(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 951 274 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
02.12.2009 Bulletin 2009/49

(21) Application number: **06806214.0**

(22) Date of filing: **12.10.2006**

(51) Int Cl.:
A61K 35/76 *(2006.01)* ***A61K 31/475*** *(2006.01)*
A61K 31/337 *(2006.01)* ***A61P 35/00*** *(2006.01)*

(86) International application number:
PCT/EP2006/009855

(87) International publication number:
WO 2007/059821 (31.05.2007 Gazette 2007/22)

(54) **PARAPOXVIRUSES IN COMBINATION WITH CLASSICAL CYTOTOXIC CHEMOTHERAPEUTIC AGENTS AS BIOCHEMOTHERAPY FOR THE TREATMENT OF CANCER**

PARAPOCKEN-VIREN IN KOMBINATION MIT KLASSISCHEN ZYTOTOXISCHEN CHEMOTHERAPEUTIKA ALS BIOCHEMOTHERAPIE ZUR BEHANDLUNG VON KREBS

PARAPOXVIRUS COMBINES A DES AGENTS CHIMIOTHERAPEUTIQUES CYTOTOXIQUES CLASSIQUES COMME BIOCHIMIOTHERAPIE POUR LE TRAITEMENT DE CANCER

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR

(30) Priority: **24.11.2005 EP 05025600**

(43) Date of publication of application:
06.08.2008 Bulletin 2008/32

(73) Proprietor: **AiCuris GmbH & Co. KG**
42117 Wuppertal (DE)

(72) Inventor: **WEBER, Olaf**
42489 Wuelfrath (DE)

(74) Representative: **Findeisen, Marco et al**
Witte, Weller & Partner
Patentanwälte
Postfach 10 54 62
70047 Stuttgart (DE)

(56) References cited:
WO-A2-00/69455 **WO-A2-02/04002**
WO-A2-03/006654

- **FRIEBE A ET AL: "Immunomodulatory effects of inactivated parapoxvirus ovis (Orf virus) on human peripheral immune cells: Induction of cytokine secretion in monocytes and Th1-like cells" JOURNAL OF VIROLOGY 2004 UNITED STATES, vol. 78, no. 17, 2004, pages 9400-9411, XP002416484 ISSN: 0022-538X**

EP 1 951 274 B1

Description

[0001] In the search for novel cancer therapies that can be used in conjunction with existing treatments, the use of virus-based therapies holds some promise (1). Viruses have evolved to infect cells and often destroy these cells through diverse mechanisms. Although a number of viruses have been used in the clinic so far, this approach has suffered from toxicity, infection of unrelated tissues, immunological side effects and, therefore, was abandoned (2). However, recombinant DNA technology offered new possibilities to use viruses in therapeutic approaches. Current attempts use replication-selective viruses (reviewed in 1). Such viruses should replicate selectively in dividing cells (3). However, although these viruses may rapidly spread in cell-culture monolayers, spread within solid tumors remains an unsolved problem (1).

[0002] The use of inactivated *Parapoxvirus ovis* for cancer therapies has been suggested previously (4).

[0003] Interferon-α (IFN-α) has previously been investigated with classical chemotherapeutics, ie. cisplatin, vinclesine and dacarbacine. The combination of biological and cytotoxic chemotherapeutics is called biochemotherapy. After biochemotherapy, responder rates are up to 66% and, therefore, superior compared to cytotoxic chemotherapy (5).

[0004] The use of inactivated viruses for biochemotherapy in cancer therapies has not been reported.

[0005] As is clear from the abovementioned prior art, no therapeutic method has so far been disclosed which uses an inactivated virus as an immunomodulating agent and a classical cytotoxic chemotherapeutic agent as a biochemotherapy for cancer.

[0006] The present invention is therefore based on the technical problem of providing a therapeutic method which not only reduces the tumor burden of patients more effectively compared to cytotoxic chemotherapy but also provides a therapeutic method for the reconstitution of the immune system after cytotoxic chemotherapy. This therapeutic method should not only have fewer or no undesirable side effects, it also should be superior to current therapies.

