamoxifen and even to pure antiestrogens such as 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

The role of antiprogestin (I) in the induction of apoptosis in the cell indicates that this antiprogestin (or a pharmaceutically acceptable derivative or analogue thereof) may be useful in a host of conditions, particularly hormone-dependent conditions, where induction of apoptosis is particularly desired. Specifically, it may be used in the treatment of such diseases as breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningioma, i.e., diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways. Antiprogesterins, such as antiprogestin (I), may thus be further used for the preparation of medicaments for inducing apoptosis or cell death for the treatment of hormone-dependent diseases as already described above.

The invention is further illustrated in the examples. The following examples are not to be understood as a limitation.

Examples

Example 1:

Dose-response study in the DMBA-induced tumor model

Materials and Methods:

5 Immature female Sprague-Dawley rats (49 - 51 days old; 10 animals/group) were used in this study. Mammary tumors were induced by a single oral administration of 10 mg 7,12-dimethylbenz[a]anthracene (DMBA, Serva/Heidelberg). Rats with at least one established tumor with a size of more than 150 mm² were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 0.5 mg/kg s.c., 4) antiprogesterin (I), 2 mg/kg s.c., 5) antiprogesterin (I), 5 mg/kg s.c., 6) antiprogesterin (I), 10 mg/kg s.c., and 7) onapristone, 5 mg/kg, s.c., daily. As a parameter for growth inhibition the change of tumor area (in % with respect to initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used. For a further description and evaluation of the DMBA prevention model, see R.G. Metha, *European Journal of Cancer* 36 (2000), pp. 1275-1282.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a considerable tumor regression in 90% of the animals. Treatment with antiprogesterin (I) at doses of or above 2 mg/kg resulted in a significant induction of apoptosis resulting in inhibition of tumor growth compared with the control (see fig. 2). There was a clear dose-response relationship. Whereas treatment with 0.5 mg/kg antiprogesterin (I) did not significantly prevent the tumor from growing, at 2 mg/kg maximal induction of apoptosis and thus growth inhibition was observed. In this group a complete tumor regression was seen in 50% of the rats. The effect of the highest dose of antiprogesterin (I) tested in this experiment (10 mg/kg), was comparable to that of 2 mg/kg. Onapristone (5 mg/kg, s.c.) was distinctly less effective than antiprogesterin (I) at comparable doses.

30 Conclusion:

In the DMBA-induced mammary tumor model in the rat, antiprogesterin (I) strongly induced apoptosis in the tumor cells and thus completely suppressed the tumor growth in intact animals. It was found that 2 mg/kg antiprogesterin (I) has a maximal apoptotic effect on tumor cells. Antiprogesterin (I) was distinctly superior to onapristone regarding the inhibition of tumor growth.

Example 2:

Tumor growth inhibition in NMU-induced breast cancer model in rat

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Materials and Methods:

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Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Starting 10 days later, rats with at least one established tumor were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 1.0 mg/kg/day, 4) antiprogesterin (I), 0.5 mg/kg/day and 5) onapristone, 5 mg/kg/day. As a parameter for growth inhibition the change of tumor area (in % of initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used.

Results:

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In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor growth inhibition. Treatment with antiprogesterin (I) at doses of 0.5 or 1.0 mg/kg resulted in a significant inhibition of tumor growth due to the induction of apoptosis compared with the control (see fig. 2). Onapristone (5 mg/kg) was distinctly less effective than antiprogesterin (I) at the much lower dose of 0.5 mg/kg.

Conclusions:

In the MNU-induced mammary tumor model in the rat, due to its potent ability to induce apoptosis in tumor cells, antiprogesterin (I) completely suppresses the tumor growth in intact animals. Both doses (1.0 mg/kg as well as 0.5 mg/kg) of antiprogesterin (I) have a significant apoptotic effect on tumor cells.

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Example 3:

Human T47D breast cancer xenograft in scid mice

10 Materials and Methods:

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). T47D breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

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Results:

In the T47D breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 3 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induces apoptosis in the tumor cells. The effect of antiprogesterin (I) is almost comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human T47D breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy) which is considered to be the maximum effective method of inhibiting growth of breast cancer in this model.

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Example 4:

Human MCF-7 breast cancer xenograft in scid mice

10 Materials and Methods:

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). MCF7 breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

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Results:

In the MCF7 breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 4 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induced apoptosis in the tumor cells. The effect of antiprogesterin (I) is comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

The effect of antiprogesterone (I) in inducing apoptosis and thus inhibiting the growth of the human MCF7 breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy).

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Example 5:

NMU-induced breast cancer in rat (histology, proliferation index and TUNEL assay)

Materials and Methods:

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Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Rats with at least one established tumor with a size of more than 150 mm² were treated for 7 days by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterone (I), 3 mg/kg s.c., daily. At the end of treatment tumors were excised, fixed in formalin and embedded in paraffin. Histology, proliferation index and apoptosis induction assays were performed on these resected tumors.

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Histology: For histology tissue slides were stained with haematoxylin and analyzed by microscopy.

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Proliferation Index: To determine the proliferation index the expression of PCNA was determined. Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear protein associated with the cell cycle. Nuclear PCNA immunoreactivity is found in the proliferative compartment of normal tissues. A monoclonal antibody, that recognizes a fixation and processing resistant epitope has been used to investigate its tissue distribution.

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TUNEL (Apoptosis Test): The biochemical hallmark of apoptosis is the degradation of the genomic DNA, an irreversible event that results in cell death. This characteristic DNA fragmentation is the result of the activation of nuclear endonucleases, which selectively cleave DNA at sites located between nucleosomal units. These DNA strand breaks were

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detected by enzymatic labeling of the 3'-OH termini with fluorescein-dUTP using terminal deoxynucleotidyl transferase (TUNEL, Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling, cf. Gavrieli et al., J. Cell Biol. 119, 493, 1992). Incorporated fluorescein was detected using the anti-fluorescein antibody alkaline phosphatase conjugate followed by alkaline phosphatase substrate reaction.

Results:

Histology: After treatment with antiprogesterin (I), the tissue sections from the NMU tumors displayed dysplastic ductal and acinous formations, usually filled with secretory material (Figure 5A). Moreover, the volume fraction of glandular structures in the tumors increased compared to controls (Figure 5B). In addition, the mammary tumors of antiprogesterin (I) treated animals showed the morphological features of differentiation.

Proliferation Index: PCNA immunoreactivity is high in untreated NMU-induced breast cancer tissue (Figure 5C: Untreated control). The number of cells with PCNA immunoreactivity is reduced by induction of differentiation in NMU-induced breast cancer tissue from rats treated with antiprogesterin (I) (Figure 5D). These data demonstrate that in breast cancer, treatment with antiprogesterin reduces the proliferation index by induction of differentiation.

TUNEL (Apoptosis): Figure 5E demonstrates the appearance of apoptosis induced by antiprogesterin (I) in NMU-induced breast cancer tissue in comparison with untreated control (Figure 5F). It is clearly evident that antiprogesterin (I) alone was capable of inducing apoptosis in the NMU-induced breast cancer tissue and thus inhibited the growth of these tumors.

Example 6:

Antiproliferative activity of antiprogesterin (I) *in vitro* in the T47D cell line

Materials and Methods:

T47D cells were grown in charcoal-treated serum supplemented with 0.1 nM E2 (estradiol), plus antiprogesterin (I) for 6 days with one medium change. Following fixation and subsequent staining with crystal violet, the absorbance was recorded and values normalized to the absorbance of untreated controls as described in R.B. Lichtner, *J. Steroid Biochem. Mol. Biol.* 1999, 71;181-189. The TUNEL assay is performed analogous to above Example 5 with the only difference that instead of tissue sections cells that are cultivated on microscopic slides are used for the assay.

Results:

In this T47D cell line *in vitro* test, antiprogesterin (I) exhibited potent tumor growth inhibiting activity with an effective threshold concentration as low as 10^{-9} to 10^{-8} mol/l whereas the antiprogesterin onapristone did not show any inhibiting effect. Even the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740) was distinctly less effective than antiprogesterin (I) (see figure 6).

Conclusion:

Antiprogesterin (I) according to the present invention induces complete inhibition of estradiol-stimulated T47D cell growth at very low concentrations and is thus superior regarding potency and efficacy to other antiprogesterins tested such as onapristone and to the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol.

CLAIMS

1. Use of the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell.
2. Use according to claim 1 wherein the induction of apoptosis is caused by the initiation of terminal differentiation.
3. Use according to any preceding claim wherein the cell is a mammalian cell.
4. Use according to claim 3 wherein the mammalian cell is a human cell.
5. Use according to any preceding claim wherein the cell is a tumor cell.
6. Use according to claim 5 wherein the tumor is breast cancer.
7. Use according to any preceding claims, wherein the medicament further comprises an antiestrogen.
8. Use of the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.
9. The use according to claim 8, where the disease is breast cancer.
10. Method of inducing apoptosis in a cell, comprising administering an effective amount of the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-

pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable analogue or derivative thereof to a cell *in vitro*.

11. The method according to claim 10, wherein the cell is a mammalian tumor cell.

5 12. The method according to claims 10 or 11, wherein the cell is a breast cancer cell.

ABSTRACT

The present invention relates to methods and uses for inducing apoptosis in a cell, in particular a breast cancer cell, by the administration of antiprogestins, in particular the antiprogestin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof. The invention further relates to a treatment of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, said treatment comprising an antiprogestin, in particular the antiprogestin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof.

Fig. 1

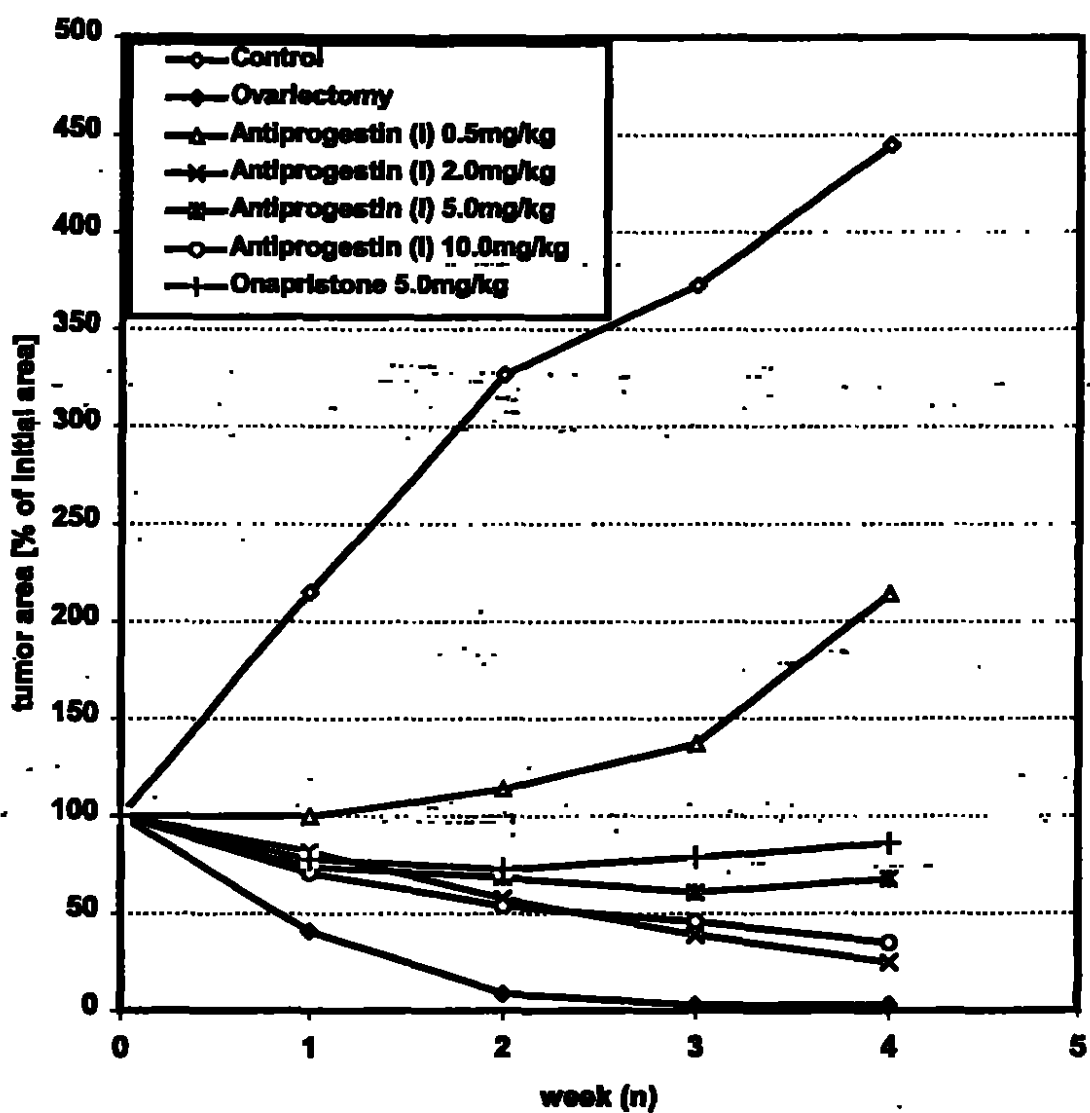


Fig. 2

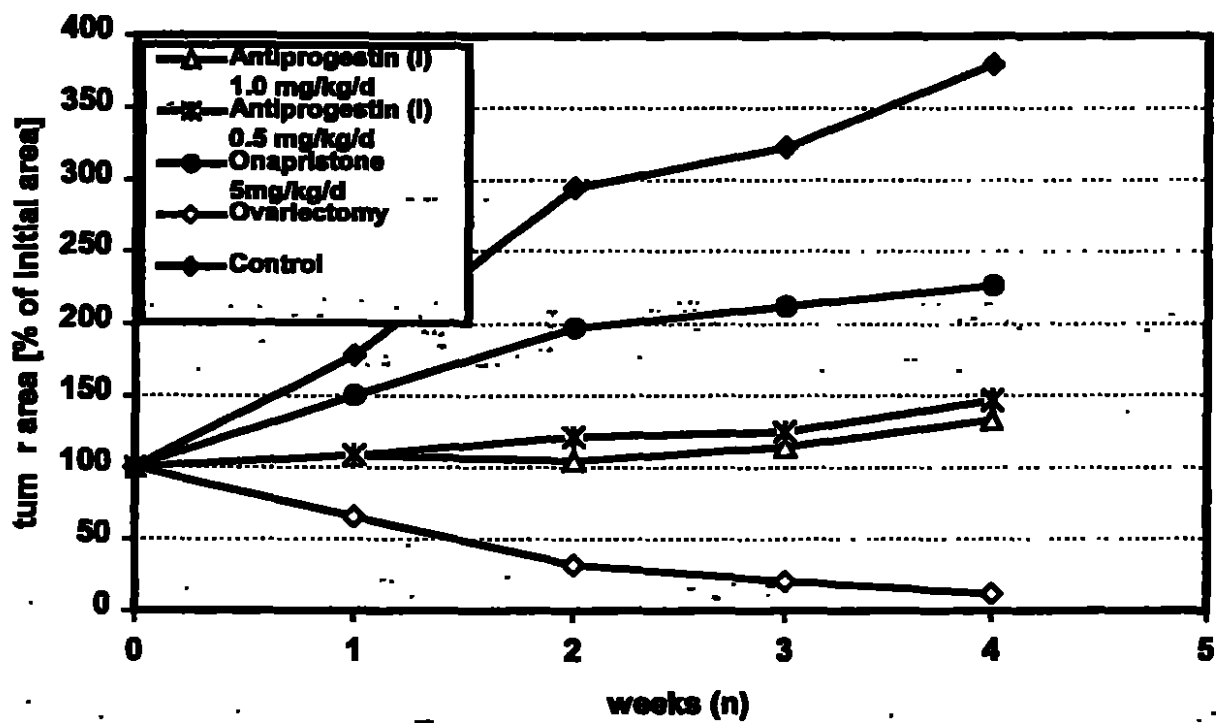


Fig. 3

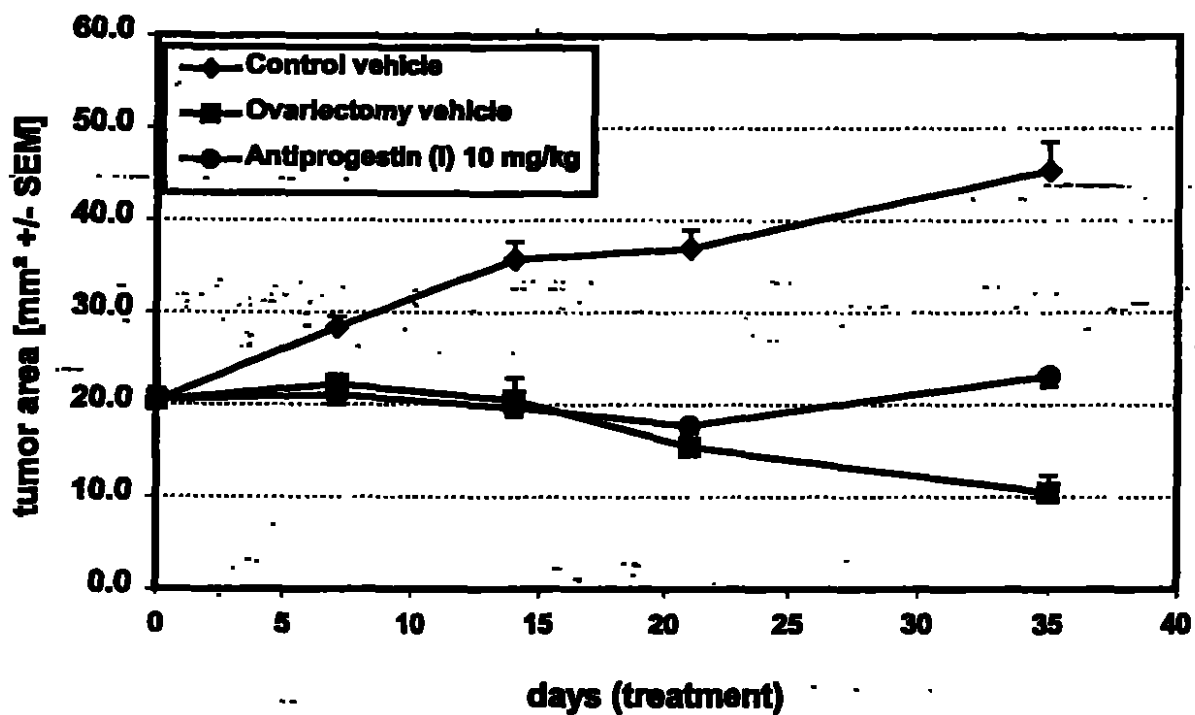


Fig. 4

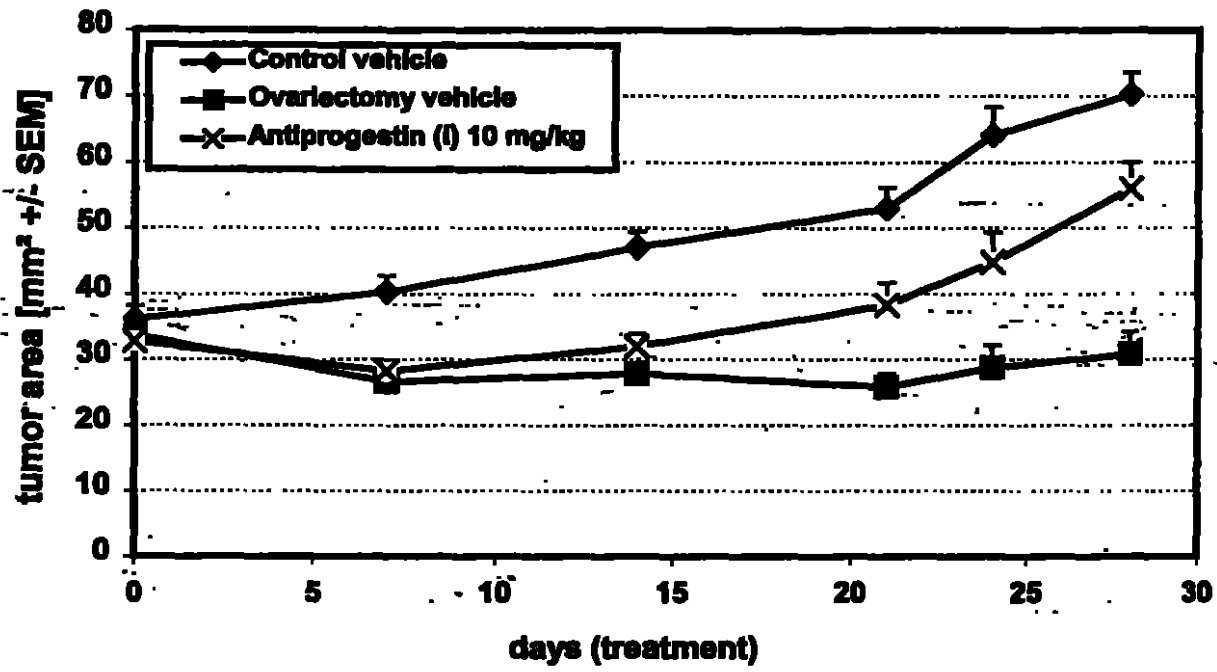
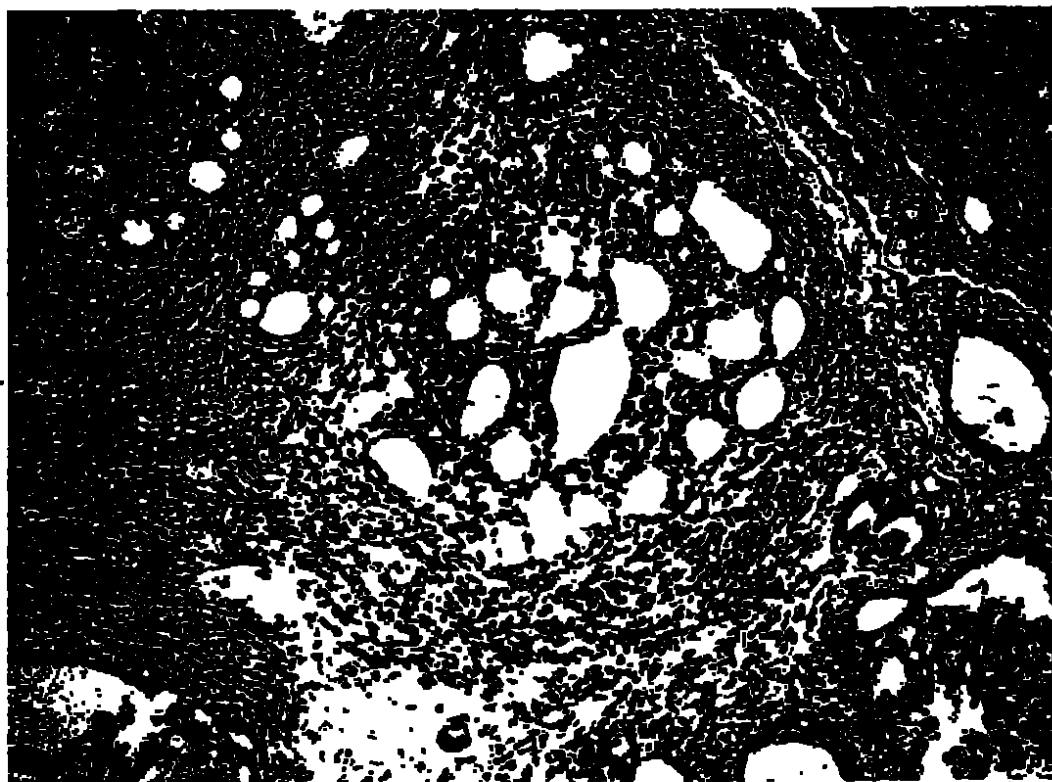


