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Use of alkyl phosphocholines in combination with antitumor medicaments

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(56) Related Art
Stekar et al. European Journal of Cancer (1995) vol. 31A, no. 3 pages 372-374
Spruss et al. Journal of Cancer Research (1993) vol. 119 no. 3 pages 142-149
Hilgard et al. Advances in Experimental Medicine and Biology (1996) vol. 416 pages 157-164

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(81) Bestimmungsstaaten *(national)*: AU, BR, BY, CA, CN, CO, GE, HR, ID, IL, IN, IS, JP, KR, KZ, LT, LV, MK, MX, NO, NZ, PH, PL, RU, SG, UA, UZ, YU, ZA.

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Erklärung gemäß Regel 4.17:
— *hinsichtlich der Berechtigung des Anmelders, die Priorität einer früheren Anmeldung zu beanspruchen (Regel 4.17 Ziffer iii) für alle Bestimmungsstaaten*

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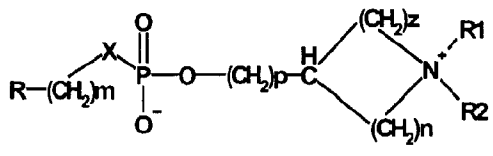
Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) **Title:** USE OF ALKYL PHOSPHOCHOLINES IN COMBINATION WITH ANTITUMOR MEDICAMENTS

(54) **Bezeichnung:** ANWENDUNG VON ALKYLPHOSPHOCHOLINEN IN KOMBINATION MIT ANTITUMORMEDIKAMENTEN



(II)

(57) **Abstract:** The invention relates to the use of alkyl phosphocholines in combination with antitumor medicaments for treating benign and malignant tumor diseases in humans and mammals. The alkyl phosphocholines can be used in an inventive combination with one or a combination of several approved cytostatics. Preferred alkyl phosphocholines are represented in formula II.

(57) **Zusammenfassung:** Die Erfindung betrifft die Verwendung von Alkylphosphocholinen in Kombination mit Antitumormedikamenten zur Behandlung gutartiger und bösartiger Tumorerkrankungen am Menschen und Säugetier. Dabei können die Alkylphosphocholine in einer erfindungsgemässen Kombination mit einem oder einer Kombination von verschiedenen zugelassenen Zytostatika eingesetzt werden. Bevorzugte Alkylphosphocholine werden durch die Formel II dargestellt.

WO 2004/012744 A1

IN THE MATTER OF an Australian
Application corresponding to
PCT Application PCT/EP2003/008346

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Date: 6 January 2005

C. E. SITCH
Deputy Managing Director - UK Translation Division
For and on behalf of RWS Group Ltd

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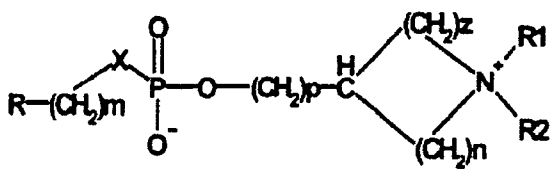

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Use of alkylphosphocholines in combination with antitumor medicaments

Alkylphosphocholines are a new class of organic compounds which show diverse antineoplastic activities (M. Lohmeyer and R. Bittman; Antitumor ether lipids and alkylphosphocholines, DOF, **19** (11), 1021-1037 (1994)). The effect of the alkylphosphocholines in this connection may be based on various molecular and biochemical mechanisms, some of which take place at the level of the plasma membrane of the cells. It is well known that alkylphosphocholines influence inositol metabolism, the interaction with phospholipases or inhibition of protein kinase C and thus that this class of substances has a general influence on cellular signal transduction (K. Maly, F. Überall, C. Schubert, E. Kindler, J. Stekar, H. Brachwitz and H. H. Grunicke, Interference of new alkylphospholipid analogues with mitogenic signal transduction, Anti-Cancer Drug Design, **10**, 411-425 (1995)). Thus, the alkylphosphocholine perifosine shows growth-inhibitory properties in relation to various melanoma, CNS, lung, colon, prostate and breast cancer cell lines with an IC_{50} in the region of 0.2 – 20 μM (P. Hilgard, T. Klenner, J. Stekar, G. Nössner, B. Kutscher and J. Engel; D-21266, a New Heterocyclic Alkylphospholipid with Antitumor Activity, Eur. J. Cancer, **33** (3), 442-446 (1997)). It is further known that perifosine blocks tumor cells in the G_1-S and G_2-M phase of the cell cycle (V. Patel, T. Lahusen, T. Sy, E. A. Sausville, J. S. Gutkind and A. M. Senderowicz; Perifosine, a Novel Alkylphospholipid, Induces $p21^{Waf1}$ Expression in Squamous Carcinoma Cells through a p53-independent Pathway, Leading to Loss in Cyclin-dependent Kinase Activity and Cell Cycle Arrest, Cancer Research **62**, 1401-1409 (2002)).