[0007] The present invention relates to:

1. The use of *Parapoxvirus ovis* in combination with at least one additional anti-cancer agent for the preparation of a medicament for treating cancer. The invention furthermore relates to the use of *Parapoxvirus ovis* for the production of a medicament for treating cancer in combination with at least one additional anti-cancer agent.
According to the invention *Parapoxvirus ovis* is understood to be *Parapoxvirus ovis* strain D1701, NZ-2, NZ-7, NZ-10 or orf-11.
Also derivatives of the abovementioned *Parapoxvirus ovis* can be used obtained by passaging or adaptation using suitable cell systems such as for example human cells such as WI-38, MRC-5, monkey cells, e.g. Vero cells, bovine cells, such as for example BL-K13A47/Reg or MDBK, and ovine cells such as MDOK, in combination with substances which are effective in anti-cancer therapy for the production of medicaments against cancer in humans and animals,
In addition, also parts or fragments of the abovementioned *Parapoxvirus ovis* and their passaging and adaption variants can be used in combination with substances which are effective in anti-cancer therapy. Parts or fragments of a virus are understood to be genomic or subgenomic fragments of the whole virus or of its genomic nucleic acid, or other components of the virus, which are expressed by means of suitable vectors such as vaccinia viruses in suitable systems such as fibroblast cell cultures. The parts or fragments of the *Parapoxvirus ovis* according to the invention can be purified by conventional methods, such as for example by filtration or chromatography. In another preferred variant the parts or fragments of the *Parapoxvirus ovis* are produced by recombination by methods known to the skilled man.
According to the invention, cancer is all human and animal diseases associated with proliferating or resting tumors.
The anti-cancer agent is a cytotoxic agent.

2. The present invention also relates to a use according to item 1, wherein the cancer is melanoma, breast cancer, prostate cancer, lung cancer, colorectal cancer, liver cancer or metastatic disease of one or more of the primary cancer.

3. The present invention also relates to the use according item 1 or 2, wherein the *Parapoxvirus ovis* is *Parapoxvirus ovis* strain D1701, NZ-2, NZ-7, NZ-10 or orf-11. In a further variant of the invention the *Parapoxvirus ovis* is a Parapoxvirus obtained by passaging of these strains.

4. The present invention also relates to the use according to one of the items 1 to 3, wherein the *Parapoxvirus ovis* is present in an inactivated form. The inactivation of the Parapoxvirus is carried out by virus inactivation methods known to the skilled man. In a preferred variant the *Parapoxvirus ovis* is inactivated by the method described in European Patent No. EP-B1-0312839.

5. The present invention also relates to the use according to one of the items 1 to 4, wherein the treatment of cancer produces a reduction of tumor size of patients, i.e. the medicament causes a reduction of the tumor size or mass, respectively.

6. The treatment of cancer reduces the number and size of metastases of the primary tumors as meas-

ured by procedures known to the skilled man.

7. The cytotoxic agent is selected from the group consisting of vinblastine, vincristine, docetaxel, Paclitaxel.

[0008] The pharmaceutical composition of the present invention may be administered in oral forms, such as, without limitation, normal and enteric coated tablets, capsules, pills, powders, granules, elixirs, tinctures, solution, suspensions, syrups, solid and liquid aerosols and emulsions. They may also be administered in parental forms, such as, without limitation, inravenous, intraperitoneal, subcutaneous, intramuscular, intratumoral and the like forms well-known to those of ordinary skill in the pharmaceutical arts. The pharmaceutical composition of the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using transdermal delivery systems well-known to those of ordinary skilled in the art.

[0009] The dosage regimen with the use of the pharmaceutical composition of the present invention is selected by one of ordinary skill in the arts; in view of a variety of factors, including, whithout limitation, age, weight, sex, and medical condition of the recipient, the severity of the condition to be treated, the route of administration, the level of metabolic and excretory function of the recipient, the dosage form employed.

