AUSTRALIA

PATENTS ACT 1990

NOTICE OF ENTITLEMENT

We, **Institut Gustave Roussy** AND **Centre National De La Recherche Scientifique,** the applicants/Nominated Persons in respect of Application No. 37570/93 state the following:-

The Nominated Persons are entitled to the grant of the patent because the Nominated Persons would, on the grant of a patent for the invention to the inventors, be entitled to have the patent assigned to the Nominated Persons.

The Nominated Persons are entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because: the Nominated Person Institut Gustave Roussy is the partial-assignee of the applicant in respect of the application listed in the declaration under Article 8 of the PCT: the Nominated Person Centre National De La Recherche Scientifique made the application listed in the declaration under Article 8 of the PCT.

The basic application listed in the declaration made under Article 8 of the PCT was the first application made in a Convention country in respect of the invention.

DATED this TWENTY FIRST day of FEBRUARY 1994

.....

a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

(DCC ref: 1636364)

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(54) Title
DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMORAL TREATMENT

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(56) Prior Art Documents
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(57) Claim

1. An adenoviral vector comprising a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but contains those sequences which carry the genetic information needed for the adenovirus to enter cells which are capable of being infected, as well as the essential sequences needed for encapsidation of this adenovirus, and an insert containing a nucleic acid sequence coding for a cytokine, wherein said insert is under the control of a promoter wherein said adenoviral vector is used as an antitumor drug.

12. A method for the production of defective adenoviruses according to claim 1, comprising the steps of:

(a) transforming cell lines of higher eukaryotes, whose cells contain a separate nucleotide sequence capable of complementing the portion of the adenovirus genome which is lacking in the adenovirus and which is essential for its replication, said separate sequence being incorporated into the genome of the cells of said cell line; and

(b) recovering the defective recombinant adenoviruses produced from the culture medium of the cells of said cell lines.

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(54) Title: DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR USE IN ANTITUMORAL TREATMENT

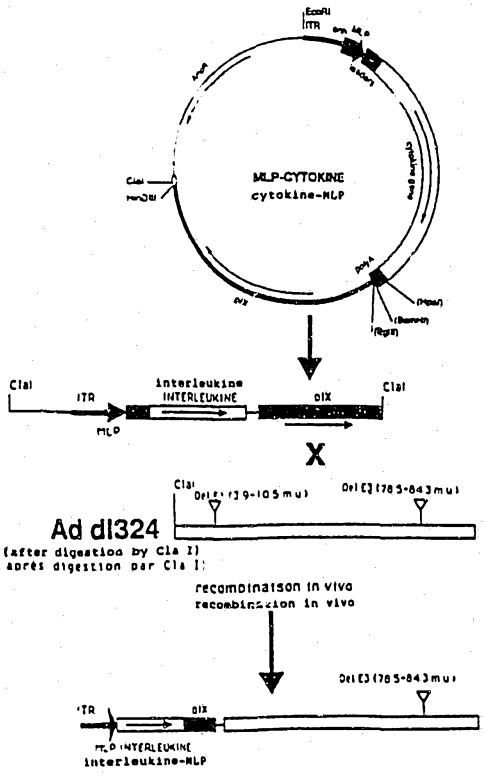
(54) Titre: ADENOVIRUS RECOMBINANTS DEFECTIFS EXPRIMANT DES CYTOKINES POUR TRAITEMENT ANTITUMORAL

(57) Abstract

Recombinant nucleic acid for use in the production of a defective adenovirus containing an inserted sequence coding for a cytokine under the control of a promotor in the genomic sequence of the recombinant adenovirus. Said recombinant adenovirus is useful in the preparation of anti-tumoral drugs capable of being directly injected into the tumour of the host.

(57) Abrégé

L'invention concerne un acide nucléique recombinant utilisable pour la production d'un adénovirus défectif contenant un insert codant pour une cytokine sous le contrôle d'un promoteur au sein de la séquence génomique de l'adénovirus recombinant. Cet adénovirus recombinant est utilisable pour la préparation de médicaments anti-tumoraux sous forme directement injectable dans la tumeur de l'hôte.



DEFECTIVE RECOMBINANT ADENOVIRUSES EXPRESSING CYTOKINES FOR ANTITUMOR TREATMENT

Cytokines are molecules (hormones) produced by cells following an antigenic stimulation or an activation by other factors. The first cytokine which will has been produced is interleukin-1 (Il-1). It permits activation of the T cells which, in turn, start producing a whole battery of lymphokines, some of which are essential for the activation of the immune system and the defenses against viral or parasitic infections.

For some years, cytokines have been used in anticancer immunotherapy. Nevertheless, when they are administered systemically, a number of problems arise. Il-2, for example, produces quite substantial side effects. it is rapidly metabolized, so that high doses have to be administered repeatedly.