Fig. 5A



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Fig. 5B

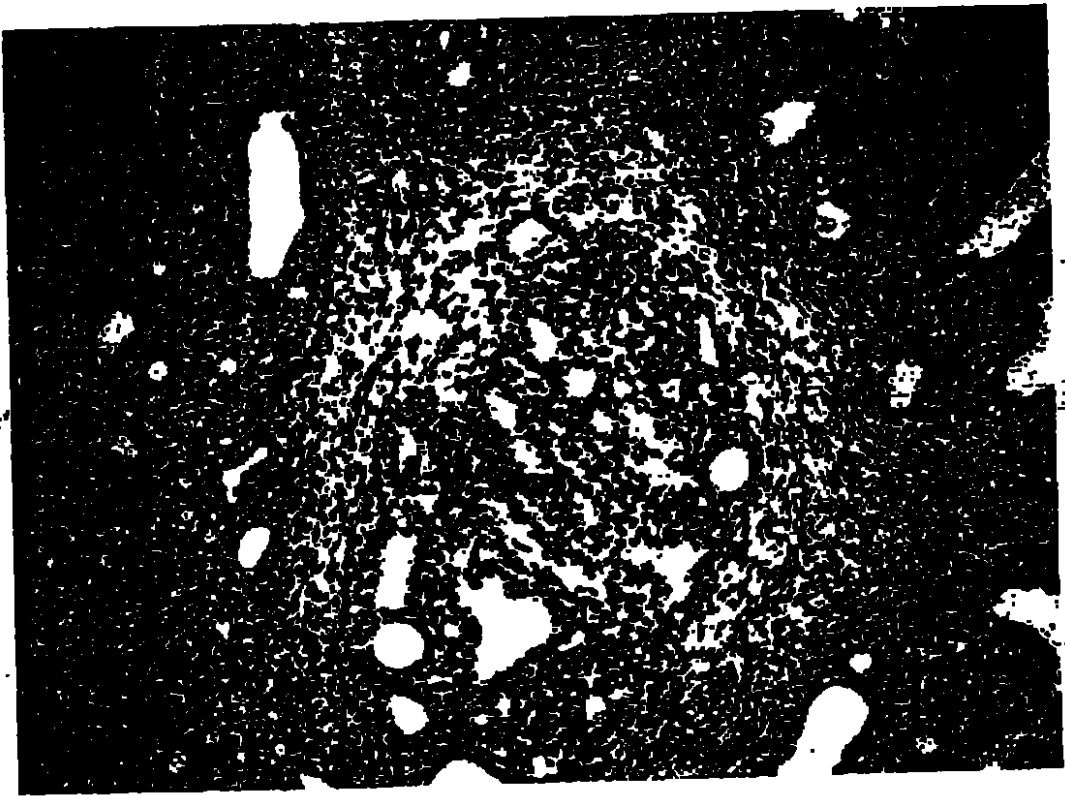


Fig. 5C

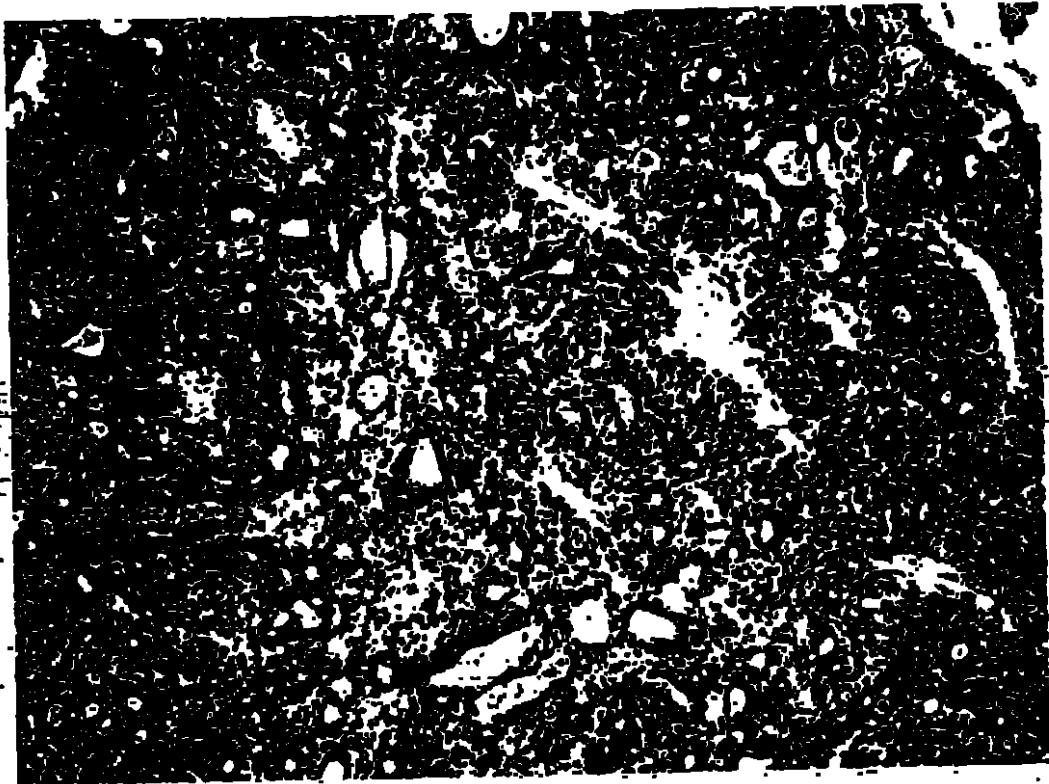


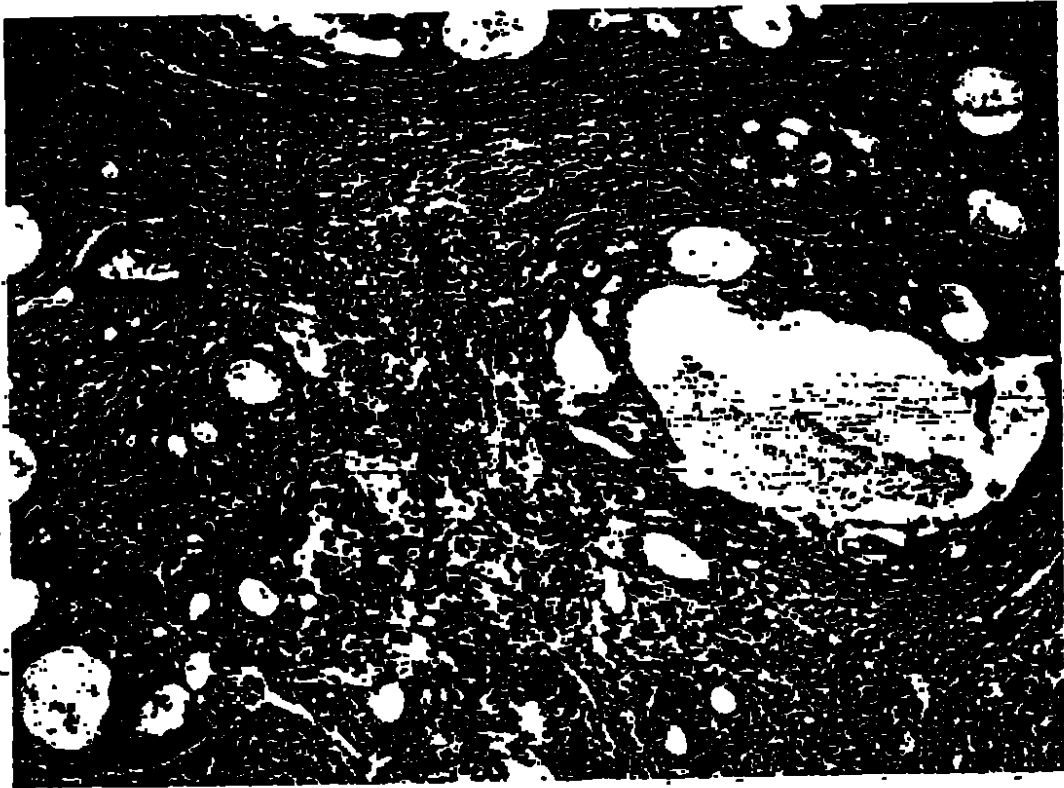
Fig. 5D

Fig. 5E

51870A

31

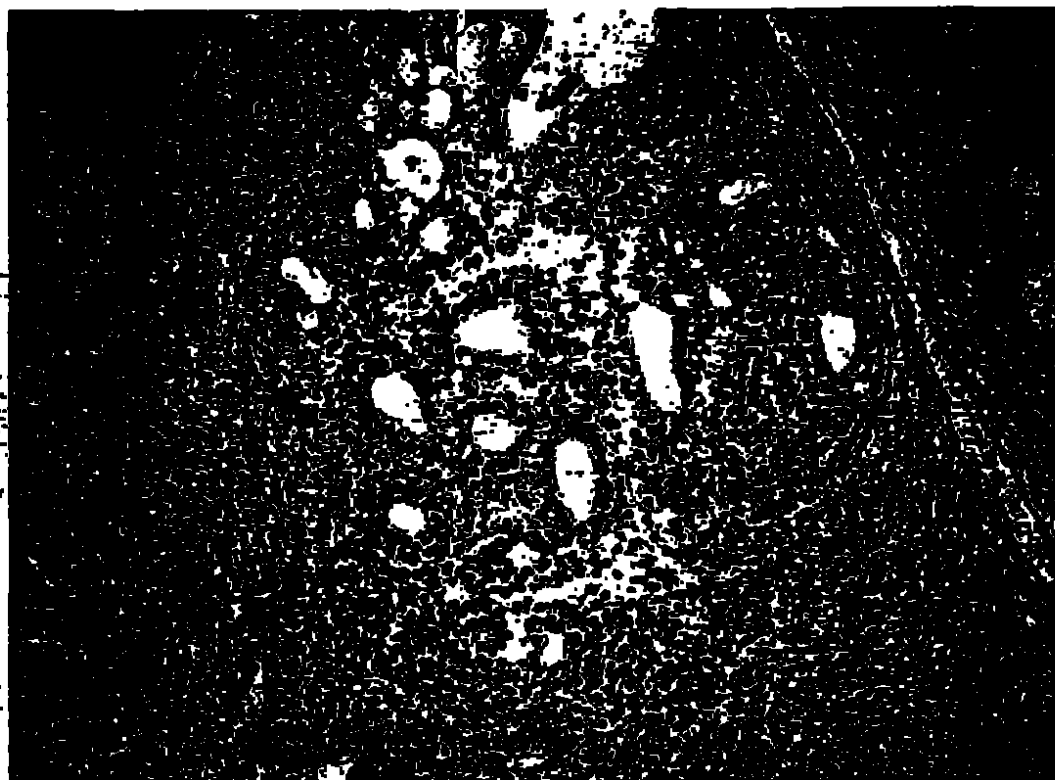
Fig. 5F

Fig. 6

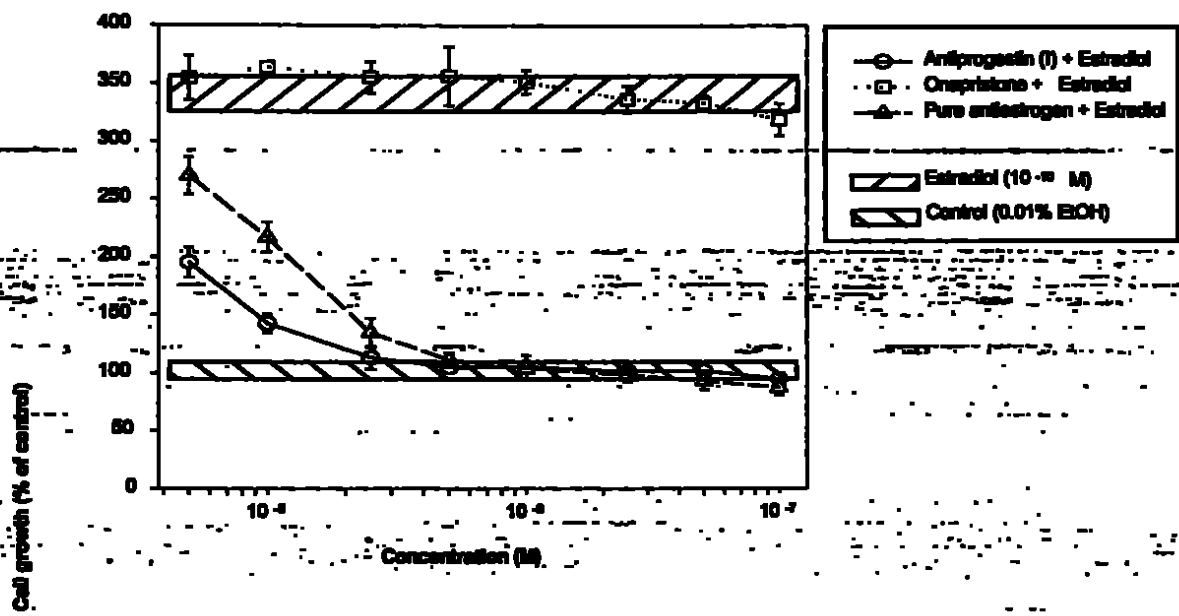


Fig. 1

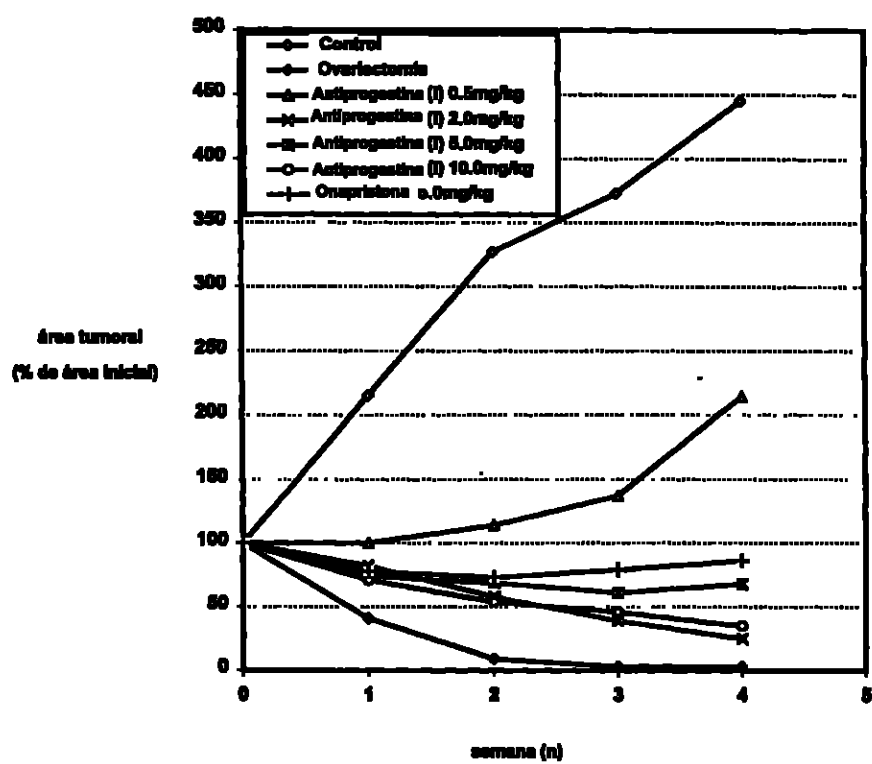


Fig. 2

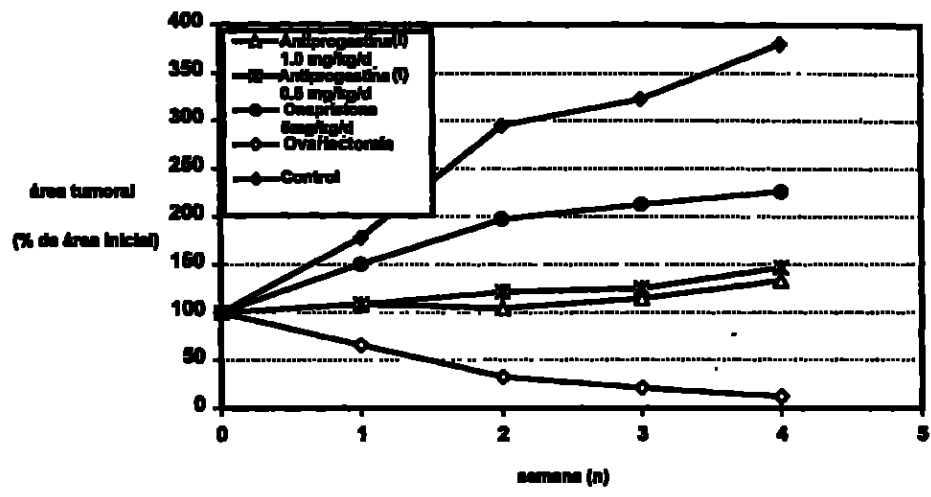


Fig. 3

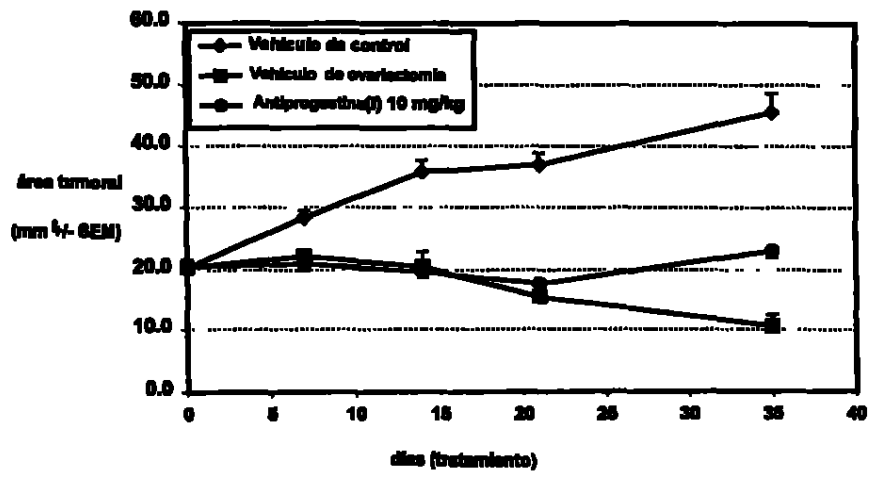


Fig. 4

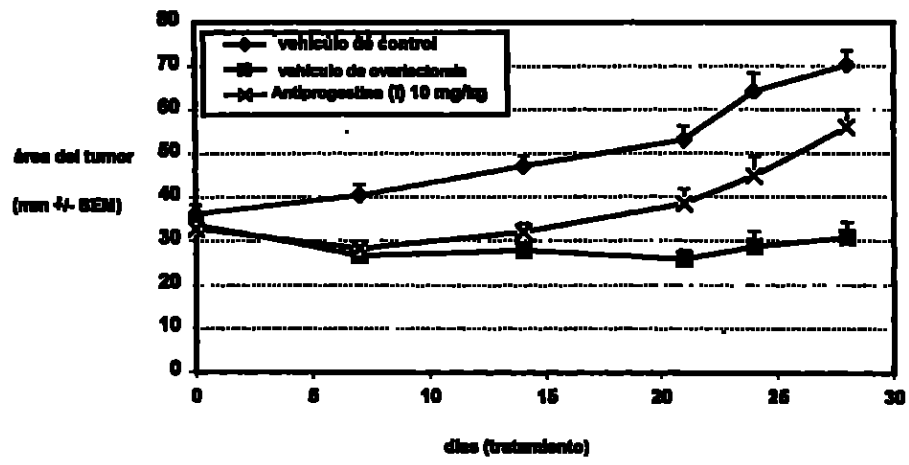


Fig. 5A

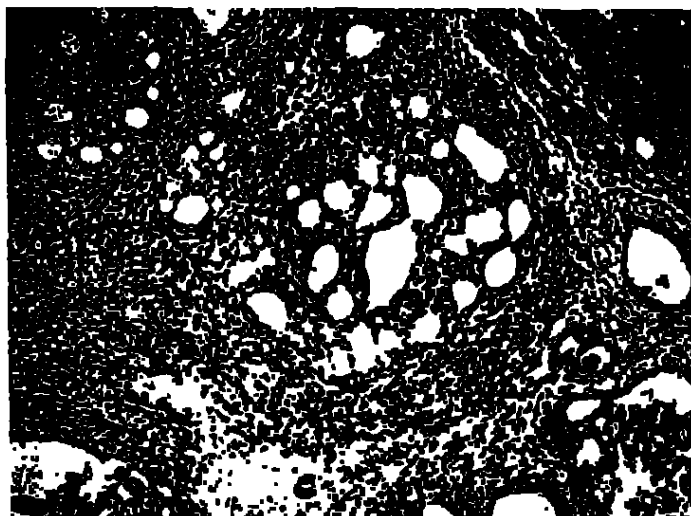
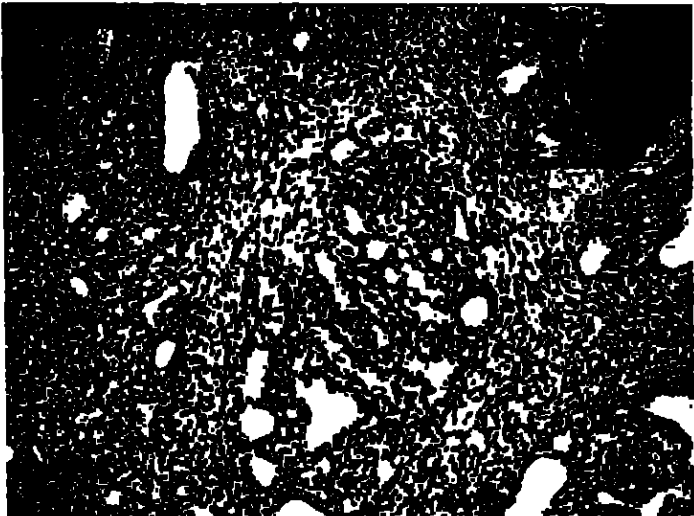


Fig. 5B



-

Fig. 5C

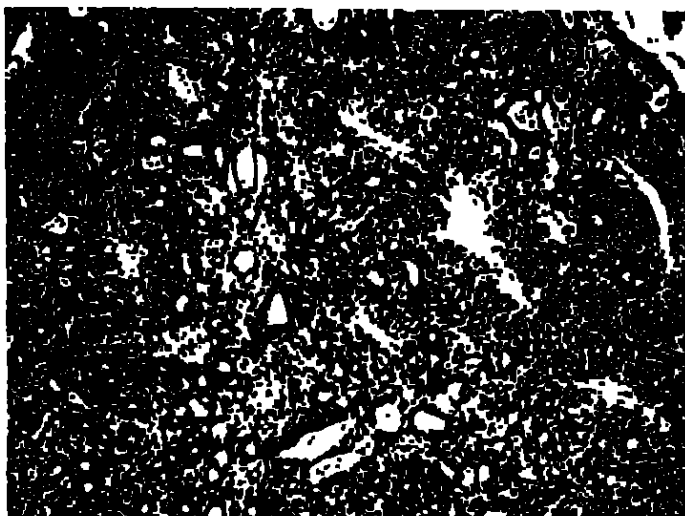


Fig. 5D

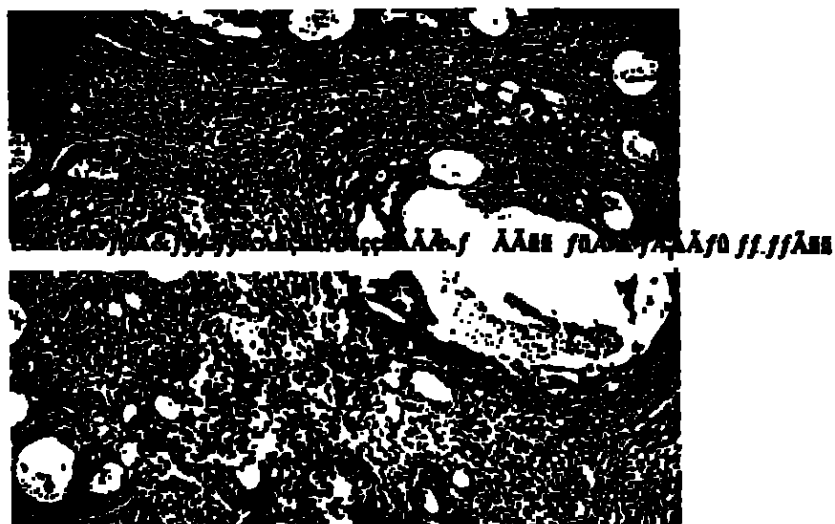
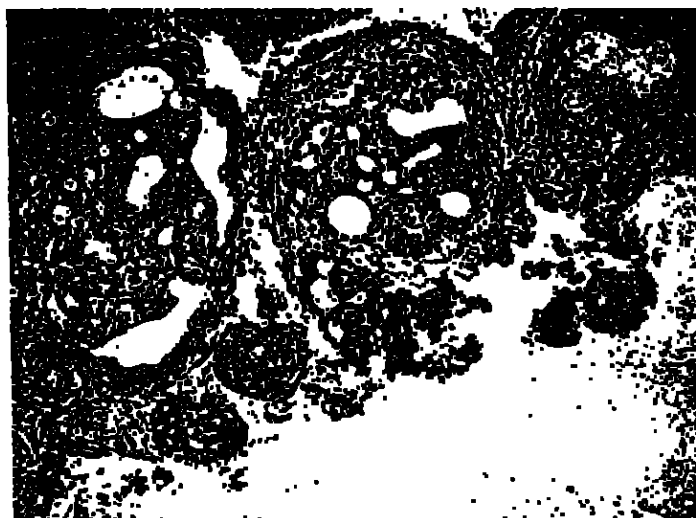


Fig. 5E



1

Fig. 5F

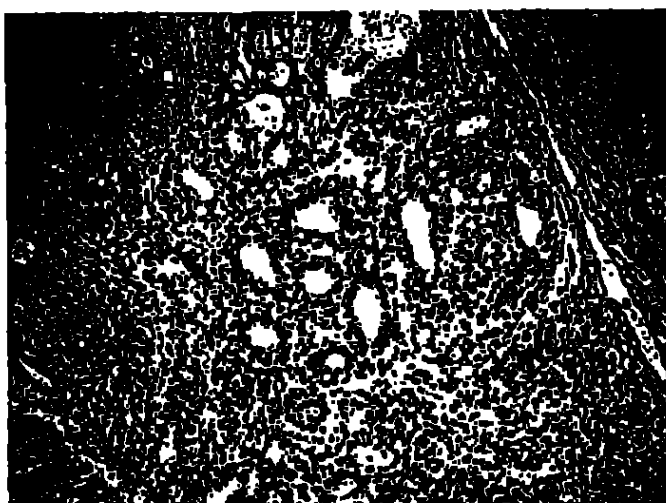
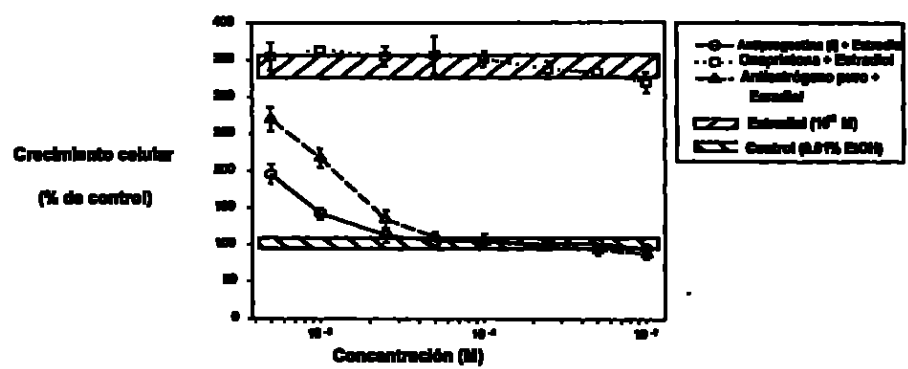


Fig. 6



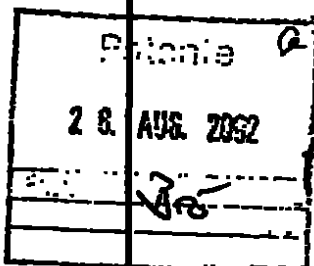
PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstrasse 178
13353 Berlin
ALLEMAGNE



WRITTEN OPINION

(PCT Rule 66)

Date of mailing
(day/month/year)

27. 08. 02

Applicant's or agent's file reference

51870AWOM1XX

REPLY DUE

within 1 / 00 months/days
from the above date of mailing

International application No.

PCT/EP 01/12006 ✓

International filing date (day/month/year)

17/10/2001 ✓

Priority date (day/month/year)

18/10/2000

International Patent Classification (IPC) or both national classification and IPC

A61K31/57

Applicant

SCHERING AKTIENGESELLSCHAFT

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.5.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 18/02/2003

Name and mailing address of the IPEA/

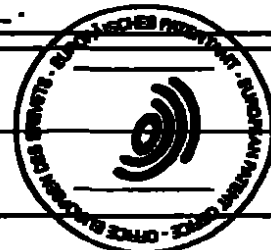


European Patent Office, Gilchiner Str. 103
D-10969 Berlin - Germany
Tel.: (+49-30) 25901-0
Fax: (+49-30) 25901-840

Authorized officer

Examiner

Formalities officer
(incl. extension of time limits)
Tel. (+49-89) 2399 2828



WRITTEN OPINION

International application No. PCT/EP 01/ 12006

I. Basis of the opinion

1. The basis of this written opinion is the application as originally filed.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

1. In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claims references).
2. If amendments are filed, the applicant should comply with the requirements of Rule 66.8 PCT and indicate the basis of the amendments in the documents of the application as originally filed (Article 34 (2) (b) PCT) otherwise these amendments may not be taken into consideration for the establishment of the international preliminary examination report. The attention of the applicant is drawn to the fact that if the application contains an unnecessary plurality of independent claims, no examination of any of the claims will be carried out.

NB: Should the applicant decide to request detailed substantive examination, then an international preliminary examination report will normally be established directly. Exceptionally the examiner may draw up a second written opinion, should this be explicitly requested.

Tratado de Cooperación en Materia de Patentes

De la Autoridad de Examen Preliminar Internacional

Fecha de envío: 27/08/2002

Referencia del solicitante o agente: 51870AWOM1XX00+P

Plazo para responder: dentro de 1 mes desde la fecha de envío mencionada.

Solicitud Internacional No. PCT/EP 01/12006

Fecha de presentación internacional: 17/10/2001

Fecha de Prioridad: 18/10/2000

Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC: A61K31/57

Solicitante: Schering Aktiengesellschaft

1. Esta opinión escrita es la primera emitida por esta Autoridad de Examen Preliminar Internacional

2. Esta opinión contiene indicaciones relacionadas con los siguientes puntos:

I (X) Base de la opinión

II Prioridad

III (X) No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

IV Falta de unidad inventiva

V(X) Declaración razonada bajo Regla 66.2 (a)(ii) con respecto a novedad, altura inventiva o aplicación industrial; citas y explicaciones que apoyan tal declaración.

VI Ciertos documentos citados

VII Ciertos defectos de la solicitud internacional

VIII Ciertas observaciones con respecto a la solicitud internacional

3. Por la presente se invita al solicitante a responder a esta opinión.

¿Cuándo? Véase el plazo indicado más arriba. El solicitante puede, antes del vencimiento del plazo, solicitar una prórroga a esta Autoridad (ver Regla 66.2(d)).

¿Cómo? Enviando una contestación escrita, acompañada por correcciones, si fuera apropiado, de acuerdo con la Regla 66.3. Para la forma y el idioma de las correcciones, ver reglas 66.8 y 66.9.

También Para una oportunidad adicional de presentar correcciones, ver Regla 66.4. Para la obligación del examinador de tomar en consideración correcciones y/o argumentos, ver Regla 66.4 bis. Para una comunicación informal con el examinador, ver Regla 66.6.

Si no se presenta una contestación, el informe de examen preliminar internacional será redactado sobre la base de esta opinión.

4. La fecha final en que el informe de examen preliminar internacional debe ser establecido, de acuerdo con la Regla 69.2, es: 18/02/2003

OPINIÓN ESCRITA

I. Base de la opinión

1. Esta opinión escrita se basa en la solicitud como fue presentada originalmente

V. Declaración razonada según Regla 66.2(a)(ii) con respecto a novedad, altura inventiva o aplicación industrial

1. En vista de los documentos citados en el informe de búsqueda internacional, se considera que la invención, como es definida en al menos algunas de las reivindicaciones, que han sido objeto de un informe de búsqueda internacional, no parece alcanzar el criterio mencionado en el Artículo 33(1)PCT, es decir que no aparenta ser nueva y/o tener altura inventiva (ver el informe de búsqueda internacional, en particular los documentos citados con "X" y/o "Y" y las correspondientes referencias a las reivindicaciones).

2. Si se presentan correcciones, el solicitante debería cumplimentar los requisitos de la Regla 66.8PCT e indicar el fundamento de las correcciones en los documentos de la solicitud como fue presentada originalmente (Artículo 34 (2) (b) PCT) de otro modo estas correcciones pueden no ser tenidas en cuenta para la confección del informe de examen preliminar internacional. Se deja en claro al solicitante que, si la solicitud contiene una pluralidad innecesaria de reivindicaciones independientes, no se llevará a cabo el examen de ninguna de estas reivindicaciones.