It is known that the use of alkylphosphocholines before or together with radiotherapy leads to synergistic effects in the treatment of tumors (G.A. Ruitter, M. Verheijl, S.F. Zerp and W.J. van Blitterswijk; Alkyl-Lysophospholipids as Anticancer Agents and Enhancers of Radiation-Induced Apoptosis, Int. J. Radiation Oncology Biol. Phys., **49** (2), 415-420, 2001). It has also been reported that various glycero-3-phospholipids, e.g. ET-18-OCH_3, in combination with various DNA-interacting substances or tubulin binders increase the antitumor activity in vitro on various tumor cell lines (A. Noseda, M.E. Berens, J.G. White and E.J. Modest; In vitro antiproliferative activity of combinations of ether lipid analogs and DNA-Interactive

2003253350 19 May 2008

agents against human tumor cells, Cancer Res., **48** (7), 1788-1791 (1988); P. Principe, H, Coulomb, C, Broquet and P. Braquet; Evaluation of combinations of antineoplastic ether phospholipids and chemotherapeutic drugs, Anti-Cancer Drugs, **3** (6), 577-587 (1992); P. Principe, H. Coulomb, J.-M. Mencia-Huerta, C. Broquet and P. Braquet; Synergistic cytoxic effect of aza-alkylphospholipids in association with chemotherapeutic drugs, J. Lipid Mediators Cell Signalling, **10** (1-2), 171-173 (1994)).

It has now been possible, surprisingly, to show that linear alkylphosphocholines of the general formula I and II, including miltefosine and perifosine, are suitable for use in a combination according to the invention with other drug products for the treatment of benign and malignant oncoses in humans and mammals. It is possible in this connection for the compounds of the general formula I and II to be employed in a combination according to the invention with antitumor substances. Antitumor substances may be alkylating agents, antimetabolites, plant alkaloids, platinum compounds, tumor antibiotics and agonists or antagonists of natural hormones. The antitumor substances may be selected from but not restricted to: cisplatin, carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, cyclophosphamide, 5-fluorouracil, fludarabine, gemcitabine and cytarabine.

It is moreover possible for the alkylphosphocholines of the general formula I and II to be employed in combination with inhibitors of signal transduction in the form of high and low molecular weight inhibitors of receptor and/or cytosolic kinases. These inhibitors may be selected from but not restricted to monoclonal antibodies and heterocyclic compounds.

In accordance with a first aspect the invention provides the use of the alkylphosphocholine miltefosine for the manufacture of a medicament for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

In accordance with a second aspect the invention provides the use of the alkylphosphocholine perifosine for the manufacture of a medicament for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

2003253350 19 May 2008

In accordance with a third aspect the invention provides a drug product comprising miltefosine, and where appropriate carriers and/or exicpients, when used in the treatment of benign or malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

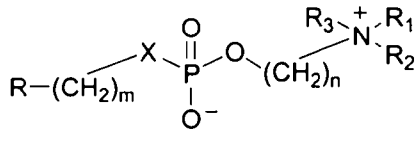
In accordance with a fourth aspect the invention provides a drug product comprising perifosine, and where appropriate carriers and/or exicpients, when used in the treatment of benign or malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

In accordance with a fifth aspect the invention provides a method of treatment of benign or malignant oncoses in a mammal, the method comprising administering to said mammal an effective amount of the alkylphosphosphochline miltefosine before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

In accordance with a sixth aspect the invention provides a method of treatment of benign or malignant oncoses in a mammal, the method comprising administering to said mammal an effective amount of the alkylphosphosphochline perifosine before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

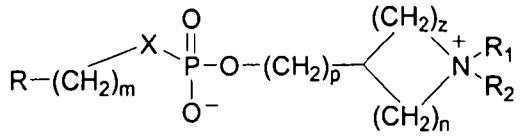
As disclosed herein, alkylphosphocholines of the general formula I and II, including miltefosine and perifosine, can be used in the form of finished drug products.