Better administration routes which would increase their efficacy while decreasing their adverse effects are hence being sought.

The subject of the invention is hence defective recombinant adenoviruses expressing one or more cytokines, as well as the use of these recombinant adenoviruses for making up pharmaceutical compositions, in particular antitumor compositions, more especially compositions which can be injected directly into solid tumors of the host.

The subject of the present invention is defective recombinant adenoviruses, characterized in that they contain a defective, non-replicable adenovirus genome

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into which one or more nucleic acid sequences coding for one more cytokines, in particular lymphokines, are inserted under the control of one or more promoters capable of being recognised by the polymerases of human cells, more especially of human tumor cells or of cells infiltrating these tumors.

The invention relates more especially to recombinant nucleic acids capable of being used for the production of such defective recombinant adenoviruses.

Such a recombinant nucleic acid is characterized in that it contains, on the one hand a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but which nevertheless contains those sequences which, in this genome, are the carrier of the genetic information needed for the corresponding adenovirus to enter the cells which the latter is capable of infecting, as well as the set of essential sequences needed for encapsidation of this adenovirus, and on the other hand an insert containing a nucleic acid sequence coding for a cytokine, this insert being under the control of a promoter present in or previously inserted into the abovementioned genomic sequence.

Adenoviruses, in particular type 2 or 5 adenoviruses capable of infecting humans (or human adenoviruses), or alternatively serotype 4 and 7 adenoviruses, represent especially preferred vectors in the context of the present invention, on account, in particular, of the large size of the foreign DNA fragment which it is

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possible to insert into the genome of these viruses.

Advantageously the abovementioned nucleic acid insertion sequence(s), coding for one or more predetermined cytokines, are contained in a defective adenovirus genome lacking the essential nucleotide sequences needed for replication of these adenoviruses, and more especially the transactivators E1A and E1B and, where appropriate, the E3 region of the adenovirus, or alternatively its E1 and E3 regions.

In other words, the invention turns to good account the capacity of these defective recombinant adenoviruses to allow the insertion sequence they contain to be expressed in the cells they invade even when, on account of their defective character, they do not multiply therein. In other words, the objective of the invention is to cause cytokines to be secreted actually within the cells of the tumour to be treated (tumor cells themselves and cells, in particular lymphocytes, which infiltrate these tumors) when these cells have been infected with these defective adenoviruses, especially when the latter are injected directly into the tumor. The cytokines produced will thus activate first and foremost, in situ, the cytotoxic cells infiltrating the tumor and those present in proximity to the tumor.

Regarding the sequence for insertion into the defective recombinant adenovirus genome, this may be chosen from all those which express a cytokine capable of exerting either a direct antitumor effect, or an activating effect on immunocompetent cells of the body, or both

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together.

Among these cytokines, the following may be mentioned as examples: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, α interferon, γ interferon, tumor necrosis factor alpha (TNFα) ([same term in French]).

The same recombinant adenoviruses may also be used in the case of certain diseases in which there is an immune deficiency and in the case of certain parasitic or viral disaeases, especially γ, α interferon and/or GM-CSF), in particular by administration systemically or via cells, preferably human cells, taken in a state that allows them to be injected into humans, these cells having previously been infected with a recombinant defective adenovirus according to the invention.

The properties of some of these cytokines are recalled below.

Interleukin-1 (IL-1):

This is produced essentially by activated macrophages and monocytes. Its molecular weight is approximately 17 kilodaltons. It displays several activities, including:

a) a chemoattractive action on polymorphonuclear cells and macrophages (1, 2),

b) an increase in the cytotoxic activity of spontaneous cytotoxic (natural killer or Nk cells),

c) an induction of fever following an infection,

d) most especially, the activation of T cells for the production of other factors.

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Interleukin-2 (IL-2), Interleukin-4 (IL-4) and Interleukin-5 (IL-5):

These are produced by activated T lymphocytes. The action of these cytokines is restricted to cells of the immune system, they cause their multiplication and their activation: IL-2 and IL-4 have been tested in antitumor immunotherapy in mice and in man. In mice, they act synergistically and cause tumor regression.

Interleukin-6 (IL-6)

This is produced by many cell types including T lymphocytes, macrophages, fibroblasts, etc. It induces the final differentiation of B lymphocytes, which become antibody producers.

Tumor Necrosis Factor α (TNFα) ([same term in French]):

This is a factor produced by macrophages. It has a dual action: a direct action on tumor cells causing their lysis and an activation of the immune system.

The use of TNFα in man must be carried out cautiously since a large number of cells possess the receptor for it: which accounts for the importance of inducing its secretion only locally, actually within the tumor, to limit its adverse effects on the other cells of the host.

Interleukin-3- (IL-3), Interleukin-7 (IL-7) and Colony stimulating Factor (CSF).