Nota: Si el solicitante decidiera solicitar un examen de fondo detallado, lo normal es que se establezca directamente. En forma excepcional el examinador puede emitir una segunda opinión escrita, si esto fuera solicitado explícitamente.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 51870AWOM1XX	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/EP01/12008	International filing date (day/month/year) 17/10/2001	Priority date (day/month/year) 18/10/2000
International Patent Classification (IPC) or national classification and IPC A61K31/57		
Applicant SCHERING AKTIENGESELLSCHAFT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 03/04/2002	Date of completion of this report 28.01.2003
Name and mailing address of the international preliminary examining authority:  European Patent Office - Glitschiner Str. 103 D-10958 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized officer Beyss, E Telephone No. +49 30 25901 344 

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP01/12006

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-19 as originally filed

Claims, No.:

1-12 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: English, which is:

- ☒ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☒ the language of publication of the international application (under Rule 48.3(b)).
- ☒ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP01/12006

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 10-12 in respect of industrial applicability.

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 - ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - ☐ no international search report has been established for the said claims Nos. .
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
 - ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims
	No:	Claims 1-12
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-12
Industrial applicability (IA)	Yes:	Claims 1-9

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/EP01/12006

No: Claims 10-12

2. Citations and explanations
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 10-12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

- D1: DE 197 06 061 A (SCHERING AG) 13 August 1998 (1998-08-13) cited in the application

1. Clarity

1.1.

Claims 1 and 2 do not meet the requirements of Article 6 PCT in that the matter for which, protection is sought is not clearly defined. The claims attempt to define the therapeutic application in terms of the result to be achieved (claim 1: "induction of apoptosis" and claim 2: "initiation of terminal differentiation") which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added.

1.2

The term "type of cancer...the cell cycle" used in claim 8 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers,

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP01/12006

thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

2. Novelty

D1 discloses (claim 1; page 3, line 30; page 4, line 56-57) 17 alpha-fluoralkylsteroides according to formula I and their use in the treatment of hormon-dependent cancers. The subject-matter of claims 1-7 of present application discloses the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the induction of apoptosis, i. e. cancer.

The subject-matter of claim 8 of present application discloses the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the treatment of a type of cancer wherein an indicator of high risk is an increased amount of tumor cells.

Both, D1 and present application disclose the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the treatment of cancer:

One could assume that an indicator of high risk which is an increased amount of tumor cells renders claim 8 novel but a functional feature is not limiting as such.

The subject-matter of claims 1-12 is therefore not new (Article 54(1) and (2) EPC).

3. Inventive Step

Document D1, which is considered to represent the most relevant state of the art, discloses a subject-matter described under point 2 of present application.

The problem to be solved by the present invention may be regarded as the provision of agents for the treatment of cancer (induction of apoptosis in a cell).

The solution proposed in claims 1-12 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) since D1 suggests that 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-

one (page 3, line 30) can be used for the treatment of cancer (page 4, line 56-57).

Thus the subject-matter of claims 1-12 of present application does not meet the requirements of Article 33(3) PCT.

4. Industrial Applicability

Claims 1-9 meet the requirements of Article 33(4) PCT.

Tratado de Cooperación en Materia de Patentes

Informe de Examen Preliminar Internacional

(Artículo PCT 36 y Regla 70)

Referencia del solicitante o agente: 51870AWOM1XX

Para acción futura: Véase la Notificación de Comunicación del Informe de Examen Preliminar Internacional (Formulario PCT/IPEA/416)

Solicitud Internacional No. PCT/EP01/12006

Fecha de presentación internacional: 17/10/2001

Fecha de Prioridad: 18/10/2000

Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC: A61K31/57

Solicitante: Schering Aktiengesellschaft y otros

1. Este Informe de Examen Preliminar Internacional ha sido preparado por esta Autoridad de Examen Preliminar Internacional y es comunicado al solicitante de acuerdo con el Artículo 36.

2. Este Informe consiste de un total de 5 hojas, incluyendo esta carátula

Este informe también es acompañado de anexos, p.ej. hojas de la descripción, reivindicaciones y/o dibujos que han sido corregidos y que son la base para este informe y/o hojas que contienen rectificaciones hechas ante esta Autoridad (véase Regla 70.16 y Sección 607 de las Instrucciones Administrativas bajo el PCT).

Estos anexos consisten de un total de hojas.

3. Este informe contiene indicaciones en relación con los siguientes ítems:

I (X) Base del informe

II Prioridad

III (X) No emite opinión en relación con la novedad, altura inventiva y aplicabilidad industrial

IV Falta de unidad inventiva

V (X) Declaración razonada bajo el Artículo 35(2) en relación con la novedad, altura inventiva o aplicabilidad industrial; citas y explicaciones que sustentan tal declaración

VI Ciertos documentos citados

VII Ciertos defectos en la solicitud internacional

VIII Ciertas observaciones con respecto a la solicitud internacional.

Fecha de comunicación de la solicitud: 03/04/2002

Fecha de terminación de este informe: 28.01.2003

Informe de Examen preliminar Internacional

Solicitud internacional No. PCT/EP01/12006

I. Base del Informe

1. Con respecto a los elementos de la solicitud internacional (las hojas de reemplazo que han sido provistas a la oficina receptora en respuesta a una invitación bajo Artículo 14 son mencionadas en este informe como "originalmente presentadas" y no están anexadas a este informe ya que no contienen modificaciones (Reglas 70.16 y 70.17))

Descripción, hojas

1-19 como originalmente presentadas

Reivindicación, Nr.

1-12 como originalmente presentadas

2. Con respecto al idioma, todos los elementos arriba indicados estaban disponibles o fueron provistos a esta Autoridad en el idioma en el cual fue presentada la solicitud internacional, a menos que esté indicado en este ítem.

Estos elementos estaban disponibles o fueron provistos a esta Autoridad en el siguiente idioma: inglés:

(X) El idioma de una traducción provista a los fines de la búsqueda internacional (bajo Regla 23.1(b)).

(X) El idioma de la publicación de la solicitud internacional (bajo Regla 48.3(b)).

(X) El idioma de una traducción provista a los fines del examen preliminar internacional (bajo Regla 55.2 y/o 55.3).

3. Con respecto a cualquier nucleótido y/o secuencia de aminoácidos divulgada en la solicitud internacional, el examen

preliminar internacional fue llevado a cabo sobre la base de los listados de secuencias:

Contenido en forma escrita en la solicitud internacional

Presentado junto con la solicitud internacional en una forma descifrable por computadora

Provista subsecuentemente a esta Autoridad en forma escrita

Provista subsecuentemente a esta Autoridad en una forma descifrable por computadora

La declaración de que el listado de secuencias provisto subsecuentemente no va más allá de lo divulgado en la solicitud internacional tal como fuera presentada

La declaración de que ha sido provista la información grabada en forma descifrable por computadora es idéntica al listado de secuencias escrito

4. Las correcciones han dado como resultado la cancelación de la descripción, páginas:

las reivindicaciones Nrs.:

los dibujos, hojas:

5. Este informe fue emitido como si (algunas de) las correcciones no hubieran sido hechas, porque han sido consideradas como que van más allá de la divulgación tal cual fue presentada (Regla 70.2(c)):

(Cualquier hoja de reemplazo que contenga este tipo de correcciones debe ser mencionada bajo el ítem 1 y anexada a este informe.

6. Observaciones adicionales, de ser necesarias:

III. No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

1. La pregunta si la invención reivindicada presente novedad, altura inventiva (ser no-obvia), o tener aplicación industrial no ha sido examinada con respecto a

toda la solicitud internacional

(x) las reivindicaciones 10-12 con respecto a su aplicación industrial

porque

la mencionada solicitud internacional, o las mencionadas reivindicaciones Nr. se refieren al siguiente objeto inventivo que no requiere un examen preliminar internacional (especificar):

la descripción, reivindicaciones o dibujos (indicar elementos particulares más abajo) o las mencionadas reivindicaciones Nr. son tan poco claras que no se pudo formar una opinión que tenga sentido (especificar):

las reivindicaciones o las mencionadas reivindicaciones Nr. están tan inadecuadamente sustentadas en la descripción que no se pudo formar una opinión que tenga sentido

(X) no se realizó el informe de búsqueda internacional para las mencionadas reivindicaciones Nr.

2. No se pudo llevar a cabo un significativo examen preliminar internacional ya que la lista de nucleótidos y/o secuencias de aminoácidos no cumple con los estándares previstos en el Anexo C de las instrucciones administrativas:

el formulario escrito no fue aportado o no se atiende al estándar

el formulario descifrable por computadora no ha sido aportado o no se atiende al estándar

V. Declaración razonada bajo Artículo 35(2) con respecto a novedad, altura inventiva y aplicación industrial, citas y explicaciones que avalen dicha declaración

1. Declaración

Novedad (N) Si: Reivindicaciones

No: Reivindicaciones 1-12

Altura inventiva (IS) Si: Reivindicaciones

No: Reivindicaciones 1-12

Aplicación industrial Si: Reivindicaciones 1-9

No: Reivindicaciones 10-12

2. Citas y explicaciones

ver hoja separada

Informe de Examen Preliminar Internacional

Hoja separada

Solicitud internacional No. PCT/EP01/12006

En relación con el ítem III

No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

Las reivindicaciones 10-12 se refieren a un objeto inventivo, el cual esta Autoridad considera es conforme a las disposiciones enunciadas en la Regla 67.1(iv)PCT. En consecuencia, no se formulará opinión con respecto a la aplicación industrial del objeto inventivo de estas reivindicaciones (Artículo 34(4)(a)(i)PCT.

En relación con el ítem V

Declaración razonada bajo el Artículo 35(2) con respecto a la novedad, altura inventiva o aplicación industrial, citas y explicaciones en que se basa dicha declaración

Declaración razonada según Regla 66.2(a)(ii) con respecto a la novedad, altura inventiva o aplicación industrial: citas y explicaciones que avalan dicha declaración.

Se hace referencia al siguiente documento:

D1: DE 197 06 061 A (Schering AG) 13 de agosto de 1998 (1998-08-13) citado en la solicitud

1. Claridad

1.1

Las reivindicaciones 1 y 2 no cumplen con los requisitos del Artículo 6 PCT debido a que el objeto que se busca proteger no está definido claramente. Las reivindicaciones intentan definir

la aplicación terapéutica en términos de los resultados a ser obtenidos (reivindicación 1: "inducción de la apoptosis" y reivindicación 2: "inicio de diferenciación terminal") que meramente se suman a la declaración del problema subyacente. Debe ser agregado el rasgo técnico necesario para obtener este resultado.

1.2

El término "tipo de cáncer...el ciclo celular" usado en la reivindicación 8 es vago y poco claro y deja dudas al lector respecto del significado del rasgo técnico al que se refiere, dando como resultado que la definición del objeto inventivo de dicha reivindicación sea poco claro (Artículo 6 PCT).

2. Novedad

D1 describe (reivindicación 1; página 3, línea 30; página 4, líneas 56-57) 17alfa-fluoroalquilesteroides de acuerdo con la fórmula I y su uso en el tratamiento de cánceres hormona-dependientes. El objeto inventivo de las reivindicaciones 1-7 de la presente solicitud, describe el uso de 11-beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para la inducción de la apoptosis, es decir cáncer.

El objeto inventivo de la reivindicación 8 de la presente invención describe el uso de 11-beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para el tratamiento de un tipo de cáncer en el cual un indicador de alto riesgo es una cantidad incrementada de células tumorales.

Tanto D1 como la presente solicitud describen el uso de 11-beta-(4-acetilfenil)-17-beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para el tratamiento de cáncer

Uno podría asumir que un indicador de alto riesgo que es una cantidad incrementada de células tumorales otorgaría novedad a la reivindicación 8 pero un rasgo funcional no es limitativo como tal.

El objeto inventivo de las reivindicaciones 1-12 por lo tanto no es nuevo (Artículo 54(1) y (2) EPC).

3. Altura inventiva

El documento D1, considerado como el representante de mayor relevante del estado de la técnica, es descrito como objeto inventivo en el punto 2 de la presente solicitud.

El problema a ser resuelto por la presente invención puede ser contemplado como la provisión de agentes para el tratamiento del cáncer (inducción de la apoptosis en una célula).

La solución propuesta en las reivindicaciones 1-12 de la presente solicitud no pueden ser considerada poseedoras de altura inventiva (Artículo 33(3) PCT) debido a que D1 sugiere que 11-beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona- (página 3, línea 30) puede ser usado para el tratamiento de cáncer (página 4, línea 56-57).

Por lo tanto, el objeto inventivo de las reivindicaciones 1-12 de la presente solicitud no cumple con los requisitos del Artículo 33(3) PCT).

4. Aplicación industrial

Las reivindicaciones 1-9 cumplen los requisitos del Artículo 33(4) PCT.

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:	
SCHERING AKTIENGESELLSCHAFT Möllerstrasse 178 13353 Berlin ALLEMAGNE	
Frist: _____	

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Applicant's or agent's file reference 51870AWOM1XX		Date of mailing (day/month/year) 28.01.2003	
International application No. PCT/EP01/12006 ✓		International filing date (day/month/year) 17/10/2001 ✓	Priority date (day/month/year) 18/10/2000
Applicant SCHERING AKTIENGESELLSCHAFT			

IMPORTANT NOTIFICATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the International application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

For the purpose of deciding whether the claimed invention is patentable or not, the elected Offices may apply criteria additional to or different from the criteria on which the international preliminary examination report is based (see Articles 27(5), 33(5)). Additional criteria may include e.g. exemptions from patentability and the requirements of enabling disclosure and of clarity and support of claims.

Name and mailing address of the IPEA/ European Patent Office - Gilchiner Str. 103 D-10858 Berlin Tel. +49 30 25901 - 0 Fax +49 30 25901 - 840	Authorized officer Geier, A Tel. +49 30 25901-706	
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Tratado de Cooperación en Materia de Patentes

De la Autoridad de Examen Preliminar Internacional

A Schering Aktiengesellschaft

Notificación de comunicación del informe de Examen Preliminar Internacional (Regla PCT 71.1)

Fecha de envío postal: 28/01/2003

Referencia del solicitante o agente: 51870AWOM1XX

Notificación Importante

Solicitud Internacional No. PCT/EP01/12006

Fecha de presentación internacional: 17/10/2001

Fecha de Prioridad: 18/10/2000

Solicitante: Schering Aktiengesellschaft

1. El solicitante es notificado por la presente que esta Autoridad de Examen Preliminar Internacional transmite el Informe de Examen Preliminar Internacional y sus anexos, en caso de haberlos, realizado sobre la solicitud internacional.
2. Una copia del informe y sus anexos, en caso de haberlos, está siendo transmitido a la Oficina Internacional para que sea comunicado a todas las oficinas elegida.
3. La Oficina Internacional preparará una traducción al inglés del informe (pero no de todos los anexos) y transmitirá dicha traducción a las oficinas elegidas que la requieran.
4. Recordatorio: El solicitante debe iniciar la fase nacional en cada oficina elegida mediante la realización de ciertos actos (presentación de traducciones y pago de impuestos nacionales) dentro de los 30 meses contados desde la fecha de prioridad

(o más tarde en algunas oficinas) Artículo 39(1)) (Véase también el recordatorio enviado por la Oficina Internacional con el Formulario PCT/IB/301).

Cuando la traducción de la solicitud internacional debe ser entregada a una oficina elegida, y esa traducción deba contener una traducción de cada anexo al informe de examen preliminar internacional, será responsabilidad del solicitante el preparar y proveer dicha traducción directamente a cada una de las oficinas elegidas.

Para más detalles a los plazos aplicables y requerimientos de las oficinas elegidas, véase el Volumen II de la Guía del solicitante de PCT.

A fin de decidir si la invención reivindicada es patentable o no, las oficinas elegidas pueden aplicar criterios adicionales o diferentes de los criterios sobre los que está basado el informe de examen preliminar internacional (véase Artículos 27(5), 33(5). Criterios adicionales pueden incluir p. ej.: excepciones a la patentabilidad y requisitos para permitir la divulgación y de claridad y sustento de las reivindicaciones.

PCT REQUEST

51870AWOM1XX

Original (for SUBMISSION) - printed on 17.10.2001 10:31:15 AM

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form - PCT/RO101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.03.2001)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	European Patent Office (EPO) (RO/EP)
0-7	Applicant's or agent's file reference	51870AWOM1XX
I	Title of invention	USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	SCHERING AKTIENGESELLSCHAFT
II-5	Address:	Müllerstr. 178 D-13353 Berlin Germany
II-6	State of nationality	DE
II-7	State of residence	DE
II-8	Telephone No.	030 4681 1532
II-9	Facsimile No.	030 4681 2058
II-10	e-mail	Andreas.Broesamle@Schering.DE
III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	HOFFMANN, Jens
III-1-5	Address:	Großstückendorf 27 D-16567 Mühlenbeck Germany
III-1-6	State of nationality	DE
III-1-7	State of residence	DE

PCT REQUEST

51870AWOM1XX

Original (for SUBMISSION) - printed on 17.10.2001 10:31:15 AM

III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	LICHTNER, Rosemarie
III-2-5	Address:	Belziger Str. 39 D-10823 Berlin Germany
III-2-6	State of nationality	DE
III-2-7	State of residence	DE
III-3	Applicant and/or inventor	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	SIEMEISTER, Gerd
III-3-5	Address:	Reinerswalder Steig 26 D-13342 Berlin Germany
III-3-6	State of nationality	DE
III-3-7	State of residence	DE
III-4	Applicant and/or inventor	
III-4-1	This person is:	applicant and inventor
III-4-2	Applicant for	US only
III-4-4	Name (LAST, First)	SCHNEIDER, Martin
III-4-5	Address:	Schluchseestr. 6a D-13469 Berlin Germany
III-4-6	State of nationality	DE
III-4-7	State of residence	DE
III-5	Applicant and/or inventor	
III-5-1	This person is:	applicant and inventor
III-5-2	Applicant for	US only
III-5-4	Name (LAST, First)	FUEHRMANN, Ulrike
III-5-5	Address:	Charlottenburger Ufer 6a D-10587 Berlin Germany
III-5-6	State of nationality	DE
III-5-7	State of residence	DE

PCT REQUEST

51870AWOM1XX

Original (for SUBMISSION) - printed on 17.10.2001 10:31:15 AM

IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent international Authorities as:	common representative
IV-1-1	Name	SCHERING AKTIENGESELLSCHAFT
IV-1-2	Address:	Müllerstr. 178 D-13353 Berlin Germany
IV-1-3	Telephone No.	030 4681 1532
IV-1-4	Facsimile No.	030 4681 2058
IV-1-5	e-mail	Andreas.Broesamle@Schering.DE
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW ME SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST

51870AWOM1XX

Original (for SUBMISSION) - printed on 17.10.2001 10:31:15 AM

V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	Exclusion(s) from precautionary designations	NONE
VI-1	Priority claim of earlier regional application	
VI-1-1	Filing date	18 October 2000 (18.10.2000)
VI-1-2	Number	00250342.3
VI-1-3	Regional Office	EP
VI-2	Priority claim of earlier national application	
VI-2-1	Filing date	18 October 2000 (18.10.2000)
VI-2-2	Number	60/240,991
VI-2-3	Country	US
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)
VIII	Declarations	Number of declarations
VIII-1	Declaration as to the identity of the inventor	-
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-
IX	Check list	number of sheets electronic file(s) attached
IX-1	Request (including declaration sheets)	5 -
IX-2	Description	19 -
IX-3	Claims	2 -
IX-4	Abstract	1 EZABST00.TXT
IX-5	Drawings	11 -
IX-7	TOTAL	38

5/5

PCT REQUEST

51870AWOM1XX

Original (for SUBMISSION) - printed on 17.10.2001 10:31:15 AM

	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-11	Copy of general power of attorney	✓	-
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract		
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative	<i>A. Brös - G</i>	
X-1-1	Name	SCHERING AKTIENGESELLSCHAFT	
X-1-2	Name of signatory	i.V. Dr. Brösamle	
X-2	Signature of applicant, agent or common representative	<i>J. Kurilla</i>	
X-2-1	Name	SCHERING AKTIENGESELLSCHAFT	
X-2-2	Name of signatory	i.A. S. Kurilla	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	
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PATENT COOPERATION TREATY

133

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstrasse 178
13353 Berlin
ALLEMAGNE

AB 31. OCT. 2002
an 30.12.02
HIPS 2002/14

PC

WRITTEN OPINION

(PCT Rule 66)

Date of mailing (day/month/year)		30.10.2002
Applicant's or agent's file reference 51870AWOM1XX		REPLY DUE within 2 month(s) from the above date of mailing
International application No. PCT/EP01/12006	International filing date (day/month/year) 17/10/2001	Priority date (day/month/year) 18/10/2000
International Patent Classification (IPC) or both national classification and IPC A61K31/57		
Applicant SCHERING AKTIENGESELLSCHAFT		

1. This written opinion is the second drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement
- VI ☐ Certain document cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 18/02/2003.

Name and mailing address of the international preliminary examining authority:  European Patent Office - Glueckiner Str. 103 D-10055 Berlin Tel. +49 30 25901 - 0 Fax: +49 30 25901 - 840	Authorized officer / Examiner Beyss, E	
	Formalities officer (incl. extension of time limits) Geier, A Telephone No. +49 30 25901 706	

WRITTEN OPINION

International application No. PCT/EP01/12006

I. Basis of the opinion

1. With regard to the elements of the International application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"):

Description, pages:

1-19 as originally filed

Claims, No.:

1-12 as originally filed

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: English, which is:

- ☒ the language of a translation furnished for the purposes of the International search (under Rule 23.1(b)).
 - ☒ the language of publication of the international application (under Rule 48.3(b)).
 - ☒ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).
3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
- ☐ contained in the international application in written form.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority in written form.
 - ☐ furnished subsequently to this Authority in computer readable form.
 - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, Inventive step and Industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:
- ☐ the entire international application,
 - ☒ claims Nos. 10-12 in respect of industrial applicability,

because:

- ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 - ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - ☐ no international search report has been established for the said claims Nos. .
2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the standard.
 - ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement		
Novelty (N)	Claims	1-12
Inventive step (IS)	Claims	
Industrial applicability (IA)	Claims	10-12

WRITTEN OPINIONInternational application No. **PCT/EP01/12006**

2. Citations and explanations
see separate sheet

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/EP01/12006

Re Item III**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Claims 10-12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following document:

D1: DE 197 06 061 A (SCHERING AG) 13 August 1998 (1998-08-13) cited in the application

1. Clarity**1.1.**

Claims 1 and 2 do not meet the requirements of Article 6 PCT in that the matter for which, protection is sought is not clearly defined. The claims attempt to define the therapeutic application in terms of the result to be achieved (claim 1: "induction of apoptosis" and claim 2: "initiation of terminal differentiation") which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added.

1.2

The term "type of cancer...the cell cycle" used in claim 8 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

**WRITTEN OPINION
SEPARATE SHEET**

International application No. PCT/EP01/12006

2. Novelty

D1 discloses (claim 1; page 3, line 30; page 4, line 56-57) 17 alpha-fluoralkylsteroides according to formula I and their use in the treatment of hormon-dependent cancers. The subject-matter of claims 1-7 of present application discloses the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the induction of apoptosis, i. e. cancer.

The subject-matter of claim 8 of present application discloses the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the treatment of a type of cancer wherein an indicator of high risk is an increased amount of tumor cells.

Both, D1 and present application disclose the use of 11-beta-(4-acetylphenyl)-17 beta-hydroxy-17 alpha-(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for the treatment of cancer.

One could assume that an indicator of high risk is an increased amount of tumor cells renders claim 8 novel but a functional feature is not limiting as such.

The subject-matter of claims 1-12 is therefore not new (Article 54(1) and (2) EPC).

Tratado de Cooperación en Materia de Patentes

De la Autoridad de Examen Preliminar Internacional

Fecha de envío: 30/10/2002

Referencia del solicitante o agente: 51870AWOM1XX00+P

Plazo para responder: dentro de 2 meses desde la fecha de envío mencionada.

Solicitud Internacional No. PCT/EP 01/12006

Fecha de presentación internacional: 17/10/2001

Fecha de Prioridad: 18/10/2000

Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC: A61K31/57

Solicitante: Schering Aktiengesellschaft

1. Esta opinión escrita es la segunda emitida por esta Autoridad de Examen Preliminar Internacional

2. Esta opinión contiene indicaciones relacionadas con los siguientes puntos:

I (X) Base de la opinión

II Prioridad

III (X) No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

IV Falta de unidad inventiva

V(X) Declaración razonada bajo Regla 66.2 (a)(ii) con respecto a novedad, altura inventiva o aplicación industrial; citas y explicaciones que apoyan tal declaración.

VI Ciertos documentos citados

VII Ciertos defectos de la solicitud internacional

VIII Ciertas observaciones con respecto a la solicitud internacional

3. Por la presente se invita al solicitante a responder a esta opinión.

¿Cuándo? Véase el plazo indicado más arriba. El solicitante puede, antes del vencimiento del plazo, solicitar una extensión a esta Autoridad (ver Regla 66.2(d)).

¿Cómo? Enviando una contestación escrita, acompañada por correcciones, si fuera apropiado, de acuerdo con la Regla 66.3. Para la forma y el idioma de las correcciones, ver reglas 66.8 y 66.9.

También Para una oportunidad adicional de presentar correcciones, ver Regla 66.4. Para la obligación del examinador de tomar en consideración correcciones y/o argumentos, ver Regla 66.4bis. Para una comunicación informal con el examinador, ver Regla 66.6.

Si no se presenta una contestación, el informe de examen preliminar internacional será confeccionado sobre la base de esta opinión.

La fecha final en que el informe de examen preliminar internacional debe ser establecido, de acuerdo con la Regla 69.2, es: 18/02/2003

OPINIÓN ESCRITA

I. Base del Informe

1. Con respecto a los elementos de la solicitud internacional (hojas de reemplazo que han sido provistas a la oficina

receptora en respuesta a una invitación bajo Artículo 14 son mencionadas en este informe como "originalmente presentadas")

Descripción, hojas

1-19 como originalmente presentadas

Reivindicación, Nr.

1-12 como originalmente presentadas

2. Con respecto al idioma, todos los elementos indicados más arriba estaban disponibles o fueron provistos a esta Autoridad en el idioma en el cual fue presentada la solicitud internacional, a menos que esté indicado bajo este ítem.

Estos elementos estaban disponibles o fueron provistos a esta Autoridad en el siguiente idioma: inglés, que es:

(X) El idioma de una traducción provista a los fines de la búsqueda internacional (bajo Regla 23.1(b)).

(X) El idioma de la publicación de la solicitud internacional (bajo Regla 48.3(b)).

(X) El idioma de una traducción provista a los fines del examen preliminar internacional (bajo Regla 55.2 y/o 55.3).

3. Con respecto a cualquier nucleótido y/o secuencia de aminoácidos divulgada en la solicitud internacional, el examen preliminar internacional fue llevado a cabo sobre la base de los listados de secuencias:

Contenido en forma escrita en la solicitud internacional

Presentado junto con la solicitud internacional en una forma descifrable por computadora

Provista subsecuentemente a esta Autoridad en forma escrita

Provista subsecuentemente a esta Autoridad en una forma descifrable por computadora

La declaración de que el listado de secuencias provisto subsecuentemente no va más allá de lo divulgado en la solicitud internacional tal como fuera presentada

La declaración de que ha sido provista la información grabada en forma descifrable por computadora es idéntica al listado de secuencias escrito

4. Las correcciones han dado como resultado la cancelación de:

la descripción, páginas:

las reivindicación, Nrs.:

los dibujos, hojas:

5. Este informe fue emitido como si (algunas de) las correcciones no hubieran sido hechas, porque han sido consideradas como que van más allá de la divulgación tal cual fue presentada (Regla 70.2(c):

(Cualquier hoja de reemplazo que contenga este tipo de correcciones debe ser mencionada bajo el ítem 1 y anexada a este informe.)

6. Observaciones adicionales, de ser necesario:

III. No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

1. La pregunta si la invención reivindicada presenta novedad, altura inventiva (ser no-obvia), o tener aplicación industrial no han sido examinada con respecto a

toda la solicitud

(x) las reivindicaciones 1-12 con respecto a su aplicabilidad industrial

porque

la mencionada solicitud internacional, o las mencionadas reivindicación Nr. se refieren al siguiente objeto inventivo que no requiere un examen preliminar internacional (especificar):

la descripción, reivindicaciones o dibujos (indicar elementos particulares más abajo) o las mencionadas reivindicaciones No. son tan poco claras que no se pudo formar una opinión que tenga sentido (especificar):

las reivindicaciones o las mencionadas reivindicaciones No. están inadecuadamente sustentadas en la descripción que no se pudo formar una opinión que tenga sentido

no se realizó el informe de búsqueda internacional para las mencionadas reivindicaciones Nr.