The compounds of the general formulae 1 and II are defined as follows:



formula I

2003253350 19 May 2008



formula II

where, independently of one another:

n, m, p, z are an integer between 0 and 4;

X is O, S, NH;

R is H, a straight-chain or branched (C_1-C_{20})-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals.

R_1, R_2, R_3 is, independently of one another, H, a straight-chain or branched (C_1-C_6)-alkyl radical, preferably methyl and ethyl, a (C_3-C_7)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals.

Also disclosed herein is a method for controlling tumors in humans and in mammals, which comprises administering at least one of the compounds of the general formula I and II to the human or mammal in an amount effective for tumor treatment before or during a treatment with approved antitumor substances.

The therapeutically effective dose, to be administered for the treatment, of the particular compound of the general formula I and II depends inter alia on the nature and the stage of the oncosis, the age and sex of the patient, the mode of administration and the duration of treatment.

The compounds of formula I and II, including miltefosine and perifosine, can be administered in a drug product as liquid, semisolid and solid drug forms. This takes place in the manner suitable in each case in the form of aerosols, oral powders, dusting powders and epipastics, uncoated tablets, coated tablets, emulsions, foams, solutions, suspensions, gels, ointments, pastes, pills, pastilles, capsules or suppositories.

2003253350 19 May 2008

Exemplary embodiments:

1. Administration of perifosine (D-21 266) in combination with cisplatin

In vivo test:	DMBA-induced rat mammary carcinoma model
Experimental animal:	Sprague-Dawley rat, female
Procedure:	The mammary carcinoma was induced by a single oral does of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.
Administration:	Perifosine 14 x 6.81 mg/kg p.o. Cisplatin 4 x 1 mg/kg i.p.
Effect:	Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

Treatment	Tumor Initial weight [g]	Day 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Cisplatin	0.9	410	0.120
Perifosine (D-21266) + Cisplatin	0.8	-75	<0.001

2. Administration of perifosine in combination with cyclophosphamide

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.
Cyclophosphamide 100 mg/kg, VZ 0, i.v.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

Treatment	Tumor Initial weight [g]	Day 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Cyclophosphamide	0.9	500	0.011
Perifosine (D-21266) + Cyclophosphamide	0.8	-83.3	<0.001

3. Administration of perifosine in combination with Adriamycin

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor was mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.
Adriamycin 4 x 2.15 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

Treatment	Tumor Initial weight [g]	Tag 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Adriamycin	1.0	781.3	0.197
Perifosine (D-21266) + Adriamycin	01.0	-70	<0.001

2003253350 19 May 2008

The claims defining the invention are as follows:

1. Use of the alkylphosphocholine miltefosine for the manufacture of a medicament for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

2. Use of the alkylphosphocholine perifosine for the manufacture of a medicament for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

3. The use according to claim 1 or 2, wherein the alkylphosphocholine is in a therapeutic dose that is effective for said treatment of benign and malignant oncoses before and/or during treatment with the antitumor agent.

4. The use according to any one of claims 1 to 3, wherein the medicament comprises customary pharmaceutical carriers, excipients and/or diluents.

5. A drug product comprising miltefosine, and where appropriate carriers and/or exicpients, when used in the treatment of benign or malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

6. A drug product comprising perifosine, and where appropriate carriers and/or exicpients, when used in the treatment of benign or malignant oncoses before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

7. A method of treatment of benign or malignant oncoses in a mammal, the method comprising administering to said mammal an effective amount of the alkylphosphosphochline miltefosine before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, and fludarabine, or a combination thereof.

2003253350 19 May 2008

8. A method of treatment of benign or malignant oncoses in a mammal, the method comprising administering to said mammal an effective amount of the alkylphosphosphochline perifosine before and/or during treatment with an antitumor agent selected from carboplatin, oxaliplatin, bleomycin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and gemcitabine, or a combination thereof.

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