These are hematopoietic growth factors. They are produced essentially by lymphocytes, monocytes and macrophages. They act at different levels of hematopoiesis, that is to say of the different stages of

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differentiation of marrow cells to blood cells. In addition, CSF exerts very substantial effects on the body's primary defences as regards the bacterial defences, luring the macrophages to the sites of infection and increasing their capacity for phagocytosis.

In combination with IL-2 and IL-4, GM-CSF proves to be an important antitumor factor.

γ Interferon (IFN-γ)

This is a factor produced by activated T cells; it is endowed with antiviral properties; it inhibits the multiplication of viruses and parasites and causes the lysis of infected cells and some tumor cells.

α Interferon (IFN-α)

Produced by T cells and monocytes, this displays an antiviral and lytic effect on infected cells. IFN-α has been used in immunotherapy against some types of cancer, including mesothelium [sic].

Naturally, the invention is not limited, as regards the choice of insertion sequences which can be used in adenoviruses according to the invention, to those sequences which have been identified above. Nevertheless, the latter are illustrative of the palette of possibilities which are afforded to the therapist, who is responsible for making the choice of the most suitable defective recombinant adenovirus to be used in the light of the nature of the tumours to be combated.

The invention also relates to pharmaceutical compositions comprising one or more recombinant vectors as described above, in combination with a

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pharmaceutically acceptable vehicle, especially sterile, isotonic compositions which can be injected directly into the tumors to be treated, or dry, in particular lyophilized, compositions which, by the addition of sterilized water or of physiological saline as the case may be, enable solutions which can be injected directly into the tumors to be made up or reconstituted.

Direct injection of non-replicable, modified adenoviruses into the tumor affords the advantage, on the one hand of avoiding diffusion of the recombinant adenoviruses in the general circulation, with the consequent side effects liable to be exerted by the cytokines expressed in places other than on the sites where the manifestation of their action is sought, in this instance the tumor cells themselves or the cells, in particular lymphocytes, which infiltrate them or which are present in their immediate proximity. Preferably, the injection is carried out at the very least in at least one site of the primary tumor.

Neither is the invention limited to administration of the recombinant adenoviruses containing the sequences coding for the cytokines of the kind in question directly in the tumors. Any other administration route permitting access of these recombinant adenoviruses to the tumor to be treated may be envisaged. In particular, use may be made of cells which are compatible with the host, for example human fibroblasts, preferably ones previously removed from the host him- or herself.

The invention also relates to cell cultures, for

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example cultured fibroblasts previously infected with recombinant nucleic acids, more especially the defective adenoviruses defined above. These infected cells, where appropriate attenuated or rendered immunologically inert, for example by irradiation, contribute to the eradication of installed tumors when they are injected systemically. This injection may be envisaged either alone, or in addition to injection directly into the tumor.

The subject of the invention is also a method for obtaining the recombinant adenoviruses described above, which comprises, after the actual step of construction of a vector by introduction of one or more insertion nucleic acid(s) into the genome of the initial defective adenovirus, a step of transformation of transformable cell lines of higher eukaryotes (in particular of human or animal origin) themselves containing a separate nucleotide sequence capable of complementing the portion(s) lacking in the genome of the defective adenovirus and without which replication of the latter is prevented, said separate sequence preferably being incorporated in the genome of the cells of said cell line.

As a preferred example of such cell lines, there may be mentioned line 293, a human embryonic kidney line which contains, integrated in its genome, the first eleven percent of the left-hand end of the genome of a type 5 adenovirus (Ad5). This fraction can then complement defective recombinant viruses which carry deletions of this region. Such a production method is described, more especially, in European Patent Application

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No. 0,185,573 of 20/11/85.

After transformation of these cell lines, the defective adenoviruses thus multiplied and produced are recovered from the culture medium of the cells of these lines and purified.

Further details of the present invention will be given in the description which follows of the possibilities of construction of a recombinant vector adenovirus containing at least one sequence coding for a cytokine, especially a lymphokine.

I. METHODS

A) Cells and viruses

Ad 5-transformed human embryonic kidney cell line 293 (Graham et al., 1977) was used for the transfection of DNA as well as for the multiplication and titration of adenovirus (Ad). In effect, cell line 293 complements the functions of the genes for E1A and E1B functions and permits the replication of defective Ad recombinants. For the construction of the recombinant Ad, human Ad5-d1324, carrying deletions in the E1 region (3.9-10.5 m.u.) and E3 region (78.5-84.3 m.u.), was used (Shenk and Williams, 1984). Cell lines 293, Hela [sic] and Vero were maintained in an Eagle minimum essential culture medium with 10% of fetal calf serum.

b) Construction of plasmids permitting the expression of different cytokines

The eukaryotic expression vector pMLP10 has been described (Ballay et al., 1985). A derivative of this vector (pMLP18) was constructed by insertion of a