[0010] The pharmaceutical compositions of the present invention are preferably formulated prior to administration and include one or more pharmaceutically acceptable excipients. Excipients are inert substances such as, without limitations, carriers, diluents, flavoring agents, sweeteners, lubricants, solubilzers, suspending agents, binders, tabet disintegrating agents and encapsulating material.

[0011] The formulation may be in unit dosage form, which is a physically discrete unit containing a unit dose, suitable for administration in human or other mammals. A unit dosage form can be a capsule or tablets, or a number of capsules or tablets. A "unit dose" is a predetermined quantity of the active pharmaceutical composition of the present invention, calculated to produce the desired therapeutic effect, in association with one or more excipients. Dosages will vary from about 10^3 to about 10^{12} physical number of viral particles per application or will be based an physical number of particles/kg/day.

[0012] The pharmaceutical compositions of the present invention may be administered in a single daily dose, or the total daily dose may be administered in divided doses, two, three, or more times per day. Where delivery is via transdermal forms, of course, administration is preferably continuous.

Description of Figure 1

[0013] Anti-tumor activity of a *Parapoxvirus ovis* (PPVO) and Taxol combination is superior to a monotherapy with either taxol or PPVO in an MDAMB 231 breast cancer model in nude mice. Paclitaxel (Taxol®, Bristol Myers Squibb) was administered at 7.5mg/kg/day i.v. on three consecutive days starting day 10. A single dose of PPVO ($1x10^6$ $TCID_{50}$) or the respective placebo was administered day 13 after transplantation intraperitoneally (n = 10 mice/group).

Example

[0014] MDA-MB-231 human breast carcinoma cells (ATCC # HTB26) were cultured in standard universal growth medium (UM: DMEM, 10% FBS, 10 mM HEPES, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin) at 37°C in 5% CO_2 in a humidified incubator. The cells were harvested by trypsinization, washed, counted, adjusted to $2.5x10^7$ cells/ml with ice cold phosphate-buffered saline (PBS), and subsequently stored on ice until transplantation. Approximately 5 x 10^6 cells in a total volume of 0.2 ml PBS are injected subcutaneously (s.c.) in the flank region. Eight-to-ten week-old female NCr nude mice (Taconic, Germantown, NY) with an average body mass of about 20-25 g were used for the experiments. Tumor measurements were performed 10 days after transplantation. Tumor sizes were calculated using the formula (a x b x b)/2. Thereafter the mice were randomized and divided into several groups that reflect different treatments (n = 10 mice/group). In the first group the mice only received PBS as a control approach. In the second group Paclitaxel (Taxol®, Bristol Myers Squibb) was administered at 7.5mg/kg/day i.v. on three consecutive days starting day 10. In the third group a single dose of PPVO ($1x10^6$ $TCID_{50}$) was administered day 13 after transplantation intraperitoneally. In the fourth group the administration of Paclitaxel and PPVO according to the dosage regimen applied to groups two and three was combined. For reasons of animal welfare, animals were sacrificed when the tumors reached approximately 10-15% of the mouse body weight or when the tumors scabbed or ulcerated.

[0015] As it can be seen in Fig. 1 the mean tumor size is clearly reduced in group four (—o—) if compared to group two (—◇—) or group three (—▲—).

[0016] Therefore, the inventors clearly demonstrated for the first time that the administration of a combination of *Parapoxvirus ovis* (PPVO) and a conventional anticancer agent is superior to a monotherapy with either the anti-cancer agent or PPVO alone.