2. Un examen preliminar internacional que tenga sentido no puede ser llevado a cabo debido a que la lista de nucleótidos y/o secuencias de aminoácidos no cumple con el estándar provisto por el Anexo C de las instrucciones administrativas:

el formulario escrito no fue aportado o no es conforme al estándar

el formulario descifrable por computadora no ha sido aportado o no es conforme al estándar

V. Declaración razonada bajo Artículo 35(2) con respecto a novedad, altura inventiva y aplicación industrial, citas y explicaciones que avalen dicha declaración

1. Declaración**Novedad (N)****Reivindicaciones 1-12****Altura inventiva (IS)****Reivindicaciones****Aplicación Industrial (IA)****Reivindicaciones 10-12****2. Citas y explicaciones****ver hoja separada**

Opinión Escrita

Hoja separada

Solicitud Internacional No. PCT/EP01/12006

En relación con el ítem III

No emite opinión con respecto a la novedad, altura inventiva y aplicación industrial

Las reivindicaciones 10-12 se refieren a un objeto inventivo, el cual esta Autoridad considera es conforme a las disposiciones de la Regla 67.1(iv) PCT. En consecuencia, no se formulará opinión con respecto a la aplicación industrial del objeto inventivo de estas reivindicaciones (Artículo 34(4)(a)(i)PCT).

En relación con el ítem V

Declaración razonada bajo Regla 66.2(a)(ii) con respecto a la novedad, altura inventiva o aplicación industrial, citas y explicaciones en que se basa dicha declaración

Se hace referencia al siguiente documento:

D1: DE 197 06 061* (Schering AG) 13 de agosto de 1998 (1998-08-12) citado en la solicitud

1. Claridad

1.1

Las reivindicaciones 1 y 2 no cumplen con los requisitos del Artículo 6 PCT debido a que el objeto que se busca proteger no está definido claramente. Las reivindicaciones tratan de definir la aplicación terapéutica en términos del resultado a ser obtenido (reivindicación 1: inducción de la apoptosis" y reivindicación 2: "inicio de diferenciación terminal") que meramente se suman a una declaración del problema subyacente.

Deben ser agregados los dispositivos técnicos necesarios para alcanzar este resultado.

1.2

El término "tipo de cáncer ... el ciclo celular" usado en la reivindicación 8 es vago y poco claro y deja al lector la duda con respecto al significado de los dispositivos técnicos a los que se refiere, resultando la definición del objeto inventivo de dicha reivindicación poco clara (Artículo 6 PCT).

2. Novedad

D1 describe (reivindicación 1; página 3, línea 30, página 4, línea 56-57) 17alfa-fluoroalquilesteroides de acuerdo con la fórmula I y su uso en el tratamiento de cánceres hormona-dependientes. El objeto inventivo de las reivindicaciones 1-7 de la presente invención describe el uso de 11-beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4-dien-3-ona para la inducción de la apoptosis, p.ej. cáncer.

El objeto inventivo de la reivindicación 8 de la presente solicitud describe el uso de 11-beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para el tratamiento de un tipo de cáncer en el que un indicador de alto riesgo es una cantidad incrementada de células tumorales.

Tanto D1 como la presente solicitud describen el uso de 11-beta-4(acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para el tratamiento de cáncer.

Uno podría asumir que un indicador de alto riesgo es una cantidad incrementada de células tumorales otorga novedad a la reivindicación 8 pero un rasgo funcional no es limitativo como tal.

El objeto inventivo de las reivindicaciones 1-12 por lo tanto no es nuevo (Artículo 54(1) y (2) EPC).

PATENT COOPERATION TREATY

WO 02/32432
PCT/EP01/12006

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PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstr. 178
13353 Berlin
ALLEMAGNE

AB

OM

G. S. JAN 2002

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Date of mailing (day/month/year) 25 April 2002 (25.04.02)		IMPORTANT NOTICE	
Applicant's or agent's file reference 51870AWOM1XX00 +?			
International application No. PCT/EP01/12006	International filing date (day/month/year) 17 October 2001 (17.10.01)	Priority date (day/month/year) 18 October 2000 (18.10.00)	
Applicant SCHERING AKTIENGESELLSCHAFT et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:
KP, KR, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE, AG, AL, AM, AP, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EA, EC, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OA, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 48.1(a-bis)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 25 April 2002 (25.04.02) under No. WO 02/32432

4. TIME LIMITS for filing a demand for international preliminary examination and for entry into national phase

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph be 30 MONTHS from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 18 months from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of the designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 18828, 18832 and 18834, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the PCT Gazette, the PCT Newsletter and the PCT Applicant's Guide, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

For filing a demand for international preliminary examination, see the PCT Applicant's Guide, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II.)

It is the applicant's sole responsibility to monitor all these limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.36	Telephone No. (41-22) 338.91.11

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Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF
THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 25 April 2002 (25.04.02)	IMPORTANT NOTICE
Applicant's or agent's file reference 51870AWOM1XX <i>00+P</i>	International application No. PCT/EP01/12006
The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 48.1 for making amendments under Article 18 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.	

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(a))

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstr. 178
13353 Berlin
ALLEMAGNE

02 JAN 2002

AR

ON

Bco

Date of mailing (day/month/year)

19 December 2001 (19.12.01)

IMPORTANT NOTIFICATION

Applicant's or agent's file reference

51870AWOM1XX00+?

International application No.

PCT/EP01/12006 ✓

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

SCHERING AKTIENGESELLSCHAFT (for all designated States except US)
HOFFMANN, Jens et al (for US)

International filing date : 17 October 2001 (17.10.01) ✓

Priority date(s) claimed : 18 October 2000 (18.10.00)

18 October 2000 (18.10.00)

Date of receipt of the record copy
by the International Bureau : 10 December 2001 (10.12.01)

List of designated Offices :

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR

OA : BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ,

EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,

LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ,

UA, UG, US, UZ, VN, YU, ZA, ZW

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

Hans LEISE

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstr. 178
13353 Berlin
ALLEMAGNEPatente
18 März 2002

Date of mailing (day/month/year)

08 March 2002 (08.03.02)

Applicant's or agent's file reference

51870AWOM1XXOD+P

IMPORTANT NOTIFICATION

International application No.

PCT/EP01/12006 ✓

International filing date (day/month/year)

17 October 2001 (17.10.01) ✓

1. The following indications appeared on record concerning:



the applicant



the inventor



the agent



the common representative

Name and Address

SIEMEISTER, Gerd
Reimerswalder Steig 26
13342 Berlin
Germany

State of Nationality

DE

State of Residence

DE

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:



the person



the name



the address



the nationality



the residence

Name and Address

SIEMEISTER, Gerd
Reimerswalder Steig 26
13503 Berlin
Germany

State of Nationality

DE

State of Residence

DE

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:



the receiving Office



the designated Offices concerned



the International Searching Authority



the elected Offices concerned



the International Preliminary Examining Authority



other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Anman QIU

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

SCHERING AKTIENGESELLSCHAFT
Müllerstr. 178
13353 Berlin
ALLEMAGNE

Patente

10. APR. 2002

Frist:

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Date of mailing (day/month/year) 27 March 2002 (27.03.02)	
Applicant's or agent's file reference 51870AWOM1XXOO+P	IMPORTANT NOTIFICATION
International application No. PCT/EP01/12006 ✓	International filing date (day/month/year) 17 October 2001 (17.10.01) ✓
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 18 October 2000 (18.10.00)
Applicant SCHERING AKTIENGESELLSCHAFT et al	

- The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
- An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
18 Octo 2000 (18.10.00)	00250342.3	EP	07 Febr 2002 (07.02.02)
18 Octo 2000 (18.10.00)	60/240,991	US	19 Marc 2002 (19.03.02)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

David MALEK

Telephone No. (41-22) 338.83.38

Telefax



Schering AG

Dr. Andreas Brösamle
Corporate Patents

From (Name, Dept.)

European Patent Office
Berlin
Attn: Mr. A. Geier

To (Name, Dept.)

E: Andreas.Brösamle@Schering.de

T: +49.30.468- 11532

+49.30.468- 1111

F: +49.30.468- 12058

T: 030 25901-0

F: 030 25901-843

No of Pages 1

Date 2003-01-08

International Patent Application No. PCT/EP01/12006

Our Ref: 61870AWOM1XX00-P

The applicant herewith informs that the International Preliminary Examination Report shall be prepared on the basis of the second written opinion of October 30, 2002.

SCHERING AKTIENGESELLSCHAFT

i.V.

Dr. Brösamle

Kurilla

Postal address:
Schering AG
D-13342 Berlin, Germany
For visitors:
Möllerstr. 178, Berlin-Mitte
www.schering.de

Executive board:
Hubertus Erlen (Chairman)
Klaus Fohle (Vice-Chairman)
Ulrich Köstlin
Lutz Lingnau
Jörg Spielerkötter (Deputy)
Günter Stock

Chairman of the supervisory board:
Giuseppe Vita

Registered seat: Berlin
Trade register:
AG Charlottenburg 93 HRB 283

Commerzbank AG, Berlin
Acct. No. 108700000, Bank No. 100 400 00
ING BHF-Bank AG, Berlin
Acct. No. 70045224, Bank No. 100 202 00
Deutsche Bank AG, Berlin
Acct. No. 2416008, Bank No. 100 700 00

Telefax de Schering AG a la Oficina Europea de Patentes de fecha
8.01.2003

Solicitud de patente internacional No. PCT/EP01/12006

Nuestra referencia: 51870AWOM1XX00-P

El solicitante informa por la presente, que el Informe de Examen Preliminar Internacional deberá ser preparado sobre la base de la segunda opinión escrita del 30 de octubre de 2002.



patents • trademarks • designs

Dörries
Frank-Molnia
& Pohlman

Mail Postfach 22 16 61, D - 80506 München • Visitors Triftstrasse 13, D - 80538 München
Phone +49 89 210296 0 • Fax +49 89 210296 33 • info@df-mp.com • www.df-mp.com

European Patent Office
Erhardtstrasse 27
80331 Munich

September 19, 2002

Official Ref.: PCT/EP01/12006
Title: Use of Antiprogestins for the Induction of Apoptosis in a Cell
Applicant: Schering AG
Our Ref.: SCH10086PCT

Response to PCT Written Opinion mailed 27 August 2002

Applicant would like to provide the following comments in response to the Written Opinion of 27 August 2002:

The Written Opinion (Item V) states that the invention, at least as defined in some of the claims, does not appear to be novel or inventive in view of documents cited in the International Search Report.

The International Search Report mailed on 26 March 2002 cited one "X" document, DE 197 06 061 A ("D1"). D1 is stated as relevant for all claims 1-12. The search report refers to page 3, line 30, page 4, lines 56-57 and claim 1 of D1. Claim 1 is directed to 17 α -fluoroalkylsteroids having a general formula I, and the specific compound 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-

H. Ulrich Dörries • Dr. Sc. Nat., Dipl.-Biol., Patentanwalt, European Patent & Trademark Attorney
David Molnia • M.S. Elec. Eng., Patentanwalt, European Patent & Trademark Attorney, U.S. Patent Agent
Sandra Pohlman • B.S. Biology, U.S. Patent Attorney, Solicitor (England & Wales)

dien-3-one is mentioned in a list on page 3, line 30. Page 4, lines 56-57 states that the invention compounds may be suitable for treating hormone-dependent cancers, among several other indications. The predominant indication is contraception and post-coital fertility control (see page 4, lines 47-51). Other mentioned indications are treating hormonal abnormalities, inducing menstruation or childbirth, hormone-replacement therapy and treating dysmenorrhea and endometriosis (page 4, lines 52-57). There is no mention or suggestion in the application at all that either the compounds of the general formula or any of the specific compounds listed in D1 could play any role in inducing apoptosis in a cell or be used for treating a type of cancer where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Moreover, there is no disclosure or suggestion to employ the specific compound 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for these purposes. The application for the first time shows that 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one induces apoptosis and moreover, provides strong evidence in standard animal models that 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one may be used for the claimed purposes. Thus, the subject matter of the independent claims 1, 8 and 10 would be novel and inventive in view of D1, and accordingly, the remaining dependent claims would also involve novel and inventive subject matter. We would like to point out that D1 has basically already been cited and discussed in the application, since WO 98/34947 is discussed on page 3, lines 5-8 of the application. WO 98/34947 claims priority from D1 and contains the same disclosure.

In view of the arguments above, ~~a detailed written opinion on the merits of the application~~
~~and a detailed written opinion on the merits of the application~~ is hereby requested.

SCHERING AG - Corporate Patents

A la Oficina Europea de Patentes

Solicitud Internacional No. PCT/EP01/12006

Nuestra referencia: 51870AWOM1XX00+P

Respuesta a la Opinión Escrita enviada el 27-08-2000

Por la presente el solicitante quisiera proveer los siguientes comentarios en respuesta a la Opinión Escrita del 27 de agosto de 2002:

La Opinión Escrita (ítem V) establece que el invento, al menos tal como está definido en algunas de las reivindicaciones, aparenta no ser nuevo o no tener actividad inventiva en vista de documentos citados en el Informe de Búsqueda Internacional.

El Informe de Búsqueda Internacional enviado el 26 de marzo de 2002 citó un documento "X", DE 197 06 061 A ("D1"). D1 es considerado como relevante para todas las reivindicaciones 1-12.

El informe de búsqueda se refiere a la página 3, línea 30, página 4, líneas 56-57 y reivindicación 1 de D1. La reivindicación 1 se refiere a 17alfa fluoroalquilesteroides de fórmula general I y el compuesto específico 11beta-(4-acetilfenil)-17beta-hidroxi-17alfa(1,1,2,2,2-

pentafluoroetil)estra-4,9-dien-3-ona está mencionado en una lista en la página 3, línea 30. En la página 4, líneas 56-57 se declara que los compuestos de acuerdo con la invención pueden ser aptos para tratar cánceres hormona-dependientes, entre muchas otras indicaciones. La indicación predominante es la contracepción y el control post-coital de la fertilidad (véase página 4, líneas 47-51). Otras indicaciones mencionadas son el

tratamiento de anormalidades hormonales, inducción de la menstruación o del parto, terapias de reemplazo hormonal y el tratamiento de la dismenorrea y la endometriosis (página 4, líneas 52-57). No hay mención o sugerencia alguna en la solicitud de que los compuestos de la fórmula general o alguno de los compuestos específicos enumerados en D1 pudieran jugar algún papel en la inducción de la apoptosis en una célula o ser usado para tratar un tipo de cáncer en el que un indicador de alto riesgo es una cantidad elevada de células tumorales en la fase S del ciclo celular. Más aún, no hay descripción o sugerencia de emplear el compuesto específico, 11beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona para estos propósitos. La solicitud muestra por primera vez que 11beta-(4-acetilfenil)-17beta-hidroxi-17alfa-(1,1,2,2,2-pentafluoroetil)-estra-4,9-dien-3-ona puede ser usado para los propósitos reivindicados. Por lo tanto, el objeto inventivo de las reivindicaciones independientes 1, 8 y 10 sería nuevo y tendría altura inventiva frente a D1, y, en consecuencia, las restantes reivindicaciones dependientes también contendrían un objeto nuevo e inventivo. Nos gustaría remarcar que D1 básicamente ya ha sido citado y discutido en la solicitud, debido a que WO 98/34947 es discutido en la página 3, líneas 5-8 de la solicitud. WO 98/34947 reivindica la prioridad de D1 y contiene la misma descripción.

En vista de los argumentos precedentes, se solicita por la presente un Informe de Examen Preliminar Internacional detallado.



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ERHARDTSTRASSE 27
D-80298 MÜNCHEN

Your Ref.	Your letter dated	Our Ref. (please indicate when replying)	Telephone: +49-30-489-11332 +49-30-4 89-11 11	Date
		ab-ku	Telefax: +49-30-489-12068	2002-09-24

International Patent Application No. PCT/EP04/12006
Our Ref: 51870AWOM1XX00-P

Response to the PCT Written Opinion mailed on August 27, 2002

The applicant would like to provide the following comments in response to the Written Opinion of 27. August 2002:

The Written Opinion (Item V) states that the invention, at least as defined in some of the claims, does not appear to be novel or inventive in view of documents cited in the International Search Report.

The International Search Report mailed on March 26, 2002 cited one "X" document, DE 197 06 061 A ("D1"). D1 is stated as relevant for all claims 1 – 12. The search report refers to page 3, line 30, page 4, lines 56 – 57 and claim 1 of D1. Claim 1 is directed to 17 α -fluoroalkylsteroids having a general formula I and the specific compound 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α (1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one is mentioned in a list on page 3, line 30. Page 4, lines 56 – 57 states that the invention compounds may be suitable for treating hormone-dependent cancers, among several other indications. The predominant indication is contraception and post-coital fertility control (see page 4, lines 47 – 51). Other mentioned indications are treating hormonal abnormalities, inducing menstruation or childbirth, hormone-replacement therapy and treating dysmenorrhea and endometriosis (page 4, lines 52 – 57). There is no mention or suggestion in the application at all that either the compounds of the general formula or any of the specific compounds listed in D1 could play any role in inducing apoptosis in a cell or be used for treating a type of cancer where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Moreover, there is no disclosure or suggestion to employ the specific compound 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one for these purposes. The application for the first time shows that 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -

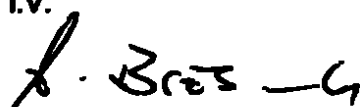
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(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one may be used for the claimed purposes. Thus, the subject matter of the independent claims 1, 8 and 10 would be novel and inventive in view of D1, and accordingly, the remaining dependent claims would also involve novel and inventive subject matter. We would like to point out that D1 has basically already been cited and discussed in the application, since WO 98/34947 is discussed on page 3, lines 5 – 8 of the application. WO 98/34947 claims priority from D1 and contains the same disclosure.

In view of the arguments above, a detailed International Preliminary Examination Report is hereby requested.

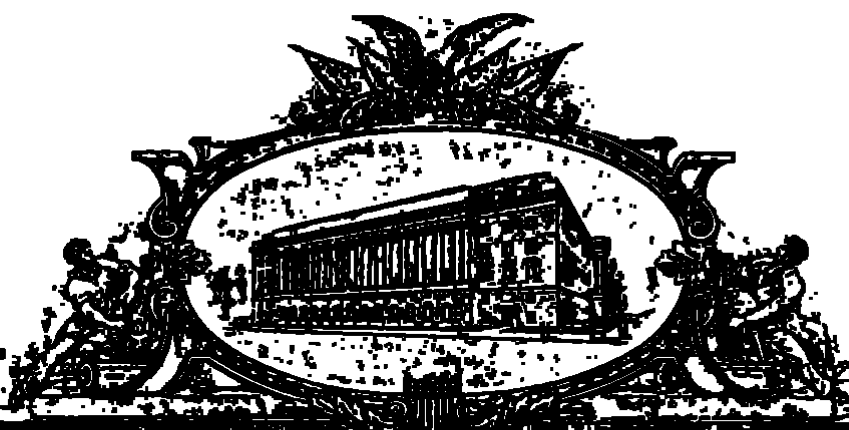
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i.V.


Dr. Brösamle


Kurilla

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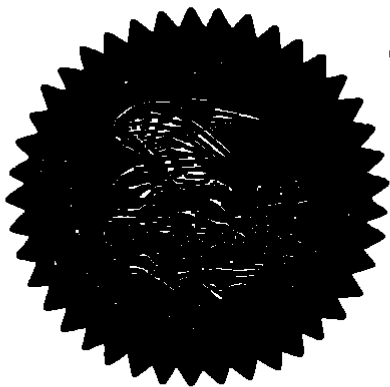
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APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.**

APPLICATION NUMBER: 60/240,991

FILING DATE: *October 18, 2000*



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

**M. LEE
Certifying Officer**



10/18/00

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(e).

Docket Number SCH-1785-V1

1c772 U.S. PTO
16007/00
60/240991

INVENTOR(s)/APPLICANT(s)			
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
HOFFMANN	Jens		Muehlenbeck, Germany

☐ Additional inventors are named on separately numbered sheets attached hereto.

TITLE OF THE INVENTION (280 CHARACTERS MAX)

USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL

CORRESPONDENCE ADDRESS

MILLEN, WHITE, ZELANO & BRANIGAN, P.C.
2200 Clarendon Blvd., Suite 1400
Arlington, Virginia 22201

Phone: (703) 243-6333 Fax: (703) 243-6410

ENCLOSED APPLICATION PARTS

☒ Abstract	☐ Small Entity Statement
☒ Specification <i>Number of Pages:</i> 19	<i>Language:</i>
☒ Claims <i>Number of Pages:</i> 1	☐ Other (specify):
☒ Drawing(s) <i>Number of Sheets:</i> 11	

METHOD OF PAYMENT

☒ A check or money order is enclosed to cover the Provisional Filing Fees	PROVISIONAL FILING FEE AMOUNT
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☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully Submitted,

October 18, 2000

DATE

Anthony J. Zelano

Registration Number 27,969

PROVISIONAL APPLICATION FILING ONLY

USE OF ANTIPROGESTINS FOR THE INDUCTION OF APOPTOSIS IN A CELL

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Field of the Invention

The present invention relates to the use of antiprogestins for the induction of apoptosis in a cell. In particular, the invention relates to use of the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell. The present invention further provides a use of antiprogestins for the preparation of a medicament for the treatment of a type of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

Background of the Invention

Antiprogestins represent a relatively new and promising class of therapeutic agents that could have significant impact on the treatment of hormone-dependent tumors and other diseases. Although antiprogestins were originally created with regard to medicinal non-surgical termination of pregnancy, certain antiprogestins have gained considerable importance, e.g., in the endocrine therapy of those breast cancers which possess receptors for progesterone (T. Mauvelont et al., in: J.G.M. Klijn et al., *Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti) Steroidal Agents*, Raven Press, New York, 1987, pp. 55-59).

This new strategy in endocrine therapy is based on the antitumor activity of antiprogestins in progesterone receptor positive human breast cancer cell lines *in vitro* and in several hormone-dependent mammary tumors of the mouse and rat *in vivo*. In particular, the antitumor mechanism of the antiprogestins onapristone and mifepristone (RU 486) has

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already been investigated using the hormone-dependent MXT mammary tumor model of the mouse as well as the DMBA- and the NMU-induced mammary tumor models of the rat (M. R. Schneider et al., *Eur. J. Cancer Clin. Oncol.*, Vol. 25, No. 4, pp. 691-701, 1989; H. Michna et al., *Breast Cancer Research and Treatment* 14:275-288, 1989; H. Michna, *J. Steroid. Biochem.* Vol. 34, Nos 1-6, pp. 447-453, 1989). However, due to low activity and adverse side effects involved with e.g. mifepristone this compound could not be recommended as a single agent in the management of breast cancer (D. Perrault et al., *J. Clin. Oncol.* 1996 Oct, 14(10), pp.2709-2712). Furthermore, mifepristone exhibits strong antiglucocorticoid side effects (cf. L.M. Kettel et al., *Fertil. Steril.* 1991 Sep, 56(3), pp. 402-407; X. Bertagna, *Psychoneuroendocrinology* 1997; 22 Suppl. 1, pp. 51-55).

The determination of the percentage of tumor cells in the respective phases of the cell cycle can be performed by the powerful DNA flow cytometry method (cf. G. M. Clark et al., *N. Engl. J. Med.* 320, 1989, March, pp.627-633; L. G. Dressler et al., *Cancer* 61(3), 1988, pp. 420-427 and literature cited therein). It has thus been shown that the stages of the cell cycle of a tumor cell, and specifically, the number of tumor cells in certain stages of the cycle, may be an important clinical predictor of disease progression and success of therapy. The number of cells in the S-phase of the cell cycle are particularly important in this regard.

EP 0 495 825 B1 discloses the use of antiprogesterins (competitive progesterone antagonists) for the production of medicaments for the treatment of mammary carcinomas having an increased content of tumor cells in the S-phase of the cell cycle, which is considered to be a high risk factor. This is based on the observation that antiprogesterins are capable of blocking the progression of tumor cells in the G₀G₁-phase of the cell cycle resulting in a substantial decrease of tumor cells in the S-phase. This effect was however not observed with the standard breast cancer therapy tamoxifen, estrogen therapy or ovariectomy. The antiprogesterins tested in EP 0 495 825 B1 are 11β-[4-N,N-dimethylamino)-phenyl]-17α-hydroxy-17β-(3-hydroxypropyl)-13α-methyl-4,9(10)-

gonadien-3-one and 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(prop-1-ynyl)-4,9(10)-estradien-3-one.

17 α -fluoroalkylsteroids having strong antiprogesterin activity as well as methods for producing them are described in WO 98/34947. WO 98/34947 does not discuss or investigate the role that the 17 α -fluoroalkylsteroids disclosed therein may play in cell apoptosis or cell cycle arrest.

Given the potential value of agents that induce apoptosis in cells, e.g., in the case of tumor cells, by blocking progression in the G_0G_1 -phase, it is desirable to identify further agents, e.g., antiprogesterins, having this specific mechanism of action. Such agents would have potential application in treating and preventing certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

Object of the Invention

It is thus an object of the present invention to further investigate the mode of action of antiprogesterins in inhibiting hormone-dependent diseases such as breast cancer and to provide a method for the targeted induction of apoptosis in cells.

Surprisingly, the inventors have discovered that the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one (or a pharmaceutically acceptable derivative or analogue thereof) may be used for the induction of apoptosis in a cell.

Summary of the Invention

The present invention is based on the unexpected observation that the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one (hereinafter referred to as "antiprogesterin (I)") induces apoptosis and cell death in the tumor cells of standard breast cancer tumor models. It was found that antiprogesterin (I) is capable of inducing apoptosis in cells via the initiation of terminal differentiation.

Thus, the present invention provides the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the induction of apoptosis in a cell. Preferably, the induction of apoptosis is caused by the initiation of terminal differentiation. The cell is preferably a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of types of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

A further aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell *in vitro*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Another aspect of the present invention is a method of inducing apoptosis in a cell by administering an effective amount of antiprogesterin (I) to the cell. This method may be applied *in vitro* or *in vivo*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

Due to the ability to induce cell apoptosis the antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be used for the treatment of certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Other types of cancer or hormone-dependent diseases that may be affected and treated by antiprogesterin (I) due to its ability to induce cell apoptosis may include, e.g., breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e. diseases which substantially originate

or are influenced by the presence of hormone receptors and/or hormone-dependent pathways.

Brief Description of the Figures

6 Figure 1 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in a dose-response study in the DMBA-induced mammary carcinoma of the rat, compared with a control, the antiprogesterin onapristone as well as ovariectomy. The study was performed with 0.5, 2.0, 5.0 and 10.0 mg/kg s.c. daily doses of antiprogesterin (I).

10

Figure 2 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in the NMU-induced mammary carcinoma of the rat, compared with a control and ovariectomy. The study was performed with 0.5 and 1.0 mg/kg s.c. daily doses of antiprogesterin (I).

15

Figure 3 shows the induction of apoptosis and thus the tumor growth inhibiting effect of antiprogesterin (I) in a 10 mg/kg s.c. dose on xenotransplanted human T47D tumors in scid mice, compared to a control and ovariectomy.

20

Figure 4 demonstrates the induction of apoptosis and thus the tumor growth inhibiting effect of a 10 mg/kg s.c. dose of antiprogesterin (I) in the MCF-7 human breast cancer model in acid mice, compared to a control and ovariectomy.

25

Figures 5A to 5F show histological data relating to the induction of apoptosis in the NMU-induced breast cancer model in rat (cf. Example 5). In particular, figure 5A shows that tumors treated with antiprogesterin (I) display ductal and acinous formations, usually filled with secretory material, compared to the control (figure 5B). Figure 5C shows untreated NMU-induced breast cancer tissue with high PCNA (proliferating cell nuclear antigen) immunoreactivity as compared to NMU-induced breast cancer tissue treated with antiprogesterin (I) (figure 5D), which exhibits low PCNA immunoreactivity. Figure 5E

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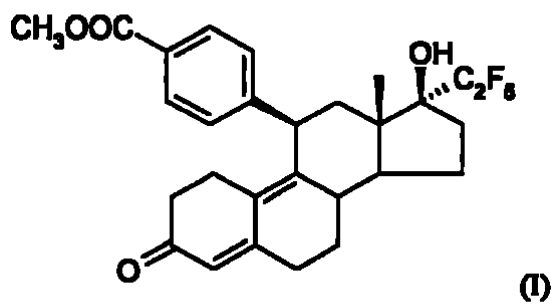
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shows the appearance of apoptosis in antiprogesterin (I)-treated NMU-induced breast cancer tissue, compared to the control (figure 5E).