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sequence containing different single restriction sites downstream of the adenovirus major late promoter. These sites thus permit cloning of the different genes coding for the chosen cytokines under the control of the viral promoter. Downstream of this sequence containing these single restriction sites, the sequence containing the polyadenylation signal of the gene coding for the early antigens of SV40 virus were placed. The BgIII - HindIII fragment of Ad5 is cloned downstream. This 3-Kbp sequence contains the gene coding for protein IX which is necessary for encapsidation of the viral genome exceeding 97% of its normal size, and permits subsequent in vivo recombination. Sequences coding for the genes for the different cytokines are isolated from plasmids obtained from different teams. These sequences, obtained after cleavage by means of different restriction enzymes, are introduced into the multiple cloning site of the expression vector described above (pMLP-18). The different plasmids designated pMLP-cytokine (IL-2, IL-4, and the like), which are used for obtaining the recombinant viruses as described in the following section, are thereby obtained.

c) Transfection of DNA and isolation of recombinant viruses

The Ad-cytokine defective recombinant adenoviruses were obtained by in vivo recombination between the straight fragment of the viral genome previously cleaved with the restriction enzyme Cla I and the homologous sequence existing on the plasmids

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pMLP-cytokine described above. The mixture of the fragment of the viral genome (2.6 m.u. - 100 m.u.), purified after cleavage, and of the plasmid linearized with the restriction enzyme Cla I or Pvu I is transfected into 293 cells using the calcium phosphate precipitation method (Graham and Van der Eb, 1973). Cell plaques showing a cytopathic effect are isolated 10 days later and the virus was amplified in culture. The viral DNA was extracted by the Hirt procedure (Graham et al., 1977) and the recombinant viruses were identified by mapping with restriction enzymes.

Figure 1 shows diagrammatically a construction of this type employing an insertion sequence coding for an interleukin (IL-2, IL-4, and the like).

In this figure:

- "leaders" corresponds to a tripartite leader
- "Del" corresponds to a "deletion"
- Ad dl 324 corresponds to an adenovirus provided with the abovementioned "deletions".

d) <u>Expression of the sequences coding for an expressed cytokine</u>

Hela or Vero cell lines are infected with the defective recombinant viruses obtained. Cells effectively transfected may be characterized essentially by means of detection of the activity of the cytokine released into their culture medium. In the case of IL-I, yields capable of reaching from 1 to 2 μg of interleukin per 10^6 cells have been observed.

Cells infected with an Ad-cytokine recombinant

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secrete variable amounts of the cytokine into the culture medium. Different methods exist for the detection and quantification of the cytokines produced.

1) Quantitative methods:

- ELISA, using specific antibodies
- RIA (radioimmunoassay)
- Western blotting

2) Qualitative methods (or biotests): based on the biological properties of cytokines

For example:

IL-2: Test of proliferation of CTL-L2 cells (CTL-L2 cells multiply and are maintained in culture only in the presence of IL-2 in the culture medium)

IL-3 and GM-CSF: Test of proliferation of TF-1 cells

IL-4: Test of proliferation of CTL-L2 cells and induction of soluble CD23 with certain cells including lymphocytes.

INF-α: Cytotoxicity test on L92-9 cells.

Neutralization test: The effect of cytokines may be blocked by incubating the target cells in the presence of cells of specific antibodies.

Some results obtained with the adenovirus vector carrying the IL-2 gene (Ad-IL-2) are described below.

1) Cells infected in vitro with Ad-IL-2 secrete significant amounts of functional IL-2.

2) Direct injection of the vector carrying the IL-2 gene into tumors already established in the animal (the tumor diameter at the time of injection is between 4 and

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7 mm) induces stimulation of the immune system which manifests itself in a stabilization of the size of the tumor, and then its regression to the point of complete disappearance in 40% to 50% of cases.

This result may be improved by treating the tumors in an earlier phase of its development or by using different vectors at the same time, for example combination of Ad-IL-2 with Ad-INF and/or Ad-IL-4, Ad-GM-CSF, Ad-IL-3. This combination has to be specified according to the type of tumor.

3) Tumor cells infected in vitro and then injected into syngeneic animals or even immunodeficient animals (Nu/Nu mice) lose their tumorigenic power (at least up to 80% of the animals reject the tumor cells; in other words, the tumor cells no longer proliferate in 80% of immunodeficient animals injected with these cells.

4) Animals which have rejected a first injection of infected tumor cells are highly immunized and are protected against parent (uninfected) tumor cells injected at different times and at different places.

When coinjected with tumor cells infected in vitro, the spleen cells of these immunized animals are, furthermore, capable of transferring the antitumor immunity to recipient animals.