References

[0017]

1. D. Kirn, R.J. Martuza, J. Zwiebel, Nat.Med. 7, 781 (2001)

2. C.M. Southam, NY Acad. Sci. 22, 656 (1960)

3. R.L. Martuza, A. Malick, J.M. Markert, K.L. Ruffner, D.M. Coen, Science 252, 856 (1991)

4. O. Weber et al. WO 02/04002

5. S.S. Legha J.Clin.Oncol. 14, 7 (1996)

Claims

1. Use of *Parapoxvirus ovis* in combination with at least one additional anti-cancer agent for the production of a medicament for treating cancer, wherein the anti-cancer agent is selected from the group consisting of vinblastine, vincristine, docetaxel, paclitaxel.

2. The use of *Parapoxvirus ovis* for the production of a medicament for treating cancer in combination with at least one additional anti-cancer agent, wherein the anti-cancer agent is selected from the group consisting of vinblastine, vincristine, docetaxel, paclitaxel.

3. The use according to any of claims 1 and 2, wherein the cancer is melanoma, breast cancer, prostate cancer, lung cancer, colorectal cancer, liver cancer, bladder cancer, cancer of the endocrine system, cancer of the nervous system, lymphoma or metastatic disease of the respective primary cancer.

4. The use according to any of claims 1 to 3, wherein *Parapoxvirus ovis* is *Parapoxvirus ovis* strain D1701, NZ-2, NZ-7, NZ-10 or orf-11 or a Parapoxvirus obtained by any massaging of these strains.

5. The use according to any of claims 1 to 4, wherein the *Parapoxvirus ovis* is present in an inactivated form.

6. The use according to any of claims 1 to 5, wherein the medicament causes a reduction of tumor size or tumor mass.

Patentansprüche

1. Verwendung von *Parapoxvirus ovis* in Kombination mit zumindest einem zusätzlichen Anti-Krebs-Agens zur Herstellung eines Arzneimittels zur Behandlung von Krebs, wobei das Anti-Krebs-Agens ausgewählt ist aus der Gruppe bestehend aus Vinblastin, Vincristin, Docetaxel, Paclitaxel.

2. Verwendung von *Parapoxvirus ovis* zur Herstellung eines Arzneimittels zur Behandlung von Krebs in Kombination mit zumindest einem zusätzlichen Anti-Krebs-Agens, wobei das Anti-Krebs-Agens ausgewählt ist aus der Gruppe bestehend aus Vinblastin, Vincristin, Docetaxel, Paclitaxel.

3. Verwendung nach einem der Ansprüche 1 oder 2, wobei es sich bei dem Krebs um ein Melanom, Brustkrebs, Prostatakrebs, Lungenkrebs, kolorektalen Krebs, Leberkrebs, Blasenkrebs, Krebs des endokrinen Systems, Krebs des Nervensystems, ein Lymphom oder eine metastatische Erkrankung des jeweiligen Primärkrebses handelt.

4. Verwendung nach einem der Ansprüche 1 bis 3, wobei es sich bei *Parapoxvirus ovis* um den *Parapoxvirus ovis* Stamm D1701, NZ-2, NZ-7, NZ-10 oder orf-11 oder ein *Parapoxvirus* handelt, das durch beliebiges Passagieren dieser Stämme erhalten wird.

5. Verwendung nach einem der Ansprüche 1 bis 4, wobei das *Parapoxvirus ovis* in einer inaktivierten Form vorliegt.

6. Verwendung nach einem der Ansprüche 1 bis 5, wobei das Arzneimittel eine Verringerung der Tumorgröße oder Tumormasse bewirkt.

Revendications

1. Utilisation de *Parapoxvirus ovis* en association avec au moins un agent anti-cancéreux supplémentaire pour la production d'un médicament destiné à traiter le cancer, dans laquelle ledit agent anticancéreux est sélectionné dans le groupe constitué de la vinblastine, de la vincristine, du docetaxel et du paclitaxel.

2. Utilisation de *Parapoxvirus ovis* pour la production d'un médicament destiné à traiter le cancer en association avec au moins un agent anticancéreux supplémentaire, dans laquelle ledit agent anticancéreux est sélectionné dans le groupe constitué de la vinblastine, de la vincristine, du docetaxel et du paclitaxel.

