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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

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12 October 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MODULATION OF REPLICATIVE FITNESS BY USING LESS FREQUENTLY USED SYNONYMOUS CODONS

(57) Abstract: Methods of producing a pathogen with reduced replicative fitness are disclosed, as are attenuated pathogens produced using the methods. In particular examples, the method includes deoptimizing one or more codons in a coding sequence, thereby reducing the replicative fitness of the pathogen. Methods of using the attenuated pathogens as immunogenic compositions are also disclosed.



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INTERNATIONAL SEARCH REPORT

In **ional application No**
PCT/US2005/036241

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N5/10

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)
C12N A61K

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)

EPO-Internal, EMBASE, BIOSIS, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KINNEY R M ET AL: "Construction of Infectious cDNA Clones for Dengue 2 Virus: Strain 16681 and Its Attenuated Vaccine Derivative, Strain PDK-53" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 230, no. 2, 14 April 1997 (1997-04-14), pages 300-308, XP004452327 ISSN: 0042-6822 page 303; figures 2-4 ----- -/--	29, 33-35, 48



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
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

2 February 2006

Date of mailing of the international search report

25. 02. 2006

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/036241

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEMM J A ET AL: "MUTATIONS WHICH ALTER THE LEVEL OR STRUCTURE OF NSP4 CAN AFFECT THE EFFICIENCY OF SINDBIS VIRUS REPLICATION IN A HOST-DEPENDENT MANNER" JOURNAL OF VIROLOGY, NEW YORK, US, US, vol. 64, no. 6, June 1990 (1990-06), pages 3001-3011, XP000826714 ISSN: 0022-538X	29, 33-35, 48
Y	page 3007 - page 3008, paragraph 1; figure 1; table 2	30-32, 36, 43, 44
X	----- CHERKASOVA ELENA A ET AL: "Long-term circulation of vaccine-derived poliovirus that causes paralytic disease" JOURNAL OF VIROLOGY, vol. 76, no. 13, July 2002 (2002-07), pages 6791-6799, XP002365441 ISSN: 0022-538X table 2	29, 33-35, 48
Y	----- STENICO MICHELE ET AL: "Codon usage in Caenorhabditis elegans: Delineation of translational selection and mutational biases" NUCLEIC ACIDS RESEARCH, vol. 22, no. 13, 1994, pages 2437-2446, XP002365419 ISSN: 0305-1048 the whole document	30-32, 36, 43, 44
A	----- RAMAKRISHNA LAKSHMI ET AL: "Codon optimization of the Tat antigen of human immunodeficiency virus type 1 generates strong immune responses in mice following genetic immunization" JOURNAL OF VIROLOGY, vol. 78, no. 17, September 2004 (2004-09), pages 9174-9189, XP002365420 ISSN: 0022-538X the whole document	
A	----- WO 02/095363 A (TAO BIOSCIENCES, LLC; MITCHELL, WAYNE; COTA, ADAM; ROBERTS, T., GUY) 28 November 2002 (2002-11-28) the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/036241

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 45-47 and 49 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15 (partly), 27-36 (partly), 43-48 (partly), 49

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15 (partly), 27-36 (partly), 43-48 (partly), 49

Method for reducing replicative fitness of human poliovirus 2 by codon "deoptimization", pharmaceutical compositions containing said attenuated virus and methods of use, sequences ID nos 5, 8, 54-58, 67-69.

2. claims: 1-15 (partly), 27-36 (partly), 43-48 (partly)

Method for reducing replicative fitness of foot and mouth disease virus by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID no 11.

3. claims: 1-15 (partly), 16, 27-36 (partly), 37, 43-48 (partly)

Method for reducing replicative fitness of SARS coronavirus urbani by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID no 14.

4. claims: 1-15(partly), 20, 27-36 (partly), 39, 43-48 (partly)

Method for reducing replicative fitness of Rubella virus by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID no 18.

5. claims: 1-15(partly), 17-19, 27-36 (partly), 38, 43-48 (partly)

Method for reducing replicative fitness of varicella zoster by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID nos 21 and 24.

6. claims: 1-11, 15 , 21-22, 27-35 , 40 , 43-48 (all partly)

Method for reducing replicative fitness of measles virus by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID nos 27 and 30.

7. claims: 1-11, 15, 21, 22, 27-35, 40, 43-48 (all partly)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Method for reducing replicative fitness of human respiratory syncytial virus by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID nos 33 and 36.

8. claims: 1-11 , 21 , 23 , 27-35, 40 , 43-48 (all partly)

Method for reducing replicative fitness of influenza A virus by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID nos 39, 42.

9. claims: 1-11(partly), 15 (partly), 21 (partly), 24-25, 27-35 (partly), 40 (partly), 41, 43-48 (partly)

Method for reducing replicative fitness of HIV-1 by codon "deoptimization", pharmaceutical compositions containing said virus and methods of use, sequence ID no 45.

10. claims: 1-11 (partly), 26, 27-35 (partly), 42, 43-48 (partly)

Method for reducing replicative fitness of E.Coli by codon "deoptimization", pharmaceutical compositions containing said bacteria and methods of use, sequence ID nos 48 and 51.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/036241

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02095363	A	28-11-2002	NONE	