It is self-evident that the descriptions of constructions of recombinant defective adenoviruses envisaged above are in no way limiting in character. Other constructions may be produced, in particular

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according to the variants also mentioned below as examples.

1) Promoter exchange

The adenovirus major late promoter may be replaced by other promoters which are ubiquitous but of exogenous origin, such as:

- promoter contained in the LTR (long terminal repeat) of RSV (Rouse sarcoma virus)
- the promoter of the IE gene of CMV (cytomegalovirus)
- The MMTV (mouse mammary tumor virus) or metallothionine inducible promoters.

Similarly, promoters permitting a more specific expression restricted to tumor cells, may be used, such as, for example:

- the promoter of the rep gene of parvovirus HI.

The invention also relates to a recombinant nucleic acid of the abovementioned type, characterized in that the genomic sequence of the adenovirus lacks its 5' end region downstream of the early promoter of the E1A region of the adenovirus, and in that the sequence coding for the cytokine is placed under the control of this early promoter. This recombinant nucleic acid may also be employed in applications mentioned more especially in connection with recombinant DNAs in which the sequence coding for the cytokine is placed under the control of the adenovirus major late promoter.

2) Simultaneous expression of several cytokine genes

3 types of constructions are described:

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- the cytokine genes are under the control of two promoters which are either identical or different (MLP and RSV, for example) and are located following one another.

- the cytokine genes are under the control of separate promoters cloned into separate regions of the virus.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An adenoviral vector comprising a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but contains those sequences which carry the genetic information needed for the adenovirus to enter cells which are capable of being infected, as well as the essential sequences needed for encapsidation of this adenovirus, and an insert containing a nucleic acid sequence coding for a cytokine, wherein said insert is under the control of a promoter wherein said adenoviral vector is used as an antitumor drug.

2. The adenoviral vector according to claim 1, wherein said vector lacks the transactivator E1A and E1B and the E3 region of the adenovirus.

3. The adenoviral vector according to claim 1, wherein the genomic sequence of the adenovirus lacks its 5′ end region downstream of the early promoter of the E1A region of the adenovirus, and the nucleic acid sequence coding for the cytokine is placed under the control of the early promoter.

4. The adenoviral vector according to claim 1 or claim 2, wherein the nucleic acid sequence coding for the cytokine is placed under the control of the adenovirus late promoter.

5. The adenovirus vector according to claim 1, wherein the genomic sequence of the adenovirus has a heterologous promoter and the nucleic acid sequence coding for the cytokine is placed under control of this heterologous promoter.

6. An adenoviral vector comprising a genomic sequence of an adenovirus which is defective in that it lacks the sequences needed for its replication, but contains those sequences which carry the genetic information needed for the adenovirus to enter cells which are capable of being infected, as well as the essential sequences needed for encapsidation of this adenovirus and comprising a nucleic acid insert containing sequences coding for several

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cytokines, or containing separate nucleic acid inserts coding for different cytokines wherein said nucleic acid inserts are placed under the control of separate promoters and wherein said adenoviral vector is used as an antitumor drug.

7. A defective adenovirus, comprising the genomic and nucleic acid sequences of any one of claims 1 to 6.

8. A cell culture infected with the adenoviral vector according to claim 1.

9. A pharmaceutical composition comprising the defective adenovirus according to claim 1, in combination with a pharmaceutically acceptable vehicle.

10. Use of the defective adenovirus as claimed in claim 9 in a form that can be injected directly into a tumor of the host.

11. The cell culture of claim 8, which is a culture of human cells injectable into humans.

12. A method for the production of defective adenoviruses according to claim 1, comprising the steps of:

(a) transforming cell lines of higher eukaryotes, whose cells contain a separate nucleotide sequence capable of complementing the portion of the adenovirus genome which is lacking in the adenovirus and which is essential for its replication, said separate sequence being incorporated into the genome of the cells of said cell line; and

(b) recovering the defective recombinant adenoviruses produced from the culture medium of the cells of said cell lines.

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13. The method according to claim 12, wherein the genome of the defective adenoviruses lacks its 5′ end region and the cell line is a human embryonic kidney line which contains, integrated in its genome, a 5′ end region of the genome of a type 5 adenovirus (Ad5) having a size corresponding to approximately 11% of that of the whole genome of this adenovirus.

14. The pharmaceutical composition of claim 9, wherein the nucleic acid sequence coding for the cytokine is placed under the control of an adenovirus late promoter.

15. The pharmaceutical composition of claim 9, wherein the genomic sequence of the adenovirus has a heterologous promoter and the nucleic acid sequence coding for the cytokine is placed under the control of said heterologous promoter.

16. The pharmaceutical composition of claim 9, wherein the nucleic acid insert contains sequences coding for several cytokines, or said nucleic acid insert contains separate inserts coding for different cytokines wherein said separate inserts are placed under the control of separate promoters.

