(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2006 (28.12.2006)

PCT

(10) International Publication Number
WO 2006/136448 A3

(51) International Patent Classification:
C12N 7/04 (2006.01) *A61P 11/00* (2006.01)
A61K 39/215 (2006.01)

(21) International Application Number:
PCT/EP2006/006091

(22) International Filing Date: 23 June 2006 (23.06.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
05013727.2 24 June 2005 (24.06.2005) EP

(71) Applicant *(for all designated States except US)*: **CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS** [ES/ES]; Serrano, 117, E-28006 Madrid (ES).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ENJUANES SANCHES, Luis** [ES/ES]; Camino Nuevo, 113, La Moraleja, E-28109 Madrid (ES). **LOPEZ DE DIEGO, Marta** [ES/ES]; C/Castilla la Mancha, n°146, E-28700 San Sebastian de los Reyes, Madrid (ES). **ALVAREZ GOMEZ, Enrique** [ES/ES]; C/Santiago de Compostela, 88 8°G, E-28035 Madrid (ES). **ALMAZAN TORAL, Fernando** [ES/ES]; C/La del Soto del Parral 1, 1A, E-28041 Madrid (ES).

(74) Agents: **VON MENGES, Albrecht** et al.; Uexküll & Stolberg, Beselerstrasse 4, 22607 Hamburg (DE).

(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, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, 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, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
19 July 2007

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: ATTENUATED SARS AND USE AS A VACCINE

(57) Abstract: The present invention relates to nucleic acids encoding attenuated SARS-CoV viruses which are capable of producing a maximum viral titer in cell culture that is reduced at least by a factor of 2 when compared to the maximum viral titer of wild-type SARS-CoV virus in the same cell culture. According to a further aspect of the present invention, the nucleic acids encoding an attenuated SARS-CoV virus, are obtainable by a method comprising steps, wherein the genome of a SARS-CoV virus is modified by amending the sequence of the gene encoding the SARS-CoV E protein so that the nucleic acid cannot express a functional E protein. The present invention further relates to the viruses encoded by these nucleic acids as well as the medical use of the nucleic acids and of the viruses.

WO 2006/136448 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/006091

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/04 A61K39/215 A61P11/00

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 A61P

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, Sequence Search, BIOSIS, EMBASE, MEDLINE, SCISEARCH, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/035712 A (UNIV NORTH CAROLINA [US]; BARIC RALPH S [US]; YOUNT BOYD [US]; CURTIS) 21 April 2005 (2005-04-21) pages 2-3, paragraph BRIDGING pages 3-4 page 19 pages 38-39 pages 40-41	1-33
Y	WO 2004/085633 A (THE UNIVERSITY OF HONG KONG) 7 October 2004 (2004-10-07) pages 20-24; sequence 15	1-3,5, 8-28,32, 33

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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	Date of mailing of the international search report
16 May 2007	01/06/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Domingues, Helena

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& DATABASE Geneseq [Online] 30 December 2004 (2004-12-30), "hSARS virus genome, SEQ ID 15." retrieved from EBI accession no. GSN:ADT36557 Database accession no. ADT36557	
Y	WO 2004/091524 A (ACAMBIS INC; MONATH, THOMAS, P; KLEANTHOUS, HAROLD) 28 October 2004 (2004-10-28) the whole document & DATABASE Geneseq [Online] 13 January 2005 (2005-01-13), "SARS coronavirus Urbani strain complete genome." retrieved from EBI accession no. GSN:ADT08491 Database accession no. ADT08491	1-3, 8-28,32, 33
Y	HUANG Y ET AL: "GENERATION OF SYNTHETIC SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS PSEUDOPARTICLES: IMPLICATIONS FOR ASSEMBLY AND VACCINE PRODUCTION" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 78, no. 22, November 2004 (2004-11), pages 12557-12565, XP009046697 ISSN: 0022-538X the whole document	1-3,5, 8-28, 31-33
Y	KUO LILI ET AL: "The small envelope protein E is not essential for murine coronavirus replication." JOURNAL OF VIROLOGY, vol. 77, no. 8, April 2003 (2003-04), pages 4597-4608, XP002359410 ISSN: 0022-538X the whole document	1-3,5, 8-28, 31-33
Y	HE Y ET AL: "Inactivated SARS-CoV vaccine elicits high titers of spike protein-specific antibodies that block receptor binding and virus entry" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 325, no. 2, 10 December 2004 (2004-12-10), pages 445-452, XP004626814 ISSN: 0006-291X the whole document	1-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHANG C-H ET AL: "Immune responses in Balb/c mice induced by a candidate SARS-CoV inactivated vaccine prepared from F69 strain" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 23, no. 24, 2 May 2005 (2005-05-02), pages 3196-3201, XP004851707 ISSN: 0264-410X the whole document	1-33
Y	CUI F -D ET AL: "Cytokine genetic adjuvant facilitates prophylactic intravascular DNA vaccine against acute and latent herpes simplex virus infection in mice" GENE THERAPY, vol. 12, no. 2, January 2005 (2005-01), pages 160-168, XP002412321 ISSN: 0969-7128 the whole document	14-23
Y	AHLERS J ET AL: "Cytokine-in-adjuvant steering of the immune response phenotype to HIV-1 vaccine constructs: Granulocyte-macrophage colony-stimulating factor and TNF-alpha synergize with IL-12 to enhance induction of cytotoxic T lymphocytes" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 158, 1997, pages 3947-3958, XP002153338 ISSN: 0022-1767 the whole document	14-23
X,P	ALMAZAN FERNANDO ET AL: "Identification of essential genes as a strategy to select a SARS candidate vaccine using a SARS-CoV infectious cDNA" ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY SPRINGER-VERLAG BERLIN, HEIDELBERGER PLATZ 3, D-14197 BERLIN, GERMANY SERIES : ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY (ISSN 0065-2598(PRINT)), 2006, pages 579-583, XP009083756 & 10TH INTERNATIONAL NIDOVIRUS SYMPOSIUM; COLORADO SPRINGS, CO, USA; JUNE 25 -30, 2005 ISSN: 0-387-26202-4(H) the whole document	1-3,5, 8-28, 31-33