Figure 6 demonstrates the tumor growth inhibiting effect of antiprogesterin (I) in the T47D breast cancer cell line (stimulated by estradiol) with an effective threshold concentration of 10^{-9} to 10^{-8} mol/l, compared with the antiprogesterin onapristone and the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

Detailed Description of the Invention

Antiprogesterin (I) — 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one — is represented below by formula (I):



Antiprogesterin (I) (or a pharmaceutically acceptable derivative or analogue thereof) is a valuable pharmaceutical agent having strong antiprogesterin activity. Antiprogesterin (I) can be used according to the present invention for the induction of apoptosis in cells.

The term "antiprogesterin" in the context of the present invention is intended to primarily comprise all compounds being capable of competitively inhibiting progesterone receptors.

However, it should also encompass compounds capable of inhibiting the biosynthesis of progestins.

Pharmaceutically acceptable derivatives or analogues of antiprogesterin (I) in the context of the present invention may include, for example, any one of the inventive compounds disclosed in WO 98/34947.

The studies performed in the context of the present invention show the potent tumor-inhibiting properties of the antiprogesterin (I) in a variety of hormone-dependent tumor models (see Examples 1 to 6). It is further demonstrated that the tumor inhibiting activity of antiprogesterin (I) as a result of the induction of apoptosis is stronger than conventional anti-tumor agents, such as, the anticestrogen tamoxifen. The treatment of breast cancer using the antiprogesterin (I) according to the present invention is even superior to ovariectomy.

Application of antiprogesterin (I) in the various tumor models as demonstrated below in the Examples revealed an accumulation of tumor cells in the G₀G₁ phase of the cell cycle together with a significant and biologically relevant reduction in the number of cells in the S and G₂M phase of the cell cycle. These results indicate an induction of differentiation. Differentiation-specific G₁ arrest has already been proposed earlier for other stem cell systems (see J.J. Wille Jr., *Cancer Res.* 1982, 42(12):5139-46; R.E. Scott, *J. Cell. Biol.* 1982, 94(2):400-405).

The experimental results obtained in the various tumor models revealed that treatment with antiprogesterin (I) seems to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with a massive sequestering of secretory products, as well as towards spindle-shaped necrobiotic subpopulations (see Example 5 and in particular figures 5A and 5B). Whereas tumor size, mitotic index and the grade of malignancy decreased distinctly, the volume fraction of glandular structures in the tumors as well as the appearance of apoptosis increased 3-fold compared to the controls (see Example 5, figures 5E and 5F).

Without limitation to any theory, these results indicate that the main mechanism of the antitumor action of antiprogesterin (I) in the tested models is a direct progesterone-receptor-mediated antiproliferative effect at the level of the tumor cells, via the induction of terminal differentiation associated with terminal cell death. In this manner, antiprogesterin (I) appears to be capable of eliminating the intrinsic block in terminal differentiation inherent in malignant tumor cells in progesterone receptor-positive tumors. This antiproliferative effect of antiprogesterin (I) seems to be dissociated from the antihormone (antiprogesterin) activity of antiprogesterin (I).

Agents such as antiprogesterin (I) that induce apoptosis in cells, for example, in the case of tumor cells, by blocking progression in the G_0G_1 -phase, have potential applications for treating and preventing numerous conditions. Such agents, including antiprogesterin (I), may be used for treating those cancers where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, such as in breast cancer.

Thus one aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for preparation of a medicament for the induction of apoptosis in a cell. In a preferred embodiment, the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof relates to a medicament for the induction of apoptosis in a tumor cell, preferably a breast tumor cell, in a human. Such medicament could be beneficial in the treatment of hormone-dependent diseases such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

The manufacture of the medicaments may be performed according to methods known in the art. Commonly known and used adjuvants as well as further suitable carriers or diluents may be used. Suitable carriers and adjuvants may be such as recommended for pharmacy, cosmetics and related fields in: *Ullmann's Encyclopedia of Technical Chemistry*, Vol. 4, (1953), pp. 1-39; *Journal of Pharmaceutical Sciences*, Vol. 52 (1963), p. 918ff; H.v.Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete";

Pharm. Ind. 2, 1961, p.72ff; Dr. H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete*, Cantor KG, Aulendorf in Württemberg, 1971.

Antiprogestins suitable for the purposes of the present invention, preferably antiprogestin
5 (I) or a pharmaceutically acceptable derivative or analogue thereof, can be incorporated
into pharmaceutical compositions according to known methods of preparing galenics for
oral or parenteral, e.g., intraperitoneal, intramuscular, subcutaneous or percutaneous
application. They can also be implanted into tissue. Implants can comprise as inert
materials e.g. biologically degradable polymers or synthetic silicones such as e.g. silicone
10 rubber.

They can be administered in the form of tablets, pills, dragees, gel capsules, granules,
suppositories, implants, injectable sterile aqueous or oily solutions, suspensions or
emulsions, ointments, creams, gels or by intravaginal (e.g., vaginal rings) or intrauterine
15 systems (e.g., diaphragms, loops).

For the preparation of a medicament for oral administration, the antiprogestins suitable for
the purposes of the present invention as defined above can be admixed with commonly
known and used adjuvants and carriers such as for example, gum arabic, talcum, starch,
20 sugars such as, e.g., mannitose, methyl cellulose, lactose, gelatin, surface-active agents,
magnesium stearate, aqueous or non-aqueous excipients, paraffin derivatives, cross-
linking agents, dispersants, emulsifiers, lubricants, conserving agents and flavoring agents
(e.g., ethereal oils). In a pharmaceutical composition, the antiprogestin may be dispersed
in a microparticle, e.g. a nanoparticulate, composition.

25 In order to further enhance the bioavailability of the active agent, the antiprogestins
suitable for the purposes of the present invention as defined above can also be formulated
as cyclodextrin clathrates by reacting them with α -, β - or γ -cyclodextrines or derivatives
thereof according to the method as disclosed in PCT/EP95/02656.

30 For parenteral administration the antiprogestins suitable for the purposes of the present
invention as defined above can be dissolved or suspended in a physiologically acceptable

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diluent, such as, e.g., oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used.

5 The amount to be administered (i.e., a "pharmaceutically effective amount") varies within a broad range and depends on the condition to be treated and the mode of administration. It can cover any amount efficient for the intended treatment. Determining a "pharmaceutically effective amount" is within the purview of the person skilled in the art.

10 One unit dose may represent about 0.1 to 100 mg active agent(s). For administration to humans, the daily dose of the active agent(s) is about 0.1 to 400 mg, preferably 10 to 100 mg, most preferably 50 mg.

The medicaments can also be administered via a depot injection or an implant preparation, optionally for sustained delivery of the active agent(s).

The preferred mode of administration is oral administration. The antiprogestins for use according to the invention, and in particular, antiprogestin (I) are particularly suitable for oral administration.

20 According to all aspects of the present invention it is also possible to combine at least one antiprogestin as defined above, in particular antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof, with at least one antiestrogen, because many hormone-dependent diseases, in particular breast cancer, exhibit not only progesterone receptors, but also estrogen receptors. The antiestrogen may be administered either simultaneously with or sequentially to the antiprogestin, and in particular with/to antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof. The amount of antiprogestin and antiestrogen may be equal or one component may be more predominant than the other, such as in an antiprogestin:antiestrogen ratio of 1:50 to 50:1, preferably 1:30 to 30:1, and most preferably 1:15 to 15:1.

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Examples of suitable antiestrogens for use according to the invention are non-steroidal antiestrogens, such as tamoxifen and nafoxidine as well as raloxifen, faslodex and EM800. Examples of steroidal antiestrogens include those disclosed in EP 0 348 341 A and those disclosed in WO 98/07740, in particular, 11 β -flouro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio-propylamino)-pentyl]-estra-1,3,5(10)-trien-3,17 β -diol, or those
5 disclosed in WO 99/33855, in particular 11 β -flouro-7 α -(5-[methyl-(7,7,8,8,9,9,10,10,10-nonafluoro-decyl)-amino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol or pharmaceutically acceptable derivatives or analogues thereof. Aromatase inhibitors having an antiestrogen effect, such as those disclosed on pages 7 to 8 of EP 0 495 825 B1 may also be used as
10 antiestrogens.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an
15 increased amount of tumor cells in the S-phase of the cell cycle. The number of tumor cells in the S-phase may be determined by DNA flow cytometry as described in Dressler et al., "DNA Flow Cytometry and Prognostic Factors in 1331 Frozen Breast Cancer Specimens," *Cancer*, Vol. 61(3), 1988, pp. 420-427; see also McGuire & Dressler, "Emerging Impact of Flow Cytometry in Predicting Recurrence and Survival in Breast
20 Cancer Patients," *JNCI*, Vol. 75(3), 1985, pp. 405-409. A high risk amount of tumor cells in the S-phase indicates a particularly suitable candidate for the use according to the invention. In the case of antiprogesterin (I), the advantage arises from both the potent anti-tumor effect, as evidenced by the standard animal models (see Examples 1 to 4), and the mechanism of action of this agent of inducing apoptosis (see in particular Example 5) and
25 cell cycle arrest.

In an alternative aspect the present invention provides a method for inducing apoptosis in a cell. The cell is preferably a mammalian cell and most preferably a human cell, and the method may be applied *in vitro* or *in vivo*. Preferably, apoptosis is induced via the
30 mechanism of initiating terminal differentiation, for example, by the administration of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof. In the

method, an effective amount of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be applied to the cells in question. For example in the T47D breast cancer cell line, whose growth is stimulated by the administration of estradiol, antiprogesterin (I) induced a complete inhibition of cell growth with an effective threshold concentration of between 10^{-9} and 10^{-8} mol (see Example 6 and figure 6). This is especially surprising as the known antiprogesterin onapristone has no reducing effect on cell growth in this tumor model. Thus, antiprogesterin (I) is superior with regard to potency and efficacy to other antiprogesterins such as onapristone and to antiestrogens such as tamoxifen and even to pure antiestrogens such as 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

The role of antiprogesterin (I) in the induction of apoptosis in the cell indicates that this antiprogesterin (or a pharmaceutically acceptable derivative or analogue thereof) may be useful in a host of conditions, particularly hormone-dependent conditions, where induction of apoptosis is particularly desired. Specifically, it may be used in the treatment of such diseases as breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e., diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways. Antiprogesterins, such as antiprogesterin (I), may thus be further used for the preparation of medicaments for inducing apoptosis or cell death for the treatment of hormone-dependent diseases as already described above.

The invention is further illustrated in the examples. The following examples are not to be understood as a limitation.

Examples

Example 1:

Dose-response study in the DMBA-induced tumor model

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Materials and Methods:

Immature female Sprague-Dawley rats (49 - 51 days old; 10 animals/group) were used in this study. Mammary tumors were induced by a single oral administration of 10 mg 7,12-dimethylbenz[a]anthracene (DMBA, Serva/Heidelberg). Rats with at least one established tumor with a size of more than 150 mm² were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 0,5 mg/kg s.c., 4) antiprogesterin (I), 2 mg/kg s.c., 5) antiprogesterin (I), 5 mg/kg s.c., 6) antiprogesterin (I), 10 mg/kg s.c., and 7) onapristone, 5 mg/kg, s.c., daily. As a parameter for growth inhibition the change of tumor area (in % with respect to initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used. For a further description and evaluation of the DMBA prevention model, see R.G. Metha, *European Journal of Cancer* 36 (2000), pp. 1275-1282.

20 **Results:**

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a considerable tumor regression in 90% of the animals. Treatment with antiprogesterin (I) at doses of or above 2 mg/kg resulted in a significant induction of apoptosis resulting in inhibition of tumor growth compared with the control (see fig. 2). There was a clear dose-response relationship. Whereas treatment with 0.5 mg/kg antiprogesterin (I) did not significantly prevent the tumor from growing, at 2 mg/kg maximal induction of apoptosis and thus growth inhibition was observed. In this group a complete tumor regression was seen in 50% of the rats. The effect of the highest dose of antiprogesterin (I) tested in this experiment (10 mg/kg), was comparable to that of 2 mg/kg. Onapristone (5 mg/kg, s.c.) was distinctly less effective than antiprogesterin (I) at comparable doses.

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Conclusion:

In the DMBA-induced mammary tumor model in the rat, antiprogesterone (I) strongly induced apoptosis in the tumor cells and thus completely suppressed the tumor growth in intact animals. It was found that 2 mg/kg antiprogesterone (I) has a maximal apoptotic effect on tumor cells. Antiprogesterone (I) was distinctly superior to onapristone regarding the inhibition of tumor growth.

Example 2:**Tumor growth inhibition in NMU-induced breast cancer model in rat****Materials and Methods:**

Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Starting 10 days later, rats with at least one established tumor were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterone (I), 1.0 mg/kg/day, 4) antiprogesterone (I), 0.5 mg/kg/day and 5) onapristone, 5 mg/kg/day. As a parameter for growth inhibition the change of tumor area (in % of initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor growth inhibition. Treatment with antiprogesterone (I) at doses of 0.5 or 1.0 mg/kg resulted in a significant inhibition of tumor growth due to the induction of apoptosis compared with the control (see fig. 2). Onapristone (5 mg/kg) was distinctly less effective than antiprogesterone (I) at the much lower dose of 0.5 mg/kg.

Conclusions:

In the MNU-induced mammary tumor model in the rat, due to its potent ability to induce apoptosis in tumor cells, antiprogesterin (I) completely suppresses the tumor growth in intact animals. Both doses (1.0 mg/kg as well as 0.5 mg/kg) of antiprogesterin (I) have a significant apoptotic effect on tumor cells.

Example 3:**Human T47D breast cancer xenograft in scid mice****Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). T47D breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

Results:

In the T47D breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 3 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induces apoptosis in the tumor cells. The effect of antiprogesterin (I) is almost comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

Conclusion:

The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human T47D breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy) which is considered to be the maximum effective method of inhibiting growth of breast cancer in this model.

Example 4:**10 Human MCF-7 breast cancer xenograft in scid mice****Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). MCF7 breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

Results:

In the MCF7 breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 4 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induced apoptosis in the tumor cells. The effect of antiprogesterin (I) is comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

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Conclusion:

The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human MCF7 breast cancer xenografted in Fox Chase acid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy).

Example 5:

NMU-induced breast cancer in rat (histology, proliferation index and TUNEL assay)

Materials and Methods:

Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Rats with at least one established tumor with a size of more than 150 mm² were treated for 7 days by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 3 mg/kg s.c., daily. At the end of treatment tumors were excised, fixed in formalin and embedded in paraffin. Histology, proliferation index and apoptosis induction assays were performed on these resected tumors.

Histology: For histology tissue slides were stained with haematoxylin and analyzed by microscopy.

Proliferation Index: To determine the proliferation index the expression of PCNA was determined. Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear protein associated with the cell cycle. Nuclear PCNA immunoreactivity is found in the proliferative compartment of normal tissues. A monoclonal antibody, that recognizes a fixation and processing resistant epitope has been used to investigate its tissue distribution.

TUNEL (Apoptosis Test): The biochemical hallmark of apoptosis is the degradation of the genomic DNA, an irreversible event that results in cell death. This characteristic DNA

fragmentation is the result of the activation of nuclear endonucleases, which selectively cleave DNA at sites located between nucleosomal units. These DNA strand breaks were detected by enzymatic labeling of the 3'-OH termini with fluorescein-dUTP using terminal deoxynucleotidyl transferase (TUNEL, Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling, cf. Gavrieli et al., J. Cell. Biol. 119, 493, 1992). Incorporated fluorescein was detected using the anti-fluorescein antibody alkaline phosphatase conjugate followed by alkaline phosphatase substrate reaction.

Results:

Histology: After treatment with antiprogesterin (I), the tissue sections from the NMU tumors displayed dysplastic ductal and acinous formations, usually filled with secretory material (Figure 5A). Moreover, the volume fraction of glandular structures in the tumors increased compared to controls (Figure 5B). In addition, the mammary tumors of antiprogesterin (I) treated animals showed the morphological features of differentiation.

Proliferation Index: PCNA immunoreactivity is high in untreated NMU-induced breast cancer tissue (Figure 5C: Untreated control). The number of cells with PCNA immunoreactivity is reduced by induction of differentiation in NMU-induced breast cancer tissue from rats treated with antiprogesterin (I) (Figure 5D). These data demonstrate that in breast cancer, treatment with antiprogesterin reduces the proliferation index by induction of differentiation.

TUNEL (Apoptosis): Figure 5E demonstrates the appearance of apoptosis induced by antiprogesterin (I) in NMU-induced breast cancer tissue in comparison with untreated control (Figure 5F). It is clearly evident that antiprogesterin (I) alone was capable of inducing apoptosis in the NMU-induced breast cancer tissue and thus inhibited the growth of these tumors.

Example 6:**Antiproliferative activity of antiprogesterin (I) *in vitro* in the T47D cell line****Materials and Methods:**

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T47D cells were grown in charcoal-treated serum supplemented with 0.1 nM E2 (estradiol) plus antiprogesterin (I) for 6 days with one medium change. Following fixation and subsequent staining with crystal violet, the absorbance was recorded and values normalized to the absorbance of untreated controls as described in R.B. Lichtner, *J.*

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Steroid Biochem. Mol. Biol. 1999, 71;181-189. The TUNEL assay is performed analogous to above Example 5 with the only difference that instead of tissue sections cells that are cultivated on microscopic slides are used for the assay.

Results:

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In this T47D cell line *in vitro* test, antiprogesterin (I) exhibited potent tumor growth inhibiting activity with an effective threshold concentration as low as 10^{-9} to 10^{-8} mol/l whereas the antiprogesterin onapristone did not show any inhibiting effect. Even the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-

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propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740) was distinctly less effective than antiprogesterin (I) (see figure 6).

Conclusion:

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Antiprogesterin (I) according to the present invention induces complete inhibition of estradiol-stimulated T47D cell growth at very low concentrations and is thus superior regarding potency and efficacy to other antiprogesterins tested such as onapristone and to the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol.

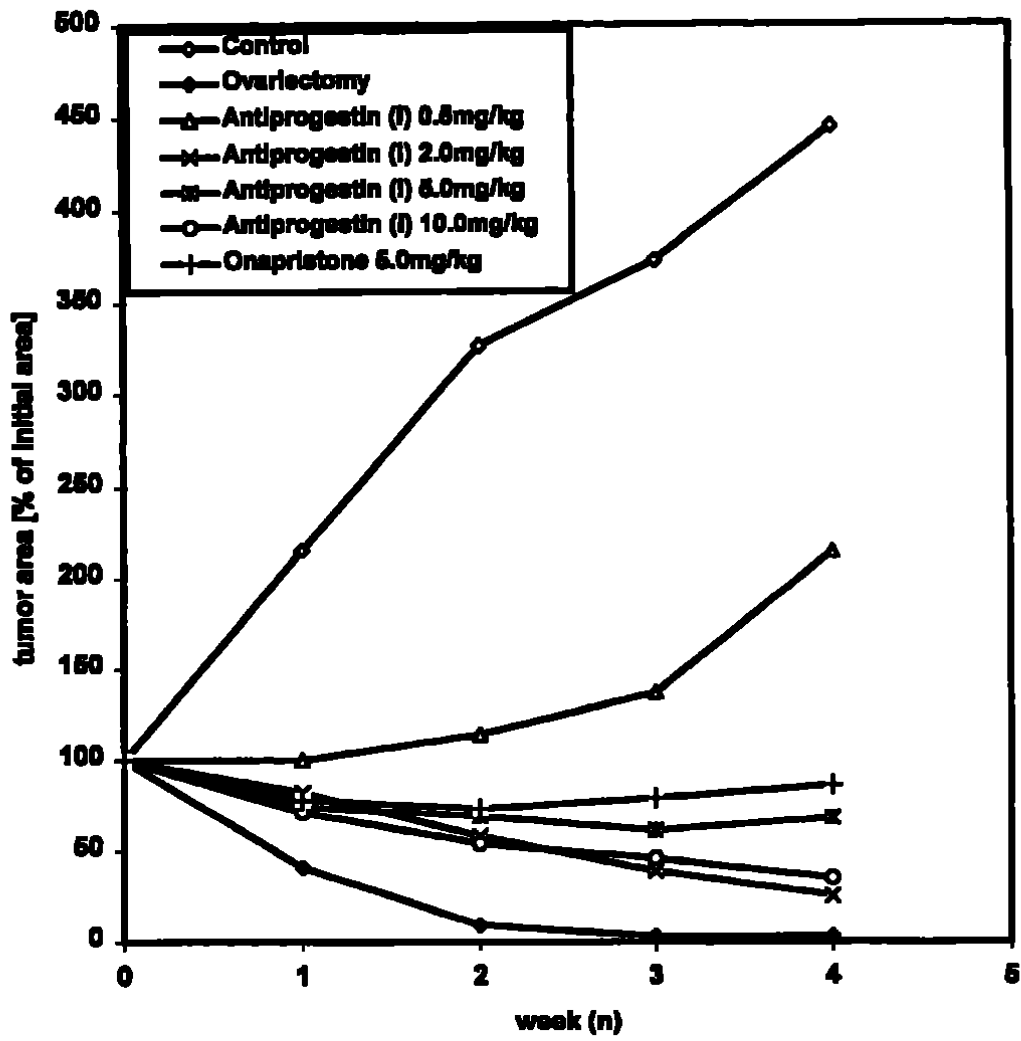
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CLAIMS

1. A method of inducing apoptosis in a cell comprising providing a pharmaceutically effective amount of the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof to such cell.
2. The method according to claim 1 wherein the induction of apoptosis is caused by the initiation of terminal differentiation.
3. The method according to claim 1 wherein the cell is a mammalian cell.
4. The method according to claim 3 wherein the mammalian cell is a human cell.
5. The method according to claim 3 wherein the cell is a tumor cell.
6. The method according to claim 5 wherein the tumor is breast cancer.
7. The method according to claim 1, wherein an antiestrogen is also administered to the cell.
8. A method for treating a disease wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle comprising providing a pharmaceutically effective amount of the antiprogesterin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof to a mammal in need thereof.
9. The method according to claim 8, wherein the disease is breast cancer.

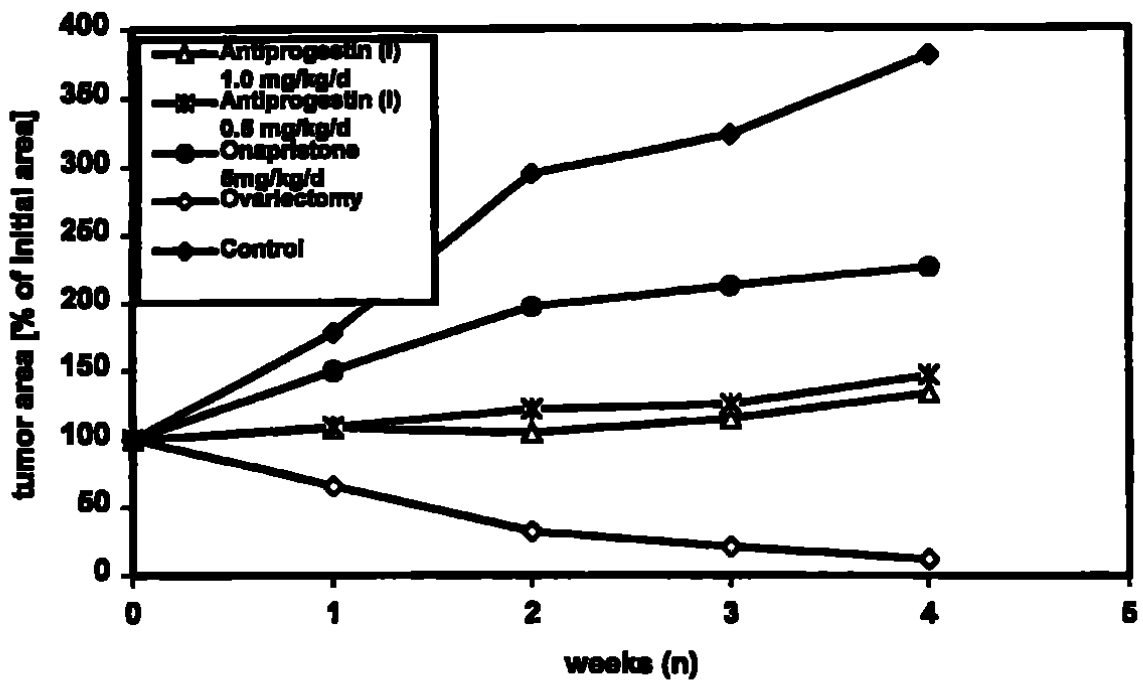
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Fig. 1



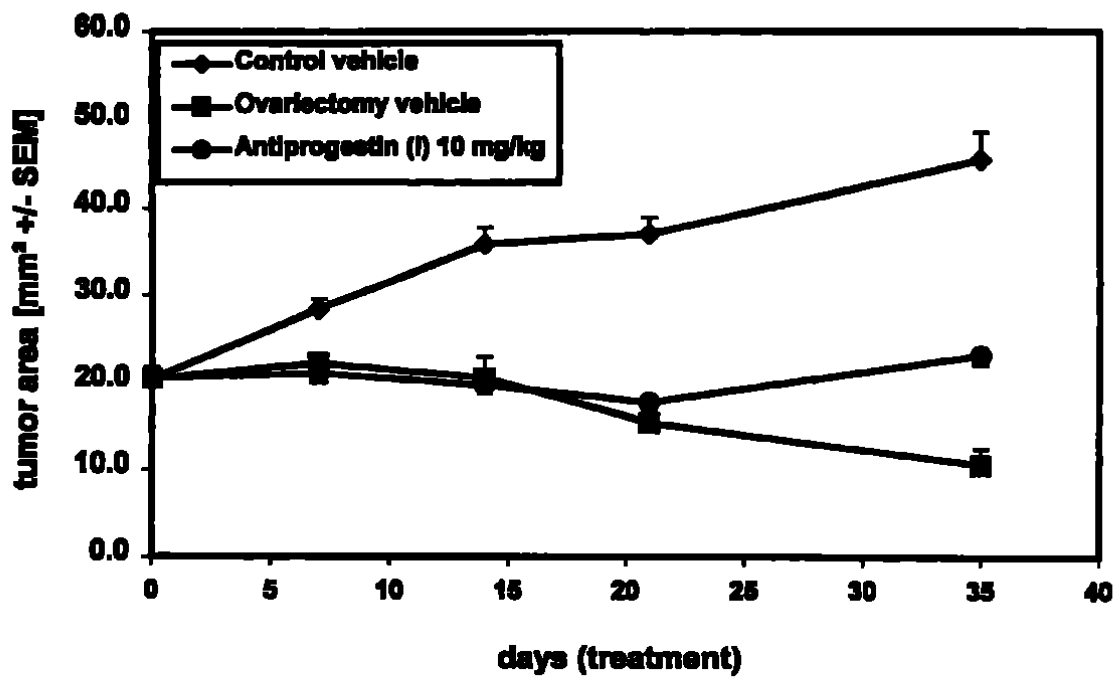
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Fig. 2



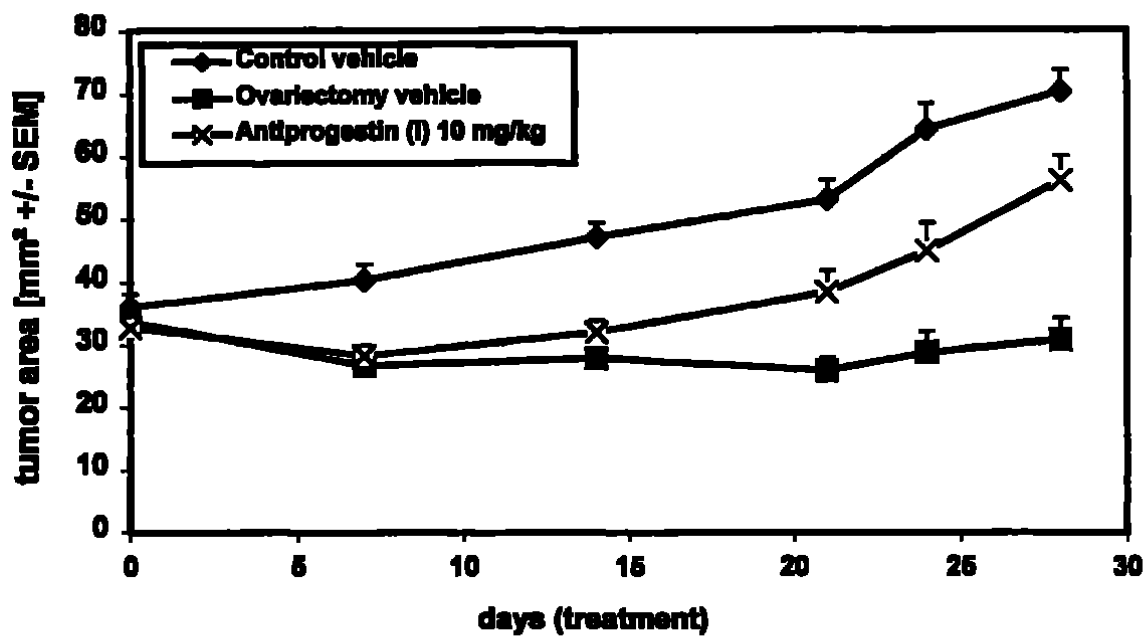
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Fig. 3