17. The pharmaceutical composition of claim 9, which can be injected directly into a tumor of the host.

18. A method for the treatment of tumors in a patient carrying a solid tumor, which method comprises injecting an effective amount of the pharmaceutical composition of claim 9 into said solid tumor.

19. The cell culture of claim 8, which is a culture of human cells injectable into humans.

20. A method for activating an immune system and the defences against viral or parasitic infections or tumor development in a patient which comprises administering systemically to said patient an effective amount of the cell culture of claim 8.

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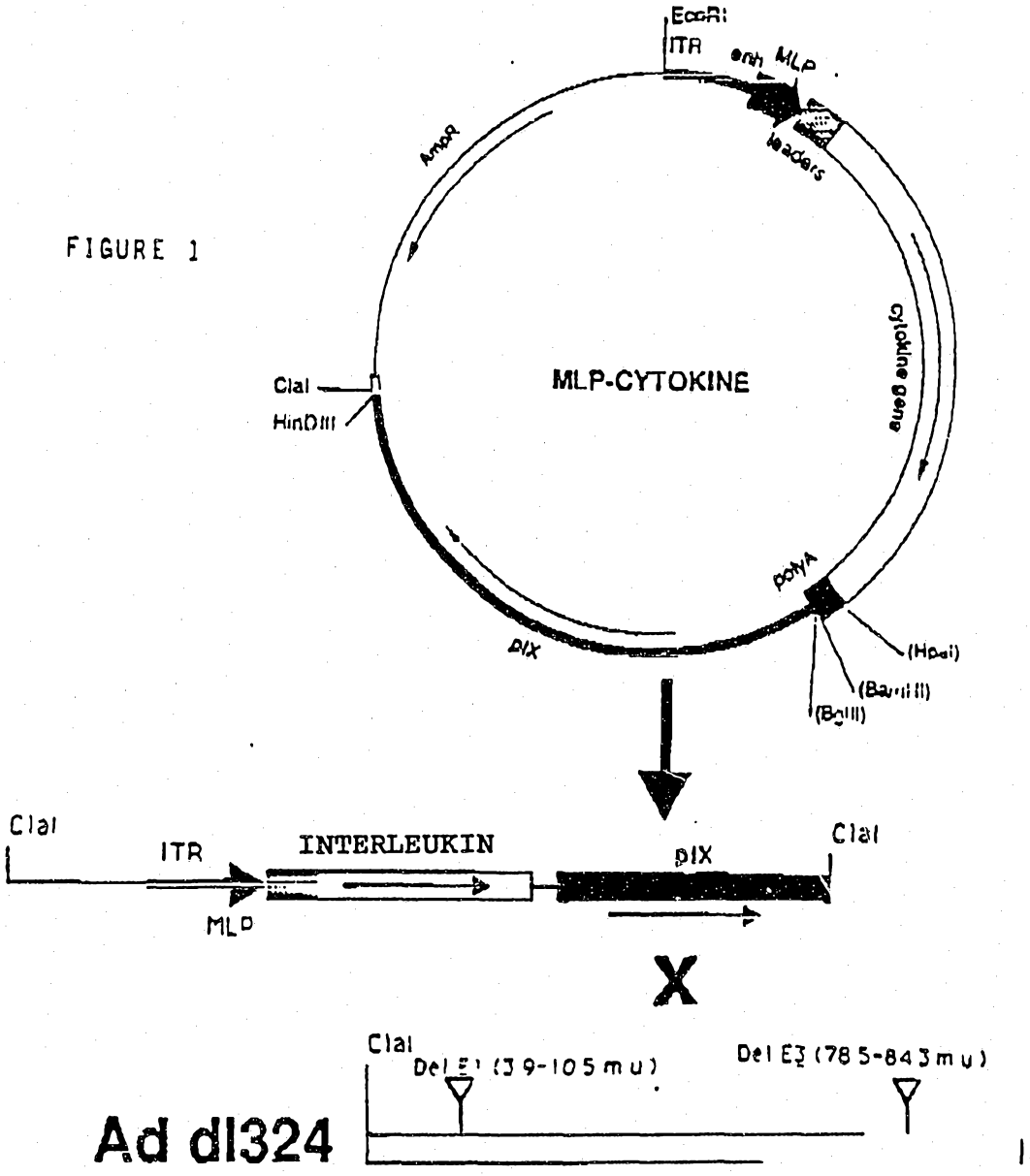
21. A method for the production of recombinant defective adenovirus according to claim 7, comprising the steps of:

(a) transforming a higher eukaryote cell line whose cells contain a separate nucleotide sequence capable of complementing the portion of the adenovirus genome which is lacking in the adenovirus and which is essential for its replication, said separate sequence being incorporated into the genome of the cells of said cell line; and

(b) recovering the defective recombinant adenoviruses produced from the culture medium of the cells of said cell lines.