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	ROBERTS RHONDA S ET AL: "Renilla Luciferase as a reporter to assess SARS-CoV mRNA transcription regulation and efficacy of anti-SARS-CoV agents" ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY SPRINGER-VERLAG BERLIN, HEIDELBERGER PLATZ 3, D-14197 BERLIN, GERMANY SERIES : ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY (ISSN 0065-2598(PRINT)), 2006, pages 597-600, XP009083865 & 10TH INTERNATIONAL NIDOVIRUS SYMPOSIUM; COLORADO SPRINGS, CO, USA; JUNE 25 -30, 2005 ISSN: 0-387-26202-4(H) the whole document ----	4-33
A	TAN ET AL: "Understanding the accessory viral proteins unique to the severe acute respiratory syndrome (SARS) coronavirus" ANTIVIRAL RESEARCH, ELSEVIER SCIENCE BV., AMSTERDAM, NL, vol. 72, no. 2, November 2006 (2006-11), pages 78-88, XP005647057 ISSN: 0166-3542 the whole document ----	1-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/006091

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:

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.:

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: 1-3, 8-24 (partially), 25-28, 32-33 (partially)

   Concerns a nucleic acid encoding an attenuated SARS-CoV that cannot express a functional E protein (represented by SEQ ID NO: 6) and that is capable of producing a maximum viral titer in cell culture that is reduced at least by a factor of 2 when compared to the maximum viral titer of wild-type SARS-CoV; SARS-CoV particles and vaccines comprising said nucleic acid; and a method for obtaining said vaccines.

   ---

2. claims: 4 (partially), 8-24 (partially), 31-33 (partially)

   Concerns a nucleic acid encoding a SARS-CoV comprising the sequences encoding the viral protein replicase, S, M and N and comprising or not the sequence encoding protein 3a; SARS-CoV particles and vaccines comprising said nucleic acid; and a method for obtaining said vaccines.

   ---

3. claims: 4 (partially), 5, 8-24 (partially), 31-33 (partially)

   Concerns a nucleic acid encoding a SARS-CoV comprising the sequences encoding the viral protein replicase, S, M and N and comprising or not the sequence encoding protein 3a, wherein the nucleic acid does not comprise the sequences encoding the viral proteins E, 6, 7a, 7b, 8a, 8b and 9b; SARS-CoV particles and vaccines comprising said nucleic acid; and a method for obtaining said vaccines.

   ---

4. claims: 6 (partially), 8-24 (partially), 29 (partially), 31-33 (partially)

   Concerns a nucleic acid encoding a SARS-CoV comprising the sequences encoding the viral protein replicase, S, M, N and E and comprising or not the sequence encoding protein 3a; SARS-CoV particles and vaccines comprising said nucleic acid; and a method for obtaining said vaccines.

   ---

5. claims: 6 (partially), 7, 8-24 (partially), 29 (partially), 30, 31-33 (partially)

   Concerns a nucleic acid encoding a SARS-CoV comprising the sequences encoding the viral protein replicase, S, M, N and E and comprising or not the sequence encoding protein 3a; wherein the nucleic acid does not comprise the sequences encoding the viral proteins 6, 7a, 7b, 8a, 8b and 9b; SARS-CoV particles and vaccines comprising said nucleic acid; and a method for obtaining said vaccines.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/006091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005035712 A	21-04-2005	NONE	
WO 2004085633 A	07-10-2004	NONE	
WO 2004091524 A	28-10-2004	NONE	