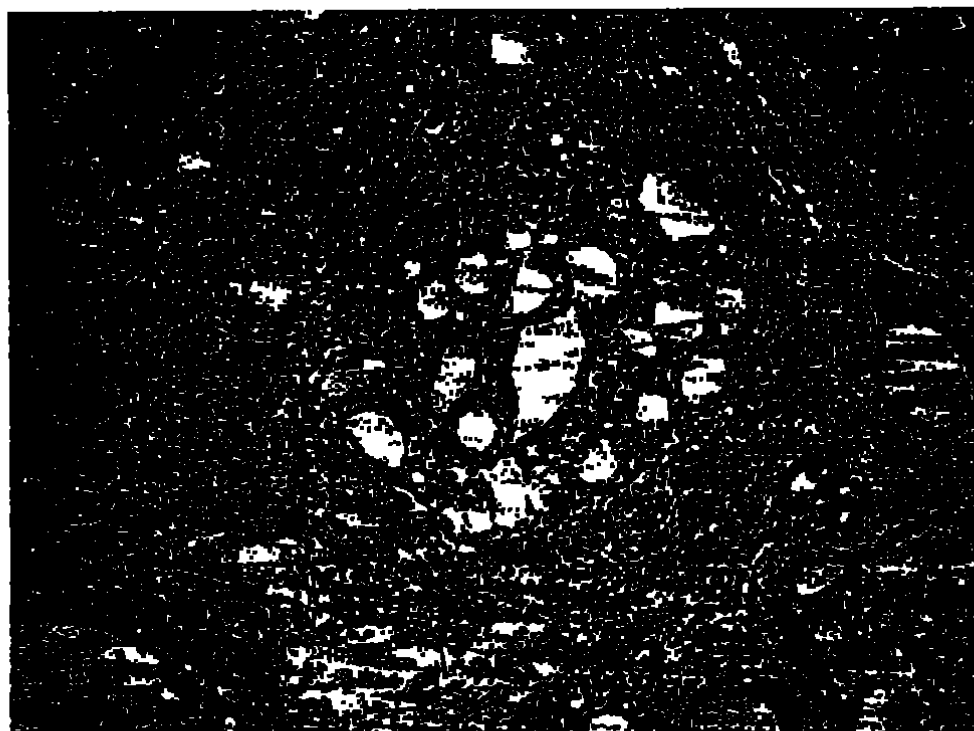
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Fig. 4



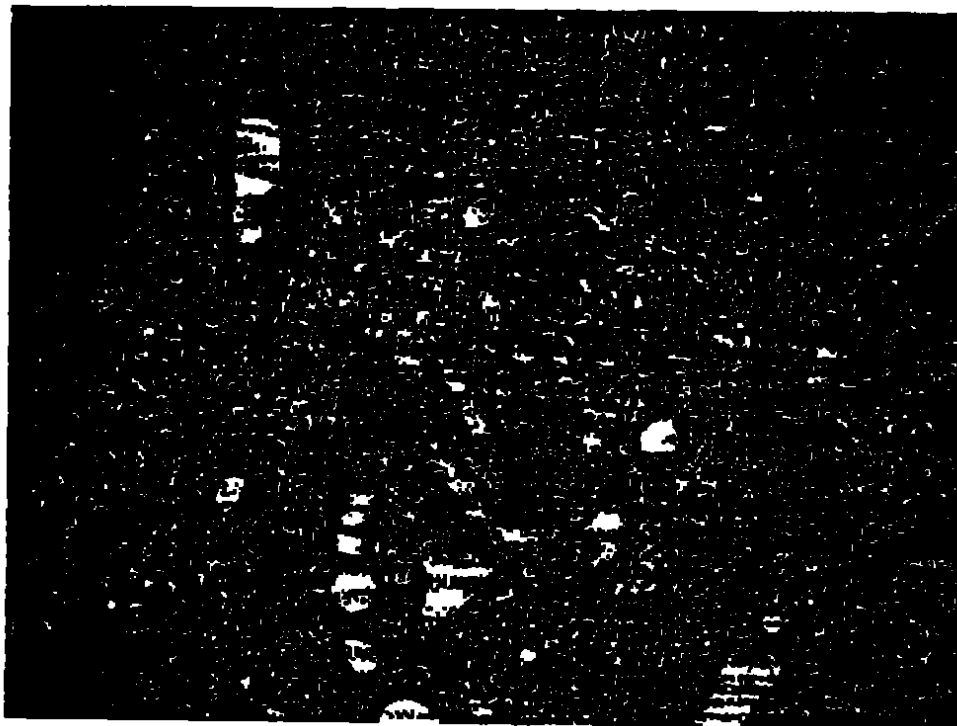
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Fig. 5A



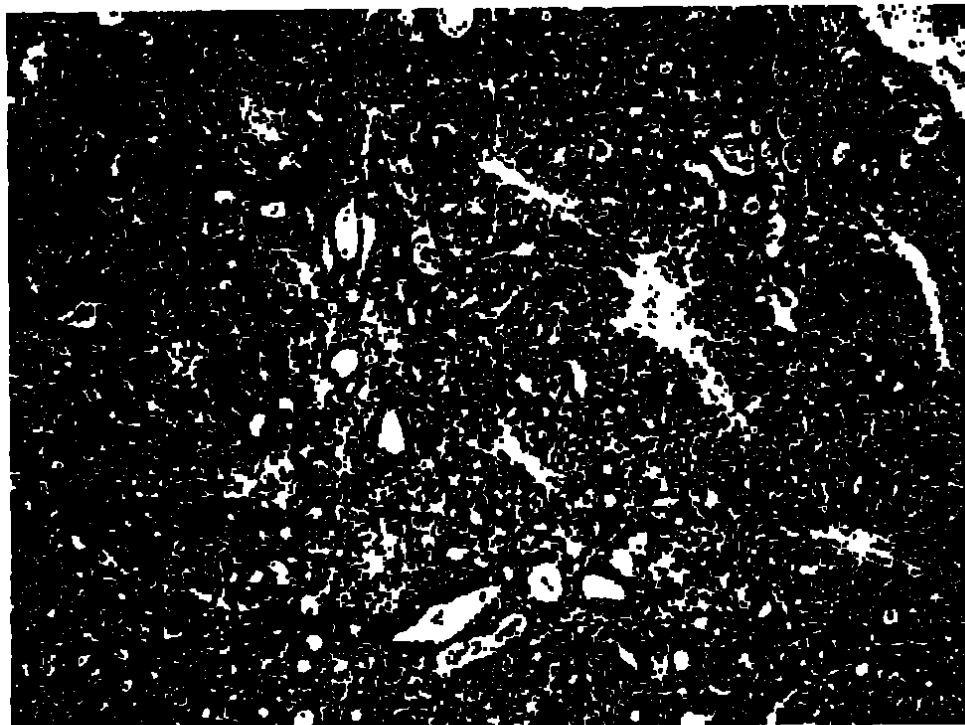
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Fig. 5B



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Fig. 5C



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Fig. 5D

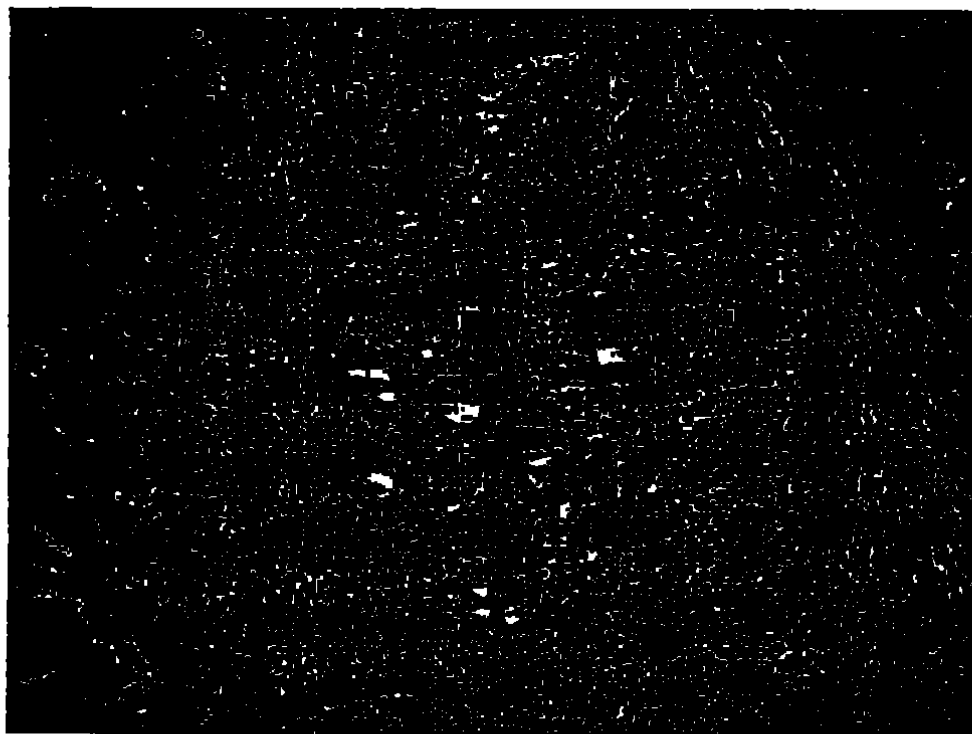


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Fig. 5E

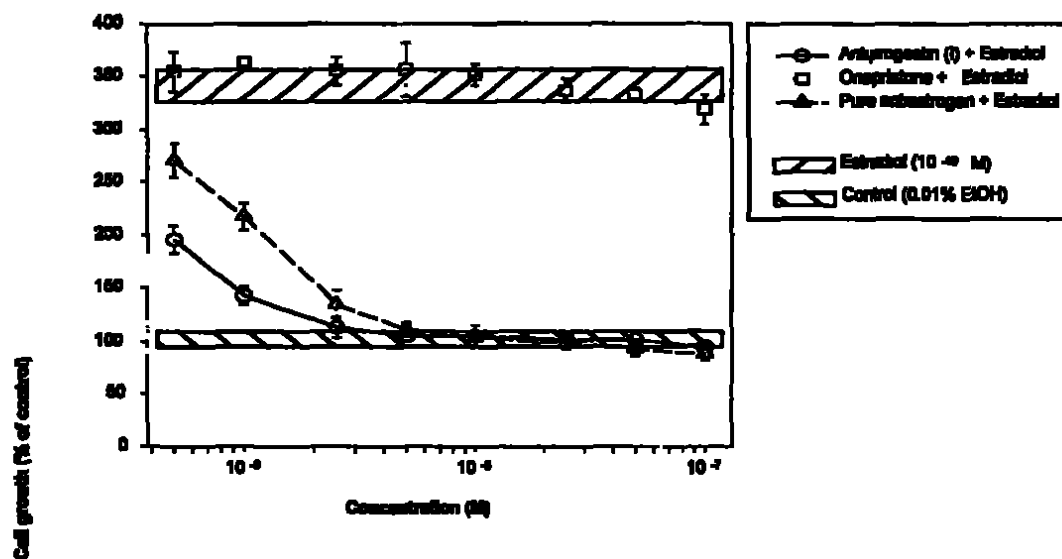


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Fig. 5F

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Fig. 6



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ABSTRACT

The present invention relates to methods and uses for inducing apoptosis in a cell, in particular a breast cancer cell, by the administration of antiprogestins, in particular the
5 antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof. The invention further relates to a treatment of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, said treatment comprising an antiprogestin, in particular the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -
10 (1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof.

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Marzo 04 del 2002

SE CERTIFICA QUE ADJUNTO SE ENCUENTRA UNA COPIA FIEL Y EXACTA DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS RESPECTO A LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA ABAJO, LA CUAL CUMPLIO CON LOS REQUISITOS PARA QUE SE LE OTORGARA UNA FECHA DE PRESENTACION DE CONFORMIDAD CON 35 USC 111.

SOLICITUD NUMERO: 60/240,991

FECHA DE PRESENTACION: *Octubre 18 del 2000*

Por Autoridad del

COMISIONADO DE PATENTES Y MARCAS

M. LEE, Funcionario de Certificaciones

(Firma y Sello Oficial)

REIVINDICACIONES

1. Un método para inducir apoptosis en una célula, método el cual comprende suministrar a dicha célula una cantidad farmacéuticamente eficaz de la antiprogestina 11 β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estr-4,9-dien-3-ona o de un derivado o de un análogo de ella farmacéuticamente aceptables.
2. El método de acuerdo con la reivindicación 1, en el cual la inducción de apoptosis es causada por la iniciación de diferenciación terminal.
3. El método de acuerdo con la reivindicación 1, en el cual la célula es una célula de mamífero.
4. El método de acuerdo con la reivindicación 3, siendo la célula de mamífero una célula humana.
5. El método de acuerdo con la reivindicación 3, siendo la célula una célula tumoral.
6. El método de acuerdo con la reivindicación 5, siendo el tumor cáncer de seno.
7. El método de acuerdo con la reivindicación 1, en el cual a la célula también se le administra un antiestrógeno.
8. Un método para el tratamiento de una enfermedad, método en el cual un indicador de riesgo elevado es una cantidad incrementada de células tumorales en la fase S de ciclo celular, método el cual comprende suministrar una cantidad farmacéuticamente eficaz de la antiprogestina 11 β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estr-4,9-dien-3-ona o de un derivado o de un análogo de ella farmacéuticamente aceptables a un mamífero que lo necesite.
9. El método de acuerdo con la reivindicación 8, siendo la enfermedad cáncer de seno.



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Bescheinigung

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Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

00250342.3

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

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**Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation**

Anmeldung Nr.:
Application no.:
Demande n°: **00250342.3**

Anmeldetag:
Date of filing:
Date de dépôt: **18/10/00**

Anmelder:
Applicant(s):
Demandeur(s):
**Schering Aktiengesellschaft
13353 Berlin
GERMANY**

Bezeichnung der Erfindung:

Title of the invention:

Titre de l'invention:

Use of antiprogestins for the induction of apoptosis in a cell

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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International Patent classification:
Classification internationale des brevets:

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Am Anmeldetag benannte Vertragsstaaten:

Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/EM/FU/FR/GB/GM/IE/IT/LU/LU/MC/NL/PT/SE/TR
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Bemerkungen:
Remarks:
Remarques:

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Europäisches Patentamt GD1 - Dienststelle Berlin 18. OKT. 2000		
Anl:		

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5 **USE OF ANTIPROGESTINS FOR THE INDUCTION OF
APOPTOSIS IN A CELL**

Field of the Invention

- 10 The present invention relates to the use of antiprogestins for the induction of apoptosis in a cell. In particular, the invention relates to use of the antiprogesterin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell. The present invention further provides a use of antiprogestins for the preparation of a
- 15 medicament for the treatment of a type of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

20 Background of the Invention

- Antiprogestins represent a relatively new and promising class of therapeutic agents that could have significant impact on the treatment of hormone-dependent tumors and other diseases. Although antiprogestins were originally created with regard to medicinal non-surgical termination of pregnancy, certain antiprogestins have gained considerable
- 25 importance, e.g., in the endocrine therapy of those breast cancers which possess receptors for progesterone (T. Maudelonde et al., in: J.G.M. Klijn et al., *Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti) Steroidal Agents*, Raven Press, New York, 1987, pp. 55-59).
- 30 This new strategy in endocrine therapy is based on the antitumor activity of antiprogestins in progesterone receptor positive human breast cancer cell lines *in vitro* and in several

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hormone-dependent mammary tumors of the mouse and rat *in vivo*. In particular, the antitumor mechanism of the antiprogestins onapristone and mifepristone (RU 486) has already been investigated using the hormone-dependent MXT mammary tumor model of the mouse as well as the DMBA- and the NMU-induced mammary tumor models of the rat (M. R. Schneider et al., *Eur. J. Cancer Clin. Oncol.*, Vol. 25, No. 4, pp. 691-701, 1989; H. Michna et al., *Breast Cancer Research and Treatment* 14:275-288, 1989; H. Michna, *J. Steroid. Biochem.* Vol. 34, Nos 1-6, pp. 447-453, 1989). However, due to low activity and adverse side effects involved with e.g. mifepristone this compound could not be recommended as a single agent in the management of breast cancer (D. Perrault et al., *J. Clin. Oncol.* 1996 Oct, 14(10), pp.2709-2712). Furthermore, mifepristone exhibits strong antiglucocorticoid side effects (cf. L.M. Kettel et al., *Fertil. Steril.* 1991 Sep, 56(3), pp. 402-407; X. Bertagna, *Psychoneuroendocrinology* 1997; 22 Suppl. 1, pp. 51-55).

The determination of the percentage of tumor cells in the respective phases of the cell cycle can be performed by the powerful DNA flow cytometry method (cf. G. M. Clark et al., *N. Engl. J. Med.* 320, 1989, March, pp.627-633; L. G. Dressler et al., *Cancer* 61(3), 1988, pp. 420-427 and literature cited therein). It has thus been shown that the stages of the cell cycle of a tumor cell, and specifically, the number of tumor cells in certain stages of the cycle, may be an important clinical predictor of disease progression and success of therapy. The number of cells in the S-phase of the cell cycle are particularly important in this regard.

EP 0 495 825 B1 discloses the use of antiprogestins (competitive progesterone antagonists) for the production of medicaments for the treatment of mammary carcinomas having an increased content of tumor cells in the S-phase of the cell cycle, which is considered to be a high risk factor. This is based on the observation that antiprogestins are capable of blocking the progression of tumor cells in the G₀G₁-phase of the cell cycle resulting in a substantial decrease of tumor cells in the S-phase. This effect was however not observed with the standard breast cancer therapy tamoxifen, estrogen therapy or ovariectomy. The antiprogestins tested in EP 0 495 825 B1 are 11β-[4-N,N-

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dimethylamino)-phenyl]-17 α -hydroxy-17 β -(3-hydroxypropyl)-13 α -methyl-4,9(10)-gonadien-3-one and 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(prop-1-ynyl)-4,9(10)-estradien-3-one.

- 5 17 α -fluoroalkylsteroids having strong antiprogestin activity as well as methods for producing them are described in WO 98/34947. WO 98/34947 does not discuss or investigate the role that the 17 α -fluoroalkylsteroids disclosed therein may play in cell apoptosis or cell cycle arrest.
- 10 Given the potential value of agents that induce apoptosis in cells, e.g., in the case of tumor cells, by blocking progression in the G₀G₁-phase, it is desirable to identify further agents, e.g., antiprogestins, having this specific mechanism of action. Such agents would have potential application in treating and preventing certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-
- 15 phase of the cell cycle.

Object of the Invention

- It is thus an object of the present invention to further investigate the mode of action of antiprogestins in inhibiting hormone-dependent diseases such as breast cancer and to
- 20 provide a method for the targeted induction of apoptosis in cells.

- Surprisingly, the inventors have discovered that the antiprogestin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one (or a pharmaceutically acceptable derivative or analogue thereof) may be used for the induction
- 25 of apoptosis in a cell.

Summary of the Invention

- The present invention is based on the unexpected observation that the antiprogestin 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one
- 30 (hereinafter referred to as "antiprogestin (I)") induces apoptosis and cell death in the

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tumor cells of standard breast cancer tumor models. It was found that antiprogesterin (I) is capable of inducing apoptosis in cells via the initiation of terminal differentiation.

Thus, the present invention provides the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the induction of apoptosis in a cell. Preferably, the induction of apoptosis is caused by the initiation of terminal differentiation. The cell is preferably a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

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Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of types of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

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A further aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell *in vitro*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

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Another aspect of the present invention is a method of inducing apoptosis in a cell by administering an effective amount of antiprogesterin (I) to the cell. This method may be applied *in vitro* or *in vivo*. Preferably, the cell is a mammalian cell, more preferably a human cell and most preferably a tumor cell, wherein the tumor is preferably breast cancer.

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Due to the ability to induce cell apoptosis the antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be used for the treatment of certain types of cancer, such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle. Other types of cancer or hormone-dependent diseases that may be affected and treated by antiprogesterin (I) due to its ability to induce

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cell apoptosis may include, e.g., breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e. diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways.

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Brief Description of the Figures

Figure 1 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in a dose-response study in the DMBA-induced mammary carcinoma of the rat, compared with a control, the antiprogesterin onapristone as well as ovariectomy.

10 The study was performed with 0.5, 2.0, 5.0 and 10.0 mg/kg s.c. daily doses of antiprogesterin (I).

Figure 2 shows the tumor growth inhibiting effect as a result of the induction of apoptosis by antiprogesterin (I) in the NMU-induced mammary carcinoma of the rat, compared with a control and ovariectomy. The study was performed with 0.5 and 1.0 mg/kg s.c. daily doses of antiprogesterin (I).

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Figure 3 shows the induction of apoptosis and thus the tumor growth inhibiting effect of antiprogesterin (I) in a 10 mg/kg s.c. dose on xenotransplanted human T47D tumors in scid mice, compared to a control and ovariectomy.

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Figure 4 demonstrates the induction of apoptosis and thus the tumor growth inhibiting effect of a 10 mg/kg s.c. dose of antiprogesterin (I) in the MCF-7 human breast cancer model in scid mice, compared to a control and ovariectomy.

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Figures 5A to 5F show histological data relating to the induction of apoptosis in the NMU-induced breast cancer model in rat (cf. Example 5). In particular, figure 5A shows that tumors treated with antiprogesterin (I) display ductal and acinous formations, usually filled with secretory material, compared to the control (figure 5B). Figure 5C shows untreated NMU-induced breast cancer tissue with high PCNA (proliferating cell nuclear antigen) immunoreactivity as compared to NMU-induced breast cancer tissue treated with

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antiprogestin (I) (figure 5D), which exhibits low PCNA immunoreactivity. Figure 5E shows the appearance of apoptosis in antiprogestin (I)-treated NMU-induced breast cancer tissue, compared to the control (figure 5E).

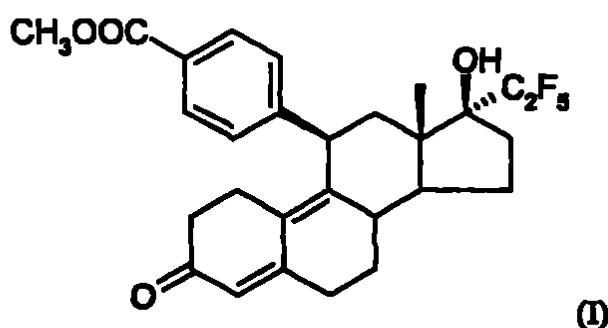
- 5 Figure 6 demonstrates the tumor growth inhibiting effect of antiprogestin (I) in the T47D breast cancer cell line (stimulated by estradiol) with an effective threshold concentration of 10^{-9} to 10^{-8} mol/L, compared with the antiprogestin onapristone and the pure antiestrogen 11 β -fluoro-7 α -(5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl)-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

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Detailed Description of the Invention

Antiprogestin (I) — 11 β -(4-acetylphenyl)-17 β -hydroxy-17 α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one — is represented below by formula (I):

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Antiprogestin (I) (or a pharmaceutically acceptable derivative or analogue thereof) is a valuable pharmaceutical agent having strong antiprogestin activity. Antiprogestin (I) can be used according to the present invention for the induction of apoptosis in cells.

- 25 The term "antiprogestin" in the context of the present invention is intended to primarily comprise all compounds being capable of competitively inhibiting progesterone receptors.

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However, it should also encompass compounds capable of inhibiting the biosynthesis of progestins.

Pharmaceutically acceptable derivatives or analogues of antiprogesterin (I) in the context of the present invention may include, for example, any one of the inventive compounds disclosed in WO 98/34947.

The studies performed in the context of the present invention show the potent tumor-inhibiting properties of the antiprogesterin (I) in a variety of hormone-dependent tumor models (see Examples 1 to 6). It is further demonstrated that the tumor inhibiting activity of antiprogesterin (I) as a result of the induction of apoptosis is stronger than conventional anti-tumor agents, such as, the antiestrogen tamoxifen. The treatment of breast cancer using the antiprogesterin (I) according to the present invention is even superior to ovariectomy.

15

Application of antiprogesterin (I) in the various tumor models as demonstrated below in the Examples revealed an accumulation of tumor cells in the G₀G₁ phase of the cell cycle together with a significant and biologically relevant reduction in the number of cells in the S and G₂M phase of the cell cycle. These results indicate an induction of differentiation. Differentiation-specific G₁ arrest has already been proposed earlier for other stem cell systems (see J.J. Wille Jr., *Cancer Res.* 1982, 42(12):5139-46; R.E. Scott, *J. Cell. Biol.* 1982, 94(2):400-405).

20

The experimental results obtained in the various tumor models revealed that treatment with antiprogesterin (I) seems to trigger differentiation of the mitotically active polygonal tumor cells towards glandular structures and acini with a massive sequestering of secretory products, as well as towards spindle-shaped necrobiotic subpopulations (see Example 5 and in particular figures 5A and 5B). Whereas tumor size, mitotic index and the grade of malignancy decreased distinctly, the volume fraction of glandular structures in the tumors as well as the appearance of apoptosis increased 3-fold compared to the controls (see Example 5, figures 5E and 5F).

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Without limitation to any theory, these results indicate that the main mechanism of the antitumor action of antiprogesterin (I) in the tested models is a direct progesterone-receptor-mediated antiproliferative effect at the level of the tumor cells, via the induction of terminal differentiation associated with terminal cell death. In this manner, antiprogesterin (I) appears to be capable of eliminating the intrinsic block in terminal differentiation inherent in malignant tumor cells in progesterone receptor-positive tumors. This antiproliferative effect of antiprogesterin (I) seems to be dissociated from the antihormone (antiprogesterin) activity of antiprogesterin (I).

10

Agents such as antiprogesterin (I) that induce apoptosis in cells, for example, in the case of tumor cells, by blocking progression in the G₀G₁-phase, have potential applications for treating and preventing numerous conditions. Such agents, including antiprogesterin (I), may be used for treating those cancers where an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, such as in breast cancer.

15

Thus one aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for preparation of a medicament for the induction of apoptosis in a cell. In a preferred embodiment, the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof relates to a medicament for the induction of apoptosis in a tumor cell, preferably a breast tumor cell, in a human. Such medicament could be beneficial in the treatment of hormone-dependent diseases such as breast cancer, wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.

20

The manufacture of the medicaments may be performed according to methods known in the art. Commonly known and used adjuvants as well as further suitable carriers or diluents may be used. Suitable carriers and adjuvants may be such as recommended for pharmacy, cosmetics and related fields in: *Ullmann's Encyclopedia of Technical Chemistry*, Vol. 4, (1953), pp. 1-39; *Journal of Pharmaceutical Sciences*, Vol. 52 (1963), p. 918ff; H.v.Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete";

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Pharm. Ind. 2, 1961, p.72ff; Dr. H.P. Fiedler, *Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete*, Cantor KG, Aulendorf in Württemberg, 1971.

Antiprogestins suitable for the purposes of the present invention, preferably antiprogestin
5 (I) or a pharmaceutically acceptable derivative or analogue thereof, can be incorporated
into pharmaceutical compositions according to known methods of preparing galenics for
oral or parenteral, e.g., intraperitoneal, intramuscular, subcutaneous or percutaneous
application. They can also be implanted into tissue. Implants can comprise as inert
materials e.g. biologically degradable polymers or synthetic silicones such as e.g. silicone
10 rubber.

They can be administered in the form of tablets, pills, dragees, gel capsules, granules,
suppositories, implants, injectable sterile aqueous or oily solutions, suspensions or
emulsions, ointments, creams, gels or by intravaginal (e.g., vaginal rings) or intrauterine
15 systems (e.g., diaphragms, loops).

For the preparation of a medicament for oral administration, the antiprogestins suitable for
the purposes of the present invention as defined above can be admixed with commonly
known and used adjuvants and carriers such as for example, gum arabic, talcum, starch,
20 sugars such as, e.g., mannitose, methyl cellulose, lactose, gelatin, surface-active agents,
magnesium stearate, aqueous or non-aqueous excipients, paraffin derivatives, cross-
linking agents, dispersants, emulsifiers, lubricants, conserving agents and flavoring agents
(e.g., ethereal oils). In a pharmaceutical composition, the antiprogestin may be dispersed
in a microparticle, e.g. a nanoparticulate, composition.