22. The method according to claim 18, wherein the defective adenovirus is a human embryonic kidney cell line which contains, integrated in its genome, a 5′ end region of the genome of a type 5 adenovirus (Ad5) and having a size corresponding to approximately 11% of that of the whole genome of said adenovirus.

FIGURE 1


EcoRI
ITR
enh MLP
leaders
AmpR
cytokine gene
ClaI
HindIII
MLP-CYTOKINE
pIX
polyA
(HpaI)
(BamHI)
(BglII)
ClaI
ITR
INTERLEUKIN
pIX
ClaI
MLP
X
ClaI
Del E1 (3 9-10 5 m u)
Del E3 (78 5-84 3 m u)
Ad dl324
(after digestion with Cla I)
in vivo recombination
Del E3 (78.5-84.3 m u.)
ITR
pIX
MLP INTERLEUKIN

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FR 93/00264

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl.[5] C12N15/86 A61K48/00; C12N15/19; C12N15/26
C12N5/06; A61K35/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl.[5] C12N ; A61K ; C07K

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 practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,8 800 971 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 11 February 1988	1,7,9
Y	see claims 1-4,6,10	1-10,12, 13

Y	COLLOQUE INSERM (HUMAN GENE TRANSFER. INTERNATIONAL WORKSHOP) Vol. 219, 11 April 1991, PARIS, FRANCE pages 271 - 272 QUANTIN, B. ET AL. "Adenovirus as an expression vector in muscle cells application to dystrophin" see the whole document	1,2,4, 6-10,12, 13

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☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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Date of the actual completion of the international search	Date of mailing of the international search report
12 May 1993 (12.05.93)	25 May 1993 (25.05.93)
Name and mailing address of the ISA/ EUROPEAN PATENT OFFICE	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No
PCT/FR93/00264

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COLLOQUE INSERM (HUMAN GENE TRANSFER, INTERNATIONAL WORKSHOP) Vol. 219, 11 April 1991, PARIS, FRANCE pages 51 - 61 STRATFORD-PERRICAUDET, L. & PERRICAUDET, M. "Gene transfer into animals : the promise of adenovirus" see the whole document	1-10,12 13
Y	EP, A, 0185573 (INSERM) 25 June 1986 (cited in the application) see the whole document	1-10,12, 13
Y	SCIENCE. Vol. 252, No. 5004, 19 April 1991, LANCASTER, PA US pages 431 - 434 ROSENFELD, M.A. ET AL. "Adenovirus-mediated transfer of a recombinant alphal-antitrypsin gene to the lung epithelium in vivo" specially figure 1 see the whole document	1,2,4, 6-10,12, 13
P,X	NUCLEIC ACIDS RESEARCH Vol. 20, No. 9, 11 May 1992, ARLINGTON, VIRGINIA US pages 2233 - 2239 WILKINSON, G.W.G. & AKRIGG, A. "Constitutive and enhanced expression from the CMV major IE promoter in a defective adenovirus vector" see the whole document	1,2, 5-10,12, 13
Y	IMMUNOLOGY TODAY Vol. 11, No. 6, June 1990, CAMBRIDGE GB pages 196 - 200 RUSSELL, S.J. "Lymphokine gene therapy for cancer" see page 197, column 1, line 10 - column 2, line 27	1-10,12, 13
A	see page 196, column 2, line 29 - line 38 see page 199, column 1, line 42 - line 47	11
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. Vol. 87, No. 22, November 1990, WASHINGTON US pages 8746 - 8750 VENKATESH, L.K. ET AL. "Selective induction of toxicity to human cells expressing human immunodeficiency virus type I Tat by a conditionally cytotoxic adenovirus vector" see figure 1	1,2, 5-10,12, 13

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

FR 9300264
SA 71741

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8800971	11-02-88	AU-B- 612983	25-07-91
		AU-A- 7789987	24-02-88
		EP-A- 0275300	27-07-88
		JP-T- 1500755	16-03-89
EP-A-0185573	25-06-86	FR-A- 2573436	23-05-86
		CA-A- 1266627	13-03-90
		DE-A- 3586092	25-06-92
		JP-A- 61158795	18-07-86

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale No PCT/FR 93/00264

I. CLASSEMENT DE L'INVENTION (si plusieurs symboles de classification sont applicables, les indiquer tous) 7

Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

CIB 5 C12N15/86; A61K48/00; C12N15/19; C12N15/26
C12N5/06; A61K35/12

II. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée 8

Système de classification	Symboles de classification
CIB 5	C12N ; A61K ; C07K

Documentation consultée autre que la documentation minimale dans la mesure où de tels documents font partie des domaines sur lesquels la recherche a porté 9