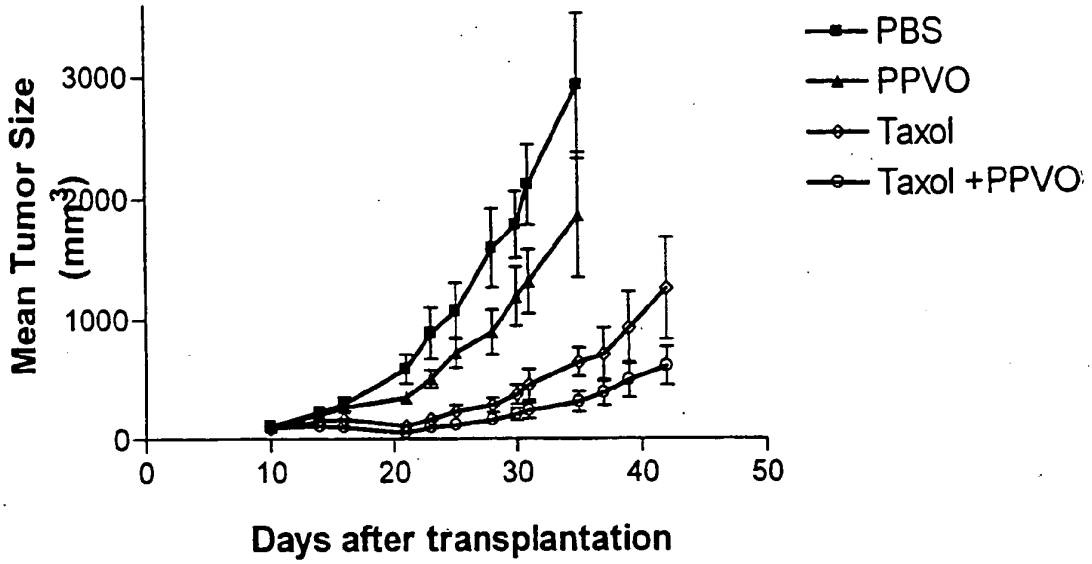
3. Utilisation selon l'une quelconque des revendications 1 ou 2, dans laquelle le cancer est un mélanome, un cancer du sein, un cancer de la prostate, un cancer des poumons, un cancer colorectal, un cancer du foie, un cancer de la vessie, un cancer du système endocrinien, un cancer du système nerveux, un lymphome ou une maladie métastatique du cancer primaire respectif.

4. Utilisation selon l'une quelconque des revendications 1 à 3, dans laquelle le *Parapoxvirus ovis* est une souche de *Parapoxvirus ovis* D1701, NZ-2, NZ-7, NZ-10 ou orf-11, ou un *Parapoxvirus* obtenu par le repiquage de l'une quelconque de ces souches.

5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le *Parapoxvirus ovis* est présent sous une forme inactivée.

6. Utilisation selon l'une quelconque des revendications 1 à 5, dans laquelle le médicament entraîne une réduction de la taille de la tumeur ou de la masse de la tumeur.

Figure 1


Mean Tumor Size (mm3)
3000
2000
1000
0
0
10
20
30
40
50
Days after transplantation
PBS
PPVO
Taxol
Taxol +PPVO

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- EP 0312839 B1 **[0007]**
- WO 0204002 A, O. Weber **[0017]**

Non-patent literature cited in the description

- **D. Kirn ; R.J. Martuza ; J. Zwiebel.** *Nat.Med.,* 2001, vol. 7, 781 **[0017]**
- **C.M. Southam.** *NY Acad. Sci.,* 1960, vol. 22, 656 **[0017]**
- **R.L. Martuza ; A. Malick ; J.M. Markert ; K.L. Ruffner ; D.M. Coen.** *Science,* 1991, vol. 252, 856 **[0017]**
- **S.S. Legha.** *J.Clin.Oncol.,* 1996, vol. 14, 7 **[0017]**