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In order to further enhance the bioavailability of the active agent, the antiprogestins
suitable for the purposes of the present invention as defined above can also be formulated
as cyclodextrin clathrates by reacting them with α -, β - or γ -cyclodextrines or derivatives
thereof according to the method as disclosed in PCT/EP95/02656.

30 For parenteral administration the antiprogestins suitable for the purposes of the present
invention as defined above can be dissolved or suspended in a physiologically acceptable

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diluent, such as, e.g., oils with or without solubilizers, surface-active agents, dispersants or emulsifiers. As oils for example and without limitation, olive oil, peanut oil, cottonseed oil, soybean oil, castor oil and sesame oil may be used.

- 5 The amount to be administered (i.e., a "pharmaceutically effective amount") varies within a broad range and depends on the condition to be treated and the mode of administration. It can cover any amount efficient for the intended treatment. Determining a "pharmaceutically effective amount" is within the purview of the person skilled in the art.
- 10 One unit dose may represent about 0.1 to 100 mg active agent(s). For administration to humans, the daily dose of the active agent(s) is about 0.1 to 400 mg, preferably 10 to 100 mg, most preferably 50 mg.

- The medicaments can also be administered via a depot injection or an implant preparation,
15 optionally for sustained delivery of the active agent(s).

The preferred mode of administration is oral administration. The antiprogestins for use according to the invention, and in particular, antiprogestin (I) are particularly suitable for oral administration.

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- According to all aspects of the present invention it is also possible to combine at least one antiprogestin as defined above, in particular antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof, with at least one antiestrogen, because many hormone-dependent diseases, in particular breast cancer, exhibit not only progesterone
25 receptors, but also estrogen receptors. The antiestrogen may be administered either simultaneously with or sequentially to the antiprogestin, and in particular with/to antiprogestin (I) or a pharmaceutically acceptable derivative or analogue thereof. The amount of antiprogestin and antiestrogen may be equal or one component may be more predominant than the other, such as in an antiprogestin:antiestrogen ratio of 1:50 to 50:1,
30 preferably 1:30 to 30:1, and most preferably 1:15 to 15:1.

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Examples of suitable antiestrogens for use according to the invention are non-steroidal antiestrogens, such as tamoxifen and nafoxidine as well as raloxifen, faslodex and EM800. Examples of steroidal antiestrogens include those disclosed in EP 0 348 341 A and those disclosed in WO 98/07740, in particular, 11 β -flouro-7 α -{5-[N-methyl-N-3-(4.4.5.5.5-
5 pentafluoropentylthio-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol, or those disclosed in WO 99/33855, in particular 11 β -flouro-7 α -{5-[methyl-(7,7,8,8,9,9,10,10,10-nonafluoro-decyl)-amino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol or pharmaceutically acceptable derivatives or analogues thereof. Aromatase inhibitors having an antiestrogen effect, such as those disclosed on pages 7 to 8 of EP 0 495 825 B1 may also be used as
10 antiestrogens.

Another aspect of the present invention is the use of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an
15 increased amount of tumor cells in the S-phase of the cell cycle. The number of tumor cells in the S-phase may be determined by DNA flow cytometry as described in Dressler et al., "DNA Flow Cytometry and Prognostic Factors in 1331 Frozen Breast Cancer Specimens," *Cancer*, Vol. 61(3), 1988, pp. 420-427; see also McGuire & Dressler, "Emerging Impact of Flow Cytometry in Predicting Recurrence and Survival in Breast
20 Cancer Patients," *JNCI*, Vol. 75(3), 1985, pp. 405-409. A high risk amount of tumor cells in the S-phase indicates a particularly suitable candidate for the use according to the invention. In the case of antiprogesterin (I), the advantage arises from both the potent anti-tumor effect, as evidenced by the standard animal models (see Examples 1 to 4), and the mechanism of action of this agent of inducing apoptosis (see in particular Example 5) and
25 cell cycle arrest.

In an alternative aspect the present invention provides a method for inducing apoptosis in a cell. The cell is preferably a mammalian cell and most preferably a human cell, and the method may be applied *in vitro* or *in vivo*. Preferably, apoptosis is induced via the
30 mechanism of initiating terminal differentiation, for example, by the administration of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof. In the

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method, an effective amount of antiprogesterin (I) or a pharmaceutically acceptable derivative or analogue thereof may be applied to the cells in question. For example in the T47D breast cancer cell line, whose growth is stimulated by the administration of estradiol, antiprogesterin (I) induced a complete inhibition of cell growth with an effective threshold concentration of between 10^{-9} and 10^{-8} mol (see Example 6 and figure 6). This is especially surprising as the known antiprogesterin onapristone has no reducing effect on cell growth in this tumor model. Thus, antiprogesterin (I) is superior with regard to potency and efficacy to other antiprogesterins such as onapristone and to antiestrogens such as tamoxifen and even to pure antiestrogens such as 11β -fluoro- 7α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740).

The role of antiprogesterin (I) in the induction of apoptosis in the cell indicates that this antiprogesterin (or a pharmaceutically acceptable derivative or analogue thereof) may be useful in a host of conditions, particularly hormone-dependent conditions, where induction of apoptosis is particularly desired. Specifically, it may be used in the treatment of such diseases as breast cancer, ovarian cancer, endometrial cancer, myeloma, anovulatory infertility, meningoma, i.e., diseases which substantially originate or are influenced by the presence of hormone receptors and/or hormone-dependent pathways. Antiprogesterins, such as antiprogesterin (I), may thus be further used for the preparation of medicaments for inducing apoptosis or cell death for the treatment of hormone-dependent diseases as already described above.

The invention is further illustrated in the examples. The following examples are not to be understood as a limitation.

Examples

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Example 1:

Dose-response study in the DMBA-induced tumor model**Materials and Methods:**

5 Immature female Sprague-Dawley rats (49 - 51 days old; 10 animals/group) were used in this study. Mammary tumors were induced by a single oral administration of 10 mg 7,12-dimethylbenz[a]anthracene (DMBA, Serva/Heidelberg). Rats with at least one established tumor with a size of more than 150 mm² were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 0,5 mg/kg s.c., 4) antiprogesterin (I), 2
10 mg/kg s.c., 5) antiprogesterin (I), 5 mg/kg s.c., 6) antiprogesterin (I), 10 mg/kg s.c., and 7) onapristone, 5 mg/kg, s.c., daily. As a parameter for growth inhibition the change of tumor area (in % with respect to initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used. For a further description and evaluation of the DMBA prevention
15 model, see R.G. Metha, *European Journal of Cancer* 36 (2000), pp. 1275-1282.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy
20 caused a considerable tumor regression in 90% of the animals. Treatment with antiprogesterin (I) at doses of or above 2 mg/kg resulted in a significant induction of apoptosis resulting in inhibition of tumor growth compared with the control (see fig. 2). There was a clear dose-response relationship. Whereas treatment with 0.5 mg/kg antiprogesterin (I) did not significantly prevent the tumor from growing, at 2 mg/kg
25 maximal induction of apoptosis and thus growth inhibition was observed. In this group a complete tumor regression was seen in 50% of the rats. The effect of the highest dose of antiprogesterin (I) tested in this experiment (10 mg/kg), was comparable to that of 2 mg/kg. Onapristone (5 mg/kg, s.c.) was distinctly less effective than antiprogesterin (I) at comparable doses.

30 **Conclusion:**

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In the DMBA-induced mammary tumor model in the rat, antiprogesterin (I) strongly induced apoptosis in the tumor cells and thus completely suppressed the tumor growth in intact animals. It was found that 2 mg/kg antiprogesterin (I) has a maximal apoptotic effect on tumor cells. Antiprogesterin (I) was distinctly superior to onapristone regarding the inhibition of tumor growth.

Example 2:**Tumor growth inhibition in NMU-induced breast cancer model in rat****Materials and Methods:**

Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Starting 10 days later, rats with at least one established tumor were treated for 4 weeks by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 1.0 mg/kg/day, 4) antiprogesterin (I), 0.5 mg/kg/day and 5) onapristone, 5 mg/kg/day. As a parameter for growth inhibition the change of tumor area (in % of initial tumor size) determined by weekly caliper measurements was used. For statistical analysis of intergroup differences of mean values the Kruskal-Wallis-test was used.

Results:

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor growth inhibition. Treatment with antiprogesterin (I) at doses of 0.5 or 1.0 mg/kg resulted in a significant inhibition of tumor growth due to the induction of apoptosis compared with the control (see fig. 2). Onapristone (5 mg/kg) was distinctly less effective than antiprogesterin (I) at the much lower dose of 0.5 mg/kg.

Conclusions:

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In the MNU-induced mammary tumor model in the rat, due to its potent ability to induce apoptosis in tumor cells, antiprogesterin (I) completely suppresses the tumor growth in intact animals. Both doses (1.0 mg/kg as well as 0.5 mg/kg) of antiprogesterin (I) have a significant apoptotic effect on tumor cells.

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Example 3:**Human T47D breast cancer xenograft in scid mice****10 Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). T47D breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started
15 when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

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Results:

In the T47D breast cancer model, ovariectomy resulted in a considerable inhibition of
25 tumor growth, compared with the rapid growth in the control. Fig. 3 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induces apoptosis in the tumor cells. The effect of antiprogesterin (I) is almost comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

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The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human T47D breast cancer xenografted in Fox Chase scid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy) which is considered to be the maximum effective method of inhibiting growth of breast cancer in this model.

5

Example 4:**Human MCF-7 breast cancer xenograft in scid mice****10 Materials and Methods:**

Female Fox Chase scid mice (M&B) were supplemented with estradiol pellets (Innovative Research of America). MCF7 breast cancer cells, obtained from cell culture and suspended in matrigel, were implanted s.c. in the inguinal region of the mice. Treatment was started when the tumors were approximately 25 mm² in size. Treatment was continued until progression of the tumors. Experimental groups were: 1) control (vehicle), 2) ovariectomy, 3) antiprogesterin (I), 10 mg/kg s.c. Tumor area was determined by caliper measurements. The Kruskal Wallis test was used for statistical analysis of intergroup differences of mean values.

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Results:

In the MCF7 breast cancer model, ovariectomy resulted in a considerable inhibition of tumor growth, compared with the rapid growth in the control. Fig. 4 clearly shows that the s.c. application of 10 mg/kg antiprogesterin (I) induced apoptosis in the tumor cells. The effect of antiprogesterin (I) is comparable to the effect of conventional estrogen deprivation therapy (ovariectomy).

30 Conclusion:

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The effect of antiprogesterin (I) in inducing apoptosis and thus inhibiting the growth of the human MCF7 breast cancer xenografted in Fox Chase acid mice is comparable to the effect of standard estrogen deprivation therapy (ovariectomy).

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Example 5:**NMU-induced breast cancer in rat (histology, proliferation index and TUNEL assay)****Materials and Methods:**

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Tumors were induced by a single intravenous injection of NMU (nitrosomethylurea, 50 mg/kg) in female Sprague-Dawley rats (obtained from Tierzucht Schönwalde, age 50-55 days). Rats with at least one established tumor with a size of more than 150 mm² were treated for 7 days by: 1) solvent control, 2) ovariectomy at treatment start, 3) antiprogesterin (I), 3 mg/kg s.c., daily. At the end of treatment tumors were excised, fixed in formalin and embedded in paraffin. Histology, proliferation index and apoptosis induction assays were performed on these resected tumors.

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Histology: For histology tissue slides were stained with haematoxylin and analyzed by microscopy.

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Proliferation Index: To determine the proliferation index the expression of PCNA was determined. Proliferating cell nuclear antigen (PCNA) is a 36 kD nuclear protein associated with the cell cycle. Nuclear PCNA immunoreactivity is found in the proliferative compartment of normal tissues. A monoclonal antibody, that recognizes a fixation and processing resistant epitope has been used to investigate its tissue distribution.

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TUNEL (Apoptosis Test): The biochemical hallmark of apoptosis is the degradation of the genomic DNA, an irreversible event that results in cell death. This characteristic DNA fragmentation is the result of the activation of nuclear endonucleases, which selectively cleave DNA at sites located between nucleosomal units. These DNA strand breaks were

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detected by enzymatic labeling of the 3'-OH termini with fluorescein-dUTP using terminal deoxynucleotidyl transferase (TUNEL, Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling, cf. Gavrieli et al., J. Cell. Biol. 119, 493, 1992). Incorporated fluorescein was detected using the anti-fluorescein antibody alkaline phosphatase conjugate followed by alkaline phosphatase substrate reaction.

Results:

Histology: After treatment with antiprogesterin (I), the tissue sections from the NMU tumors displayed dysplastic ductal and acinous formations, usually filled with secretory material (Figure 5A). Moreover, the volume fraction of glandular structures in the tumors increased compared to controls (Figure 5B). In addition, the mammary tumors of antiprogesterin (I) treated animals showed the morphological features of differentiation.

Proliferation Index: PCNA immunoreactivity is high in untreated NMU-induced breast cancer tissue (Figure 5C: Untreated control). The number of cells with PCNA immunoreactivity is reduced by induction of differentiation in NMU-induced breast cancer tissue from rats treated with antiprogesterin (I) (Figure 5D). These data demonstrate that in breast cancer, treatment with antiprogesterin reduces the proliferation index by induction of differentiation.

TUNEL (Apoptosis): Figure 5E demonstrates the appearance of apoptosis induced by antiprogesterin (I) in NMU-induced breast cancer tissue in comparison with untreated control (Figure 5F). It is clearly evident that antiprogesterin (I) alone was capable of inducing apoptosis in the NMU-induced breast cancer tissue and thus inhibited the growth of these tumors.

30 Example 6:

Antiproliferative activity of antiprogesterin (I) *in vitro* in the T47D cell line

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708**Materials and Methods:**

T47D cells were grown in charcoal-treated serum supplemented with 0.1 nM E2 (estradiol) plus antiprogesterin (I) for 6 days with one medium change. Following fixation and subsequent staining with crystal violet, the absorbance was recorded and values normalized to the absorbance of untreated controls as described in R.B. Lichtner, *J. Steroid Biochem. Mol. Biol.* 1999, 71;181-189. The TUNEL assay is performed analogous to above Example 5 with the only difference that instead of tissue sections cells that are cultivated on microscopic slides are used for the assay.

Results:

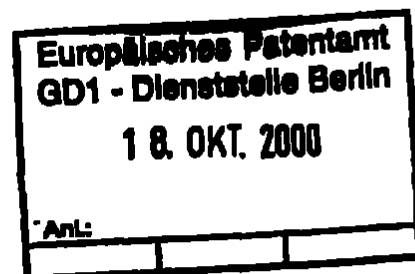
In this T47D cell line *in vitro* test, antiprogesterin (I) exhibited potent tumor growth inhibiting activity with an effective threshold concentration as low as 10^{-9} to 10^{-8} mol/l whereas the antiprogesterin onapristone did not show any inhibiting effect. Even the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol (WO 98/07740) was distinctly less effective than antiprogesterin (I) (see figure 6).

Conclusion:

Antiprogesterin (I) according to the present invention induces complete inhibition of estradiol-stimulated T47D cell growth at very low concentrations and is thus superior regarding potency and efficacy to other antiprogesterins tested such as onapristone and to the pure antiestrogen 11 β -fluoro-7 α -{5-[N-methyl-N-3-(4,4,5,5,5-pentafluoropentylthio)-propylamino]-pentyl}-estra-1,3,5(10)-trien-3,17 β -diol.

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CLAIMS

1. Use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the induction of apoptosis in a cell.
2. Use according to claim 1 wherein the induction of apoptosis is caused by the initiation of terminal differentiation.
3. Use according to any preceding claim wherein the cell is a mammalian cell.
4. Use according to claim 3 wherein the mammalian cell is a human cell.
5. Use according to any preceding claim wherein the cell is a tumor cell.
6. Use according to claim 5 wherein the tumor is breast cancer.
7. Use according to any preceding claims, wherein the medicament further comprises an antiestrogen.
8. Use of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof for the preparation of a medicament for the treatment of a type of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle.
9. The use according to claim 8, where the disease is breast cancer.
10. Method of inducing apoptosis in a cell, comprising administering an effective amount of the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-

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pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable analogue or derivative thereof to a cell *in vitro*.

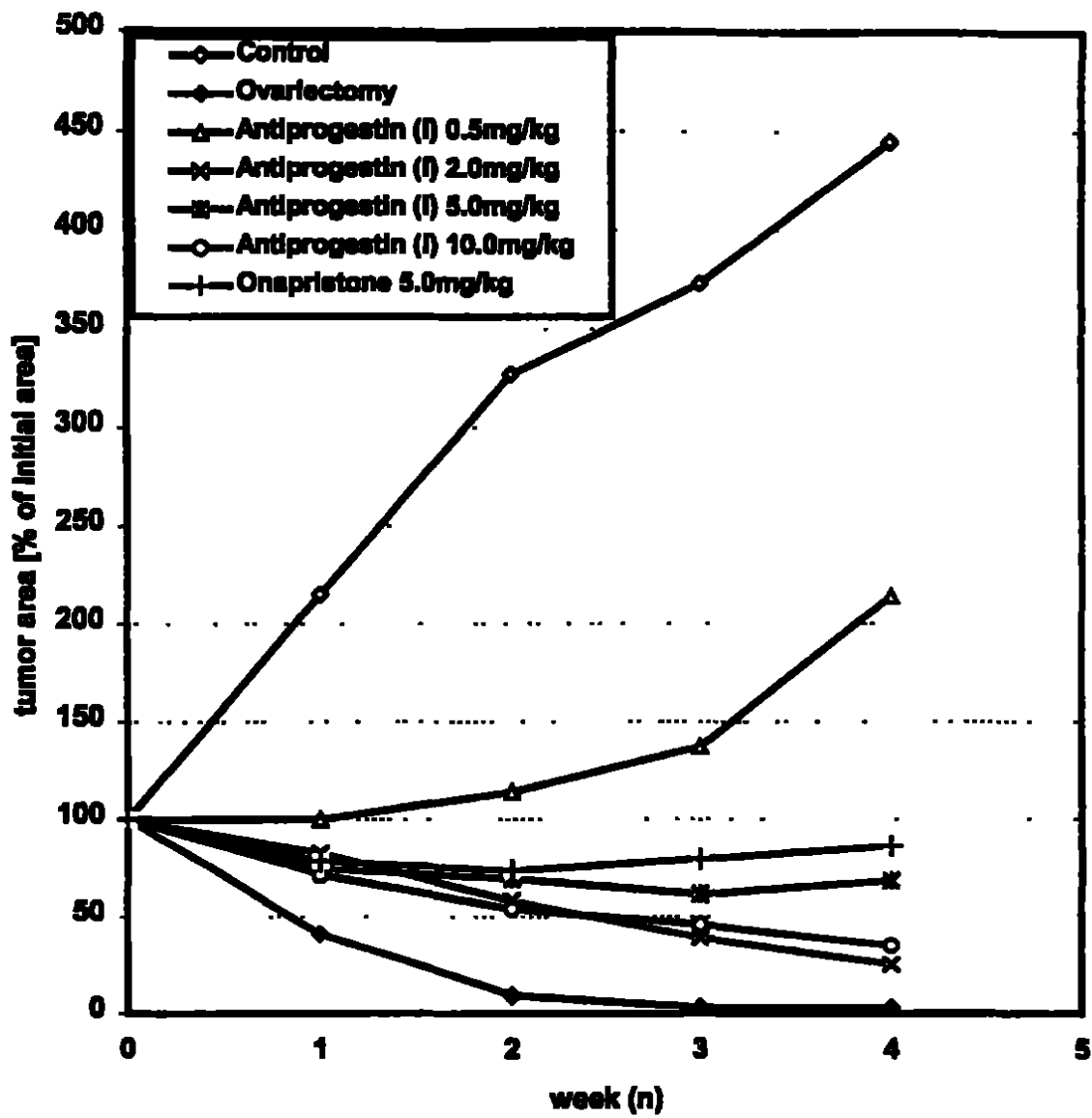
11. The method according to claim 10, wherein the cell is a mammalian tumor cell.

5 12. The method according to claims 10 or 11, wherein the cell is a breast cancer cell.

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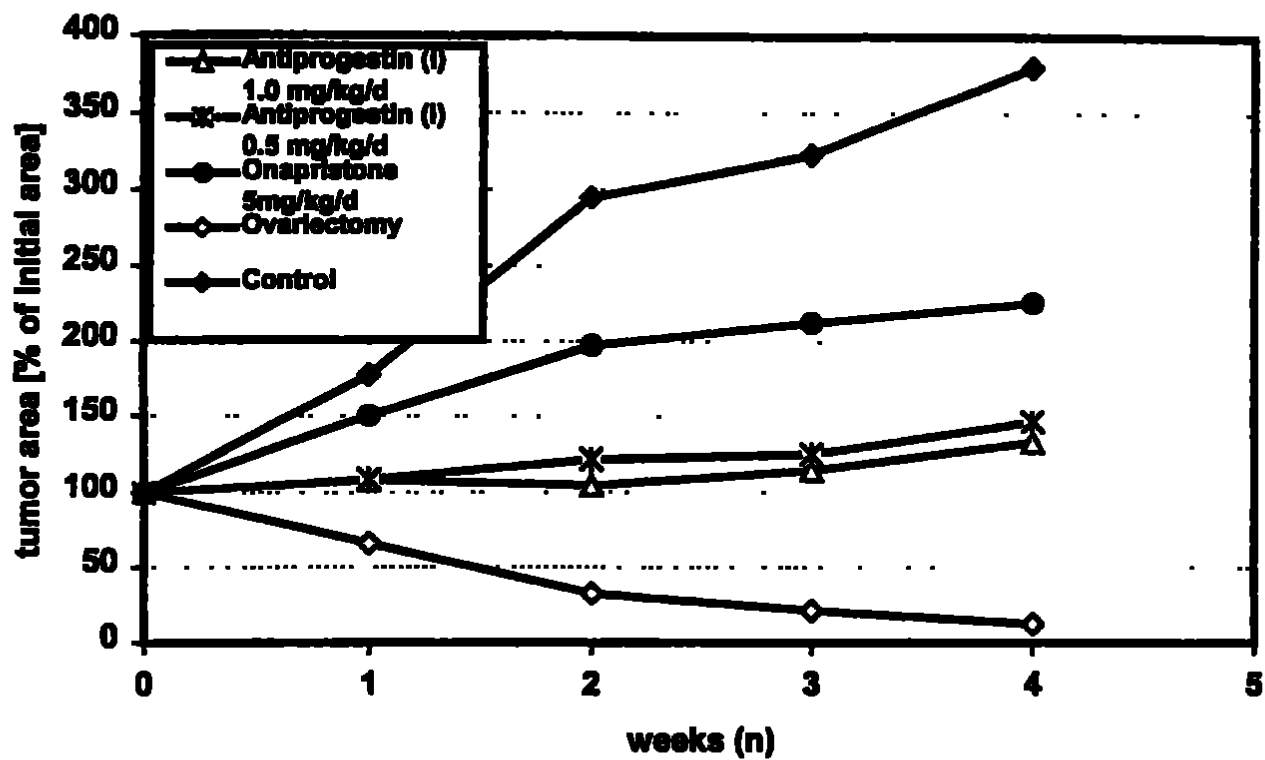
Fig. 1



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Fig. 2

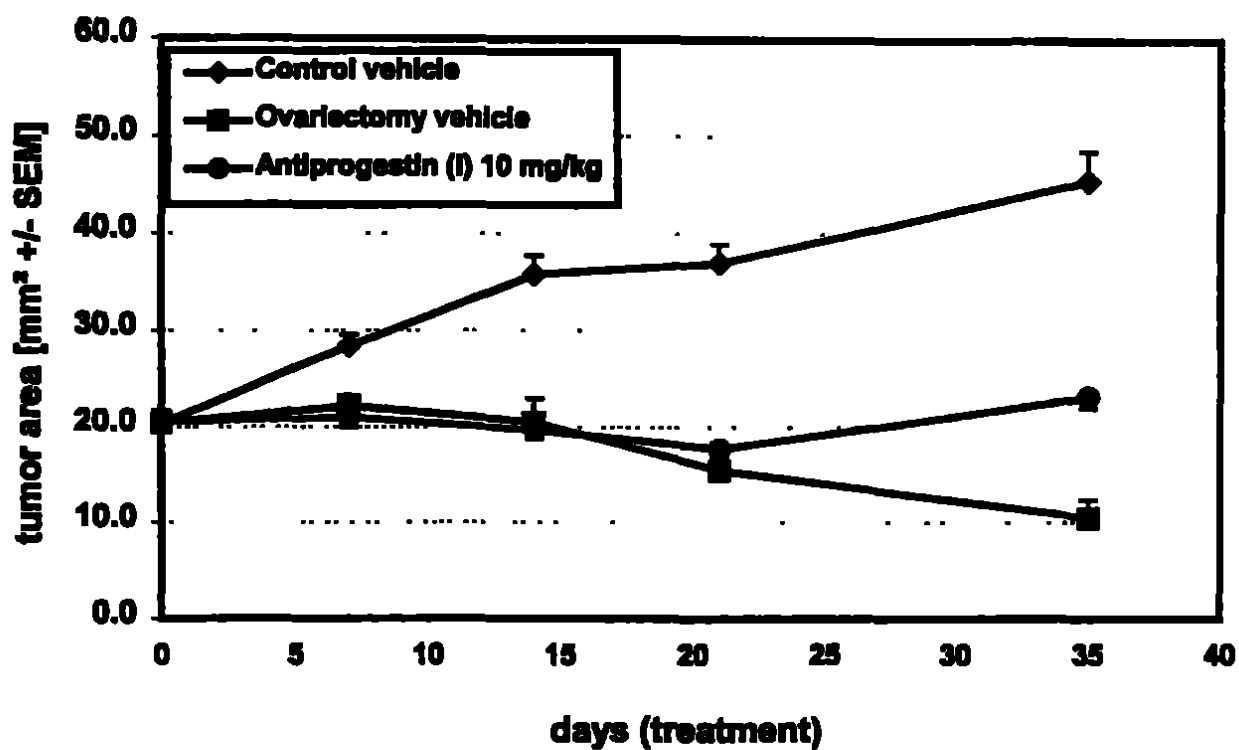


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etc
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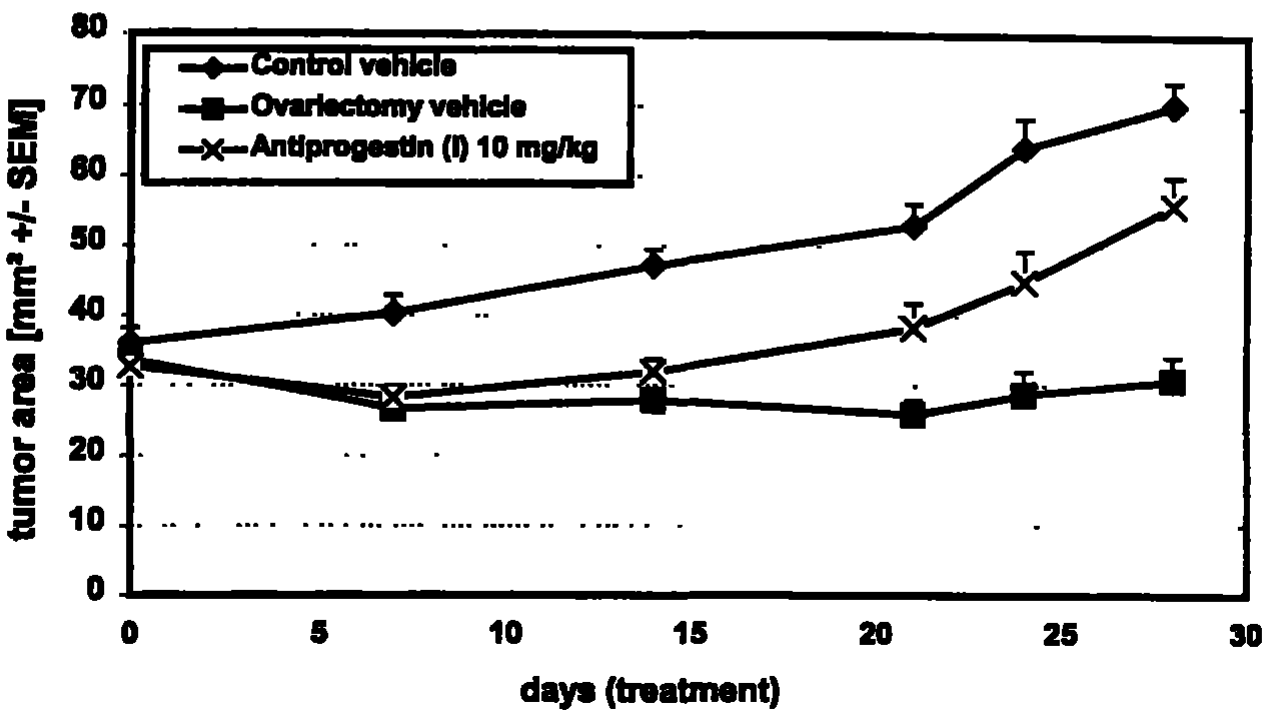
Fig. 3