III. DOCUMENTS CONSIDERES COMME PERTINENTS 10

Catégorie °	Identification des documents cités, avec indication, si nécessaire, 12 des passages pertinents 13	No. des revendications visées 14
X	WO,A,8 800 971 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 11 Février 1988	1,7,9
Y	voir revendications 1-4,6,10	1-10,12, 13

Y	COLLOQUE INSERM (HUMAN GENE TRANSFER. INTERNATIONAL WORKSHOP) vol. 219, 11 Avril 1991, PARIS, FRANCE pages 271 - 272 QUANTIN, B. ET AL. 'Adenovirus as an expression vector in muscle cells application to dystrophin' voir le document en entier	1,2,4, 6-10,12, 13

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° Catégories spéciales de documents cités: 11

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document antérieur, mais publié à la date de dépôt international ou après cette date

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"T" document ultérieur publié postérieurement à la date de dépôt international ou à la date de priorité et n'appartenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive

"Y" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant évidente pour une personne du métier.

"&" document qui fait partie de la même famille de brevets

IV. CERTIFICATION

Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
12 MAI 1993	25. 05. 93
Administration chargée de la recherche internationale	**Signature du fonctionnaire autorisé**
OFFICE EUROPEEN DES BREVETS	CHAMBONNET F.J.

III. DOCUMENTS CONSIDERES COMME PERTINENTS [14] (SUITE DES RENSEIGNEMENTS INDIQUES SUR LA DEUXIEME FEUILLE)

Catégorie °	Identification des documents cités, [16] avec indication, si nécessaire des passages pertinents [17]	No. des revendications visées [18]
Y	COLLOQUE INSERM (HUMAN GENE TRANSFER, INTERNATIONAL WORKSHOP) vol. 219, 11 Avril 1991, PARIS, FRANCE pages 51 - 61 STRATFORD-PERRICAUDET, L. & PERRICAUDET, M. 'Gene transfer into animals : the promise of adenovirus' voir le document en entier	1-10,12, 13
Y	EP,A,0 185 573 (INSERM) 25 Juin 1986 cité dans la demande voir le document en entier	1-10,12, 13
Y	SCIENCE. vol. 252, no. 5004, 19 Avril 1991, LANCASTER, PA US pages 431 - 434 ROSENFELD, M.A. ET AL. 'Adenovirus-mediated transfer of a recombinant alpha1-antitrypsin gene to the lung epithelium in vivo' specially figure 1 voir le document en entier	1,2,4, 6-10,12, 13
P,X	NUCLEIC ACIDS RESEARCH vol. 20, no. 9, 11 Mai 1992, ARLINGTON, VIRGINIA US pages 2233 - 2239 WILKINSON, G.W.G. & AKRIGG, A. 'Constitutive and enhanced expression from the CMV major IE promoter in a defective adenovirus vector' voir le document en entier	1,2, 5-10,12, 13
Y	IMMUNOLOGY TODAY vol. 11, no. 6, Juin 1990, CAMBRIDGE GB pages 196 - 200 RUSSELL, S.J. 'Lymphokine gene therapy for cancer' voir page 197, colonne 1, ligne 10 - colonne 2, ligne 27	1-10,12, 13
A	voir page 196, colonne 2, ligne 29 - ligne 38 voir page 199, colonne 1, ligne 42 - ligne 47	11

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III. DOCUMENTS CONSIDERES COMME PERTINENTS [14] (SUITE DES RENSEIGNEMENTS INDIQUES SUR LA DEUXIEME FEUILLE)

Catégorie [15]	Identification des documents cités, [16] avec indication, si nécessaire des passages pertinents [17]	No. des revendications visées [18]
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 87, no. 22, Novembre 1990, WASHINGTON US pages 8746 - 8750 VENKATESH, L.K. ET AL. 'Selective induction of toxicity to human cells expressing human immunodeficiency virus type I Tat by a conditionally cytotoxic adenovirus vector' voir figure 1 ---	1,2, 5-10,12, 13

ANNEXE AU RAPPORT DE RECHERCHE INTERNATIONALE RELATIF A LA DEMANDE INTERNATIONALE NO.

FR 9300264
SA 71741

La présente annexe indique les membres de la famille de brevets relatifs aux documents brevets cités dans le rapport de recherche internationale visé ci-dessus.
Lesdits membres sont contenus au fichier informatique de l'Office européen des brevets à la date du
Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l'Office européen des brevets. 12/05/93

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)		Date de publication
WO-A-8800971	11-02-88	AU-B-	612983	25-07-91
		AU-A-	7789987	24-02-88
		EP-A-	0275300	27-07-88
		JP-T-	1500755	16-03-89
EP-A-0185573	25-06-86	FR-A-	2573436	23-05-86
		CA-A-	1266627	13-03-90
		DE-A-	3586092	25-06-92
		JP-A-	61158795	18-07-86

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