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Fig. 4

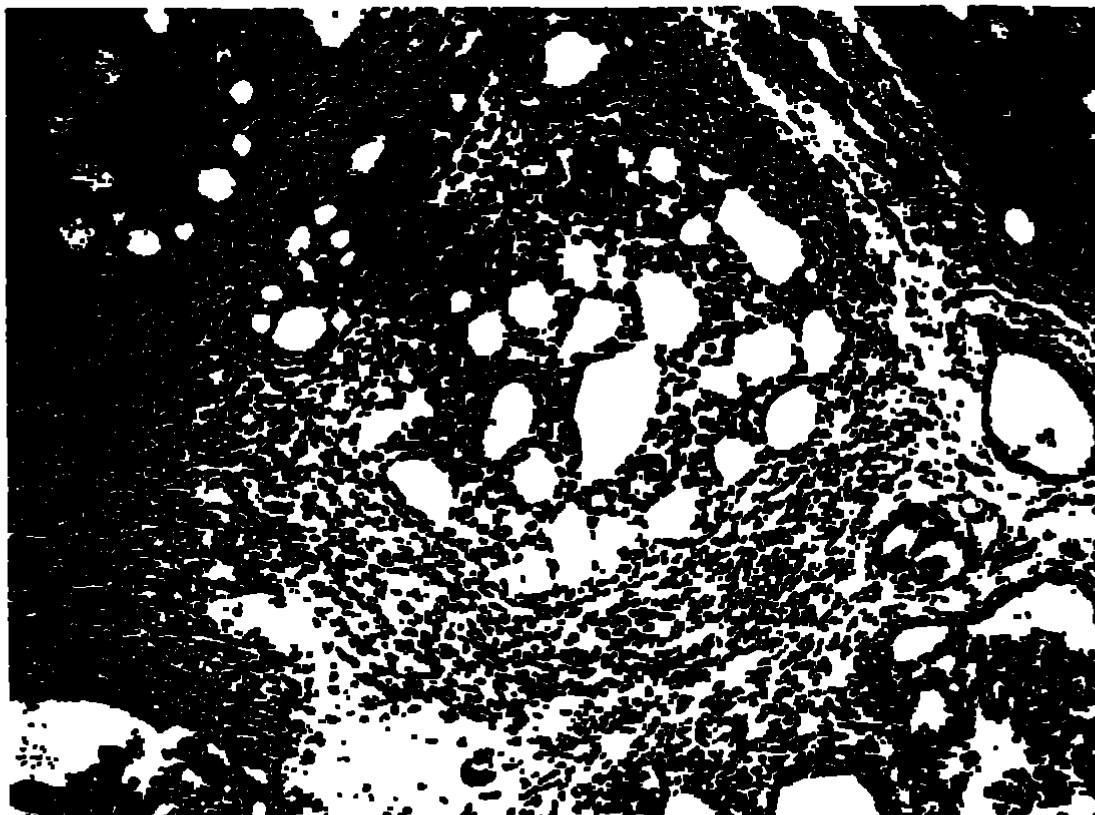


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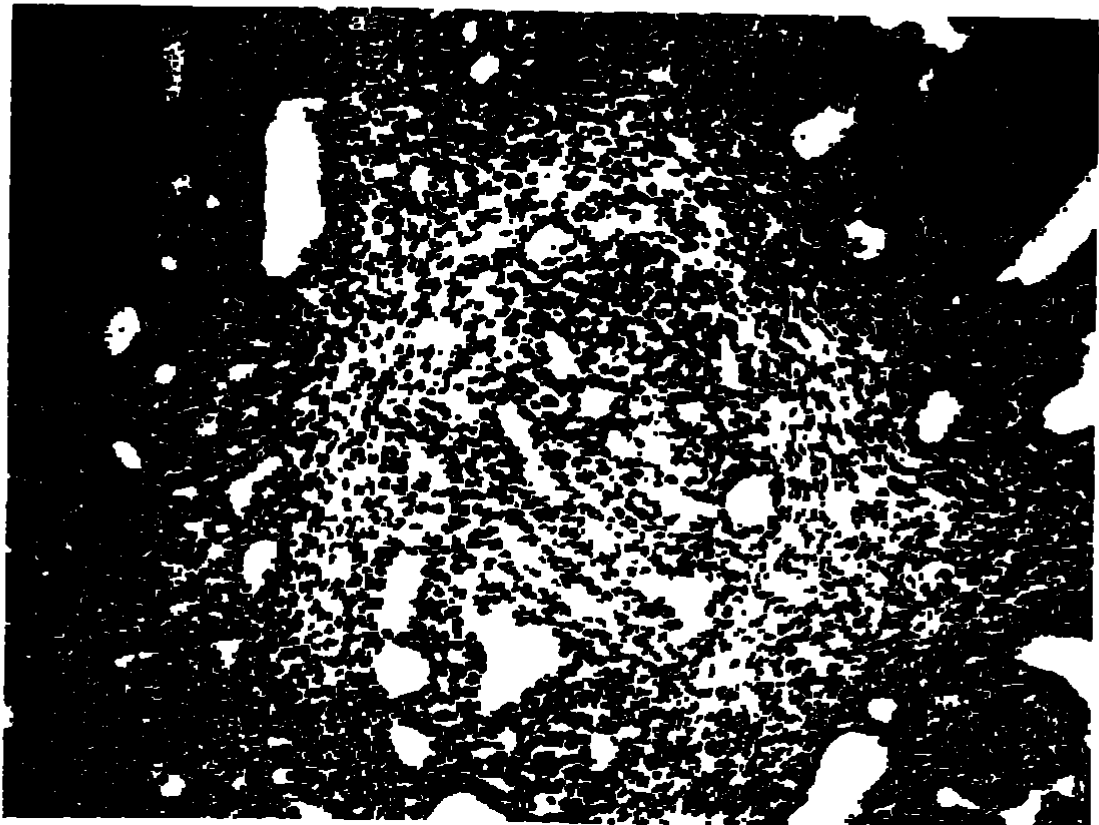
Fig. 5A



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Fig. 5B

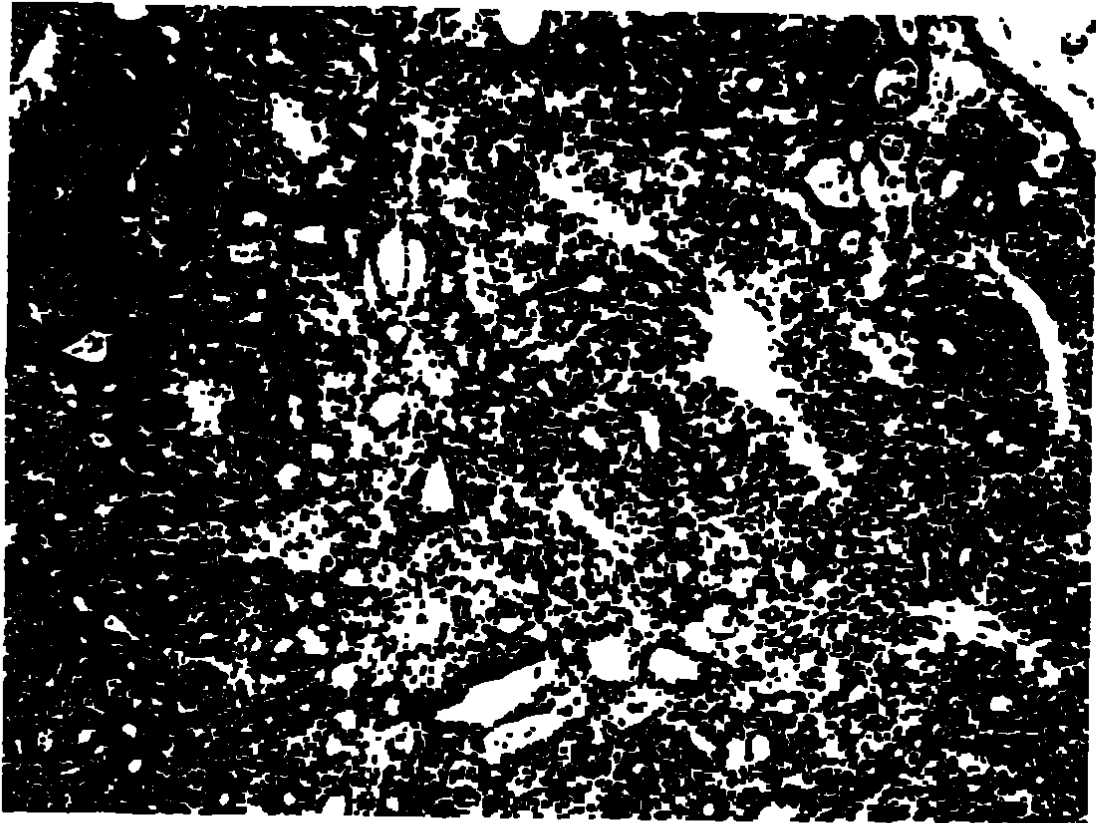


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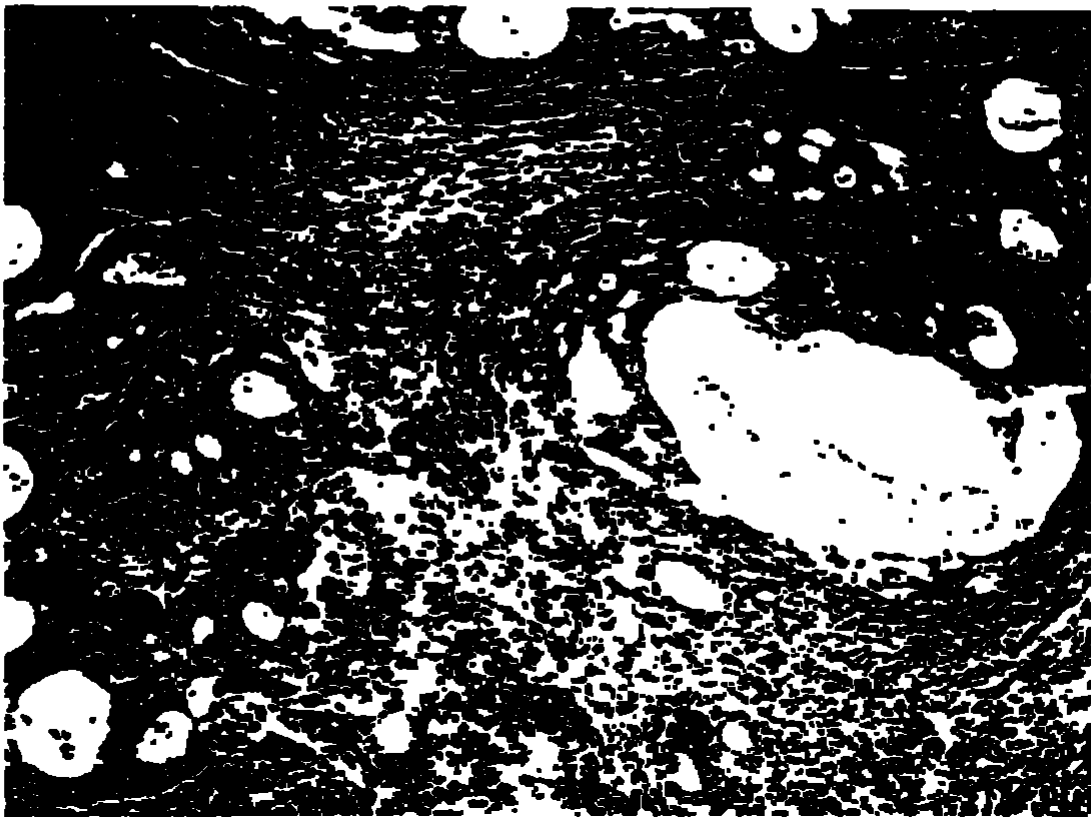
Fig. 5C



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Fig. 5D

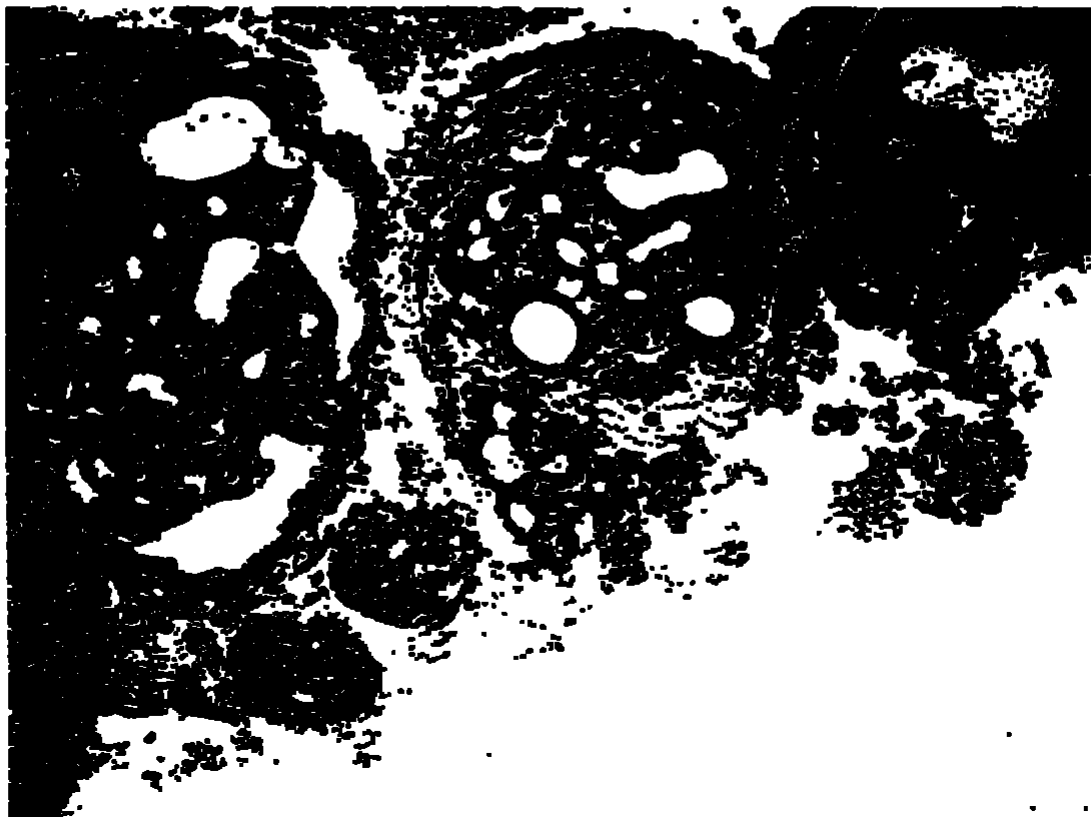


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Fig. 5E



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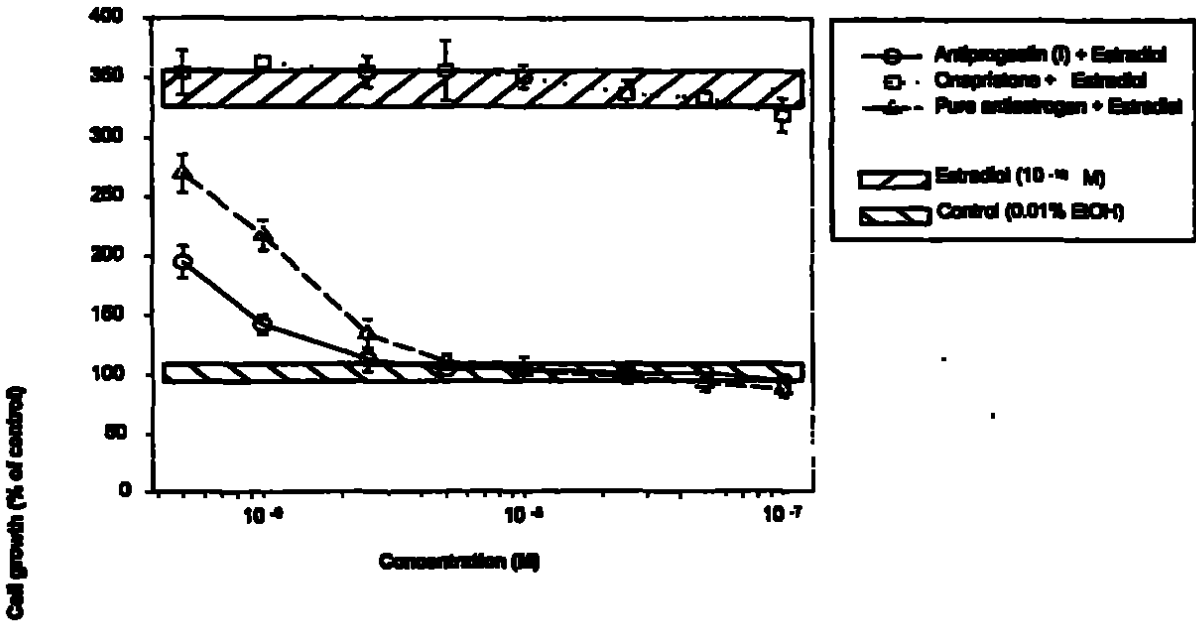
Fig. 5F



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Fig. 6



Europäisches Patentamt GD1 - Dienststelle Benin	ABST
18. OKT. 2000	
Anl:	

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ABSTRACT

The present invention relates to methods and uses for inducing apoptosis in a cell, in particular a breast cancer cell, by the administration of antiprogestins, in particular the

5 antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof. The invention further relates to a treatment of cancer wherein an indicator of high risk is an increased amount of tumor cells in the S-phase of the cell cycle, said treatment comprising an antiprogestin, in particular the antiprogestin 11β -(4-acetylphenyl)- 17β -hydroxy- 17α -

10 (1,1,2,2,2-pentafluoroethyl)-estra-4,9-dien-3-one or a pharmaceutically acceptable derivative or analogue thereof.

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Oficina Europea de Patentes

Certificado

Los documentos adjuntos son una copia fiel y exacta de la versión originalmente presentada de la solicitud de patente europea que se especifica en la siguiente página.

Solicitud de Patente No.

00250342.3

El Presidente de la Oficina de Patentes Europeas

Por encargo:

I.L.C. HATTEN-HECKMAN

(Firma y Sello Oficial)

LA HAYA, 02/03/01

Foja 2 del Certificado

Solicitud No.: 00250342.3

Fecha de presentación: 18/10/00

Solicitante:

Schering Aktiengesellschaft

13353 Berlín

ALEMANIA

Título:

Uso de antiprogestinas para la inducción de apoptosis en una célula

Prioridades reivindicadas: -

Clasificación internacional de patentes: -

Países contratantes mencionados en la fecha de la solicitud:

AT/BE/CH/CY/DE/DK/ES/FI/FR(GB/GR/IE/IT/LI/LU/MC/NL/PT/SE/TR

REIVINDICACIONES

1. Uso de la antiprogestina 11 β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estr-4,9-dien-3-ona o un derivado o de un análogo de ella farmacéuticamente aceptables para la inducción de apoptosis en una célula
2. Uso de acuerdo con la reivindicación 1, en el cual la inducción de apoptosis es causada por la iniciación de diferenciación terminal.
3. El método de acuerdo con cualquiera de las anteriores reivindicaciones, siendo la célula una célula mamífera
4. Uso de acuerdo con la reivindicación 3, siendo la célula mamífera una célula humana.
5. Uso de acuerdo con cualquiera de las anteriores reivindicaciones, siendo la célula una célula tumoral.
6. Uso de acuerdo con la reivindicación 5, siendo el tumor cáncer de seno.
7. Uso de acuerdo con cualquiera de las anteriores reivindicaciones, en el cual el medicamento comprende además un anti-estrógeno.
8. Uso de la antiprogestina 11 β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estr-4,9-dien-3-ona o de un sal o de un análogo de ella farmacéuticamente aceptables para la preparación de un medicamento para el tratamiento de un tipo de cáncer, siendo el indicador de riesgo elevado una cantidad incrementada de células tumorales en la fase S del ciclo celular.
9. El uso de acuerdo con la reivindicación 8, siendo la enfermedad cáncer de seno.
10. Método para inducir apoptosis en una célula, el cual comprende administrar una cantidad eficaz de la antiprogestina 11 β -(4-acetilfenil)-17 β -hidroxi-17 α -(1,1,2,2,2-pentafluoroetil)-estr-4,9-dien-3-ona o de un derivado o un de análogo de ella farmacéuticamente aceptables a una célula *in vitro*.
11. El método de acuerdo con la reivindicación 10, siendo la célula una célula tumoral mamífera.
12. El método de acuerdo con las reivindicaciones 10 u 11, siendo la célula una célula de cáncer de seno.

**DOCUMENTO DE CESIÓN
ASSIGNMENT DOCUMENT**

Los suscritos,
(We, the undersigned)

- 1) JENS HOFFMANN
con domicilio en Mühlenbeck, Alemania
(domiciled in)
- 2) ROSEMARIE LICHTNER
con domicilio en Berlin, Alemania
(domiciled in)
- 3) GERD SIEMEISTER
con domicilio en Berlin, Alemania
(domiciled in)
- 4) MARTIN SCHNEIDER
con domicilio en Berlin, Alemania
(domiciled in)
- 5) ULRIKE FUHRMANN
con domicilio en Berlin, Alemania
(domiciled in)

declaramos que somos los inventores de la invención que lleva el título
(declare that we are the inventors of the invention titled)

Use of Antiprogestins for Prophylaxis and Treatment of hormone-dependent Diseases

y que hemos cedido el derecho sobre esta invención en Colombia, junto con los
privilegios
(and that we have assigned our rights over this invention in Colombia, together with its
privileges)

y anexidades a favor de SCHERING AKTIENGESELLSCHAFT
(and attachments, to) Berlin, Rep. Fed. de Alemania

1) *Jens Hoffmann*

3) *[Signature]*

5) *Ulrike Fuhrmann*

2) *Rosemarie Lichtner*

4) *Martin Schneider*

Urkundenrolle-Nr. R 166/2003

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221

Ich habe das Mitwirkungsverbot nach § 3 Abs. 1 Nr. 7 BeurkG erläutert. Meine Frage, ob eine Vorbefassung im Sinne der Vorschrift vorliege, wurde verneint.

Die vor mir geleisteten Unterschriften

des Herrn Jens **H o f f m a n n**,
geboren am 9. Januar 1963,

der Frau Rosemarie **L i c h t n e r**,
geboren am 21. April 1949,

des Herrn Gerhard **S i e m e i s t e r**,
geboren am 24. März 1959,

des Herrn Martin **S c h n e i d e r**,
geboren am 23. November 1951,

der Frau Dr. Ulrike **F u h r m a n n**,
geboren am 12. Mai 1959,

- von Person bekannt -

sämtlichst geschäftsansässig in 13353 Berlin, Müllerstraße 178,

beglaubige ich.

Berlin, 31. März 2003

Notar

Kostenberechnung gem. §§ 141, 154 KostO

Geschäftswert: 12.000,00 €

Beglaubigungsgebühr §§ 32, 45 I

Wegegebühr gem. § 58

Schreibauslagen §§ 136, 152

Postauslagen §§ 137, 152

16 % Mehrwertsteuer

Berlin, 31. März 2003

Notar

15,00 €
15,00 €
1,00 €
1,44 €
32,44 €
5,19 €
37,63 €

A p o s t i l l e
(Convention de La Haye du 5 octobre 1961)

1. Land: Bundesrepublik Deutschland

Diese öffentliche Urkunde

2. ist unterschrieben von Dimitri R e m p e n

3. in seiner Eigenschaft als Notar

4. sie ist versehen mit dem Siegel
des Notars

Bestätigt

5. in Berlin

6. am 4. April 2003

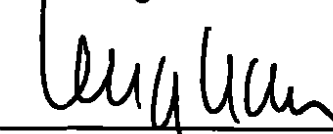
7. durch den Präsidenten des Landgerichts in Berlin

8. unter Nr. 9101 a E - F. 1019/2003

9. Siegel

10. Unterschrift

Im Auftrage



(Kupfernagel)

Vorsitzender Richter am Landgericht

223

Traducción Oficial del Alemán de la certificación la nota de gastos y la apostilla adjuntas al Documento de Cesión suscrito entre los inventores Jens Hoffmann, Rosemarie Lichtner, Gerd Siemeister, Martin Schneider y Ulrike Fuhrmann y la compañía Schering Aktiengesellschaft domiciliada en Berlín, Alemania.

Registro Notarial Número R 166/2003

Habiendo explicado la prohibición de participación conforme al Artículo 3 inciso 1 Numeral 1 de la Ley de Notarización, yo les pregunté a los participantes si existe algún impedimento en el sentido de dicha Ley. La respuesta fue negativa.

Autentico las firmas que aparecen anteriormente, las cuales fueron ejecutadas en mi presencia y pertenecen a

Señor Jens Hoffmann, nacido el 9 de Enero de 1963,
Señora Rosemarie Lichtner, nacida el 21 de Abril de 1949,
Señor Gerd Siemeister, nacido el 24 de Marzo de 1959,
Señor Martin Schneider, nacido el 23 de Noviembre de 1951,
Señora Ulrike Fuhrmann, nacida el 12 de Mayo de 1959,

todos ellos conocidos personalmente por el suscrito notario y domiciliados comercialmente en 13353 Berlín, Müllerstraße 178.

Berlín, 31 de Marzo del 2003

Dimitri Rempen, Notario en Berlín
(Firma y Sello Oficial)

----- (continúa en la 2ª página) -----

Nota de gastos según Artículos 141,154 Disposición Impuestos:

Valor del negocio: 12.000,00 EUROS

Impuesto Artículo 32, 45I Disp. Impuestos	15,00 EUROS
Impuesto Artículo 58 Disp. Impuestos	15,00 EUROS
Impuesto Artículos 136, 152 Disp. Impuestos	1,00 EUROS
Impuesto Artículos 137, 152 Disp. Impuestos	1,44 EUROS
I.V.A. 16%	5,19 EUROS
Total:	37,63 EUROS

Berlín, 31 de Marzo del 2003

Dimitri Rempen, Notario (firma)

APOSTILLA

(Convención de La Haya del 5 de Octubre de 1961)

1. País: República Federal de Alemania

Este documento público

2. Ha sido firmado por: Dimitri Rempen
3. En su calidad de: Notario
4. Y lleva el sello: Del mencionado Notario

Confirmado

5. En: Berlín
6. El día: 4 de Abril del 2003
7. Por: El Presidente del Tribunal Regional en Berlín
8. Bajo el No.: 9101 a E-F 1019/2003
9. Sello: EL PRESIDENTE DEL TRIBUNAL REGIONAL EN BERLIN
10. Firma: Por encargo: Kupfernagel,
Juez Presidente en el Tribunal Regional


ADOLF WATZKE
Traductor Oficial
Res. 2433 Minjusticia

OFFICE OF THE
ATTORNEY GENERAL
STATE OF TEXAS
SAN ANTONIO

WATSON
JULY 1937
LAS VEGAS

225

SUPERINDUSTRIA Y COMERCIO
Radiación : 03040568 00000000 Folios: 225
Fecha (MM): 2003-05-15 14:18:58
Trámite : 011 PCT-PATENT 379 FASE MCI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCION ✓ MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente.		()
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.		()
- Descripción de la invención.		()
- Dibujos de ser estos pertinentes.		()
- Comprobante de pago de las tasas establecidas.		()
COMPLETA ()		
INCOMPLETA ()		()
DISEÑO INDUSTRIAL ()		
- Indicación de que se solicita el registro de Diseño Industrial	()	()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	()	()
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	()	()
- Comprobante de pago de las tasas establecidas	()	()
COMPLETA ()		
INCOMPLETA ()	()	()
ESQUEMA DE TRAZADO ()		
- Indicación de que solicita el registro de un Esquema de trazado.	()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	()	()
- Representación gráfica del esquema de trazado	()	()
- Comprobante de pago de las tasas establecidas.	()	()
COMPLETA ()		
INCOMPLETA ()	()	()

Firma Responsable: **ANDREA POSSE**

Fecha:



2020
Bogotá, D.C.,

Doctor:
GUSTAVO HOLLMAN RESTREPO
Apoderado
Shering Aktiengesellschaft

Oficio N° 08414

ASUNTO:	Radicación N°	03-040568
	Solicitud Internacional	PCT/EP01/12008
	Fecha inicio fase nacional	18/05/03
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha 1.º OCT. 2003.