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(54) Title: REPLICATION DEFICIENT INFLUENZA VIRUS FOR THE EXPRESSION OF HETEROLOGOUS SEQUENCES

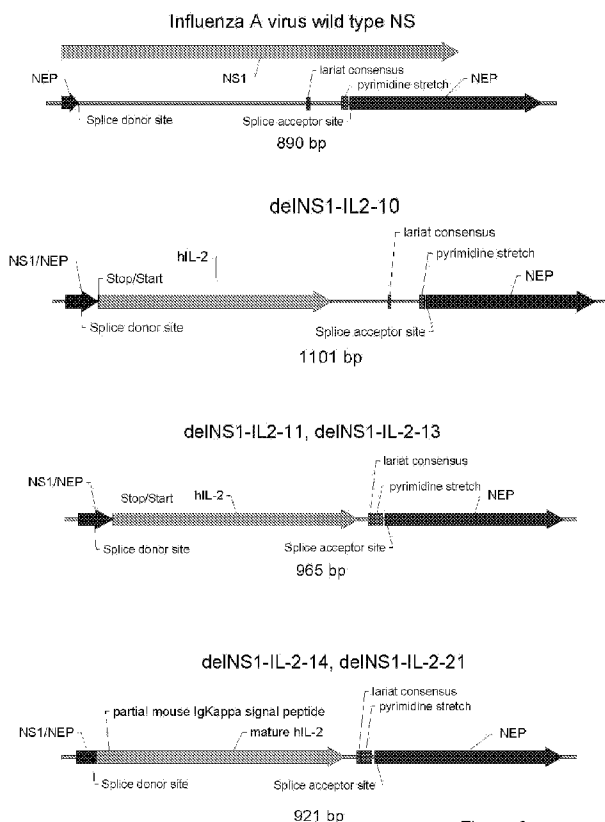


Figure 2

(57) Abstract: The present invention covers a novel replication deficient influenza virus comprising a modified NS1 segment coding for a NS1 protein lacking a functional RNA binding domain and functional effector domain and a heterologous sequence inserted between the splice donor site and the splice acceptor site of the NS gene segment. Further the use of the virus as vector for expression of various proteins like chemokines, cytokines or antigenic structures is covered, methods for producing virus particles using said virus vector as well as its use for production of vaccines. Also a fusion peptide comprising part of the N-terminus of an NS1 protein and a signal sequence fused to the C-terminus of said NS1 peptide is covered.

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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, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/86 C12N15/62 C07K14/11 A61K39/00 A61K39/145

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)

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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, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/016715 A (GREENHILLS BIOTECHNOLOGY RES D [AT]; SACHET MONIKA [AT]; BERGMANN MICH) 15 February 2007 (2007-02-15) cited in the application	1-3,6,18
Y	page 8, paragraph 5 - page 10, paragraph 2; claim 25 page 13, paragraph 1 - paragraph 3; claim 14 8 9 25 page 11, line 6 - line 23 page 12, paragraph 4 - page 13, paragraph 4 page 14, paragraph 4 - page 15; claims 1-7,9-15,22-26,31 ----- -/--	4,8,9, 26-34



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

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/058154

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2006/088481 A (SINAI SCHOOL MEDICINE [US]; UNIV KENTUCKY [US]; PALESE PETER [US]; GAR) 24 August 2006 (2006-08-24) cited in the application paragraphs [0018], [0019], [0023], [0025] - [0030], [0033], [0039], [0096], [0077], [0082] - [0087], [0102] - [0104], [0178]; claims 1-6, 8-10, 15-18, 21-24, 27-35 -----	1-7, 24-34
A Y	WO 01/64860 A (POLYMU SCIEN IMMUNBIO FORSCH [AT]; FERKO BORIS [AT]; EGOROV ANDRE [A]) 7 September 2001 (2001-09-07) page 9, line 3 - line 16; figures 1,2; example 1 page 11, line 19 - page 12, line 14 page 6, line 2 -----	1-11, 21, 23-34 3, 5-11, 15
X	KITTEL C ET AL: "Rescue of influenza virus expressing GFP from the NS1 reading frame" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 324, no. 1, 20 June 2004 (2004-06-20), pages 67-73, XP004512673 ISSN: 0042-6822 cited in the application	1-3, 6, 8, 26-34
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/058154

C(Continuation).. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FERKO B, KITTEL C, ROMANOVA J, SEREINIG S, KATINGER H, EGOROV A.: "Live attenuated influenza virus expressing human interleukin-2 reveals increased immunogenic potential in young and aged hosts." J VIROL., vol. 80, no. 26, 13 September 2006 (2006-09-13), - December 2006 (2006-12) pages 11621-11627, XP002470601 cited in the application the whole document</p>	1
A	<p>FERKO B, STASAKOVA J, ROMANOVA J, KITTEL C, SEREINIG S, KATINGER H, EGOROV A.: "Immunogenicity and protection efficacy of replication-deficient influenza A viruses with altered NS1 genes" J VIROL., vol. 78, no. 23, December 2004 (2004-12), pages 13037-13045, XP002470602 the whole document</p>	1
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A	<p>WO 99/64068 A (SINAI SCHOOL MEDICINE [US]) 16 December 1999 (1999-12-16) cited in the application page 12, line 7 - page 19, line 21; claims 1-6,21,23-28,45-51; examples 9,10 paragraphs [05.4], [05.5], [06.2]</p>	1-4
A	<p>WANG W ET AL: "The RNA-binding and effector domains of the viral NS1 protein are conserved to different extents among influenza A and B viruses" VIROLOGY, ACADEMIC PRESS, ORLANDO, US, vol. 223, 1996, pages 41-50, XP002978957 ISSN: 0042-6822 the whole document</p>	1

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/058154

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>BERGMANN MICHAEL; ET AL.: "A genetically engineered influenza A virus with ras-dependent oncolytic properties" CANCER RESEARCH, vol. 61, no. 22, 15 November 2001 (2001-11-15), pages 8188-8193, XP002470603 the whole document</p>	27,28
A	<p>TAKASUKA N ET AL: "Intranasal inoculation of a recombinant influenza virus containing exogenous nucleotides in the NS segment induces mucosal immune response against the exogenous gene product in mice" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 20, no. 11-12, 22 February 2002 (2002-02-22), pages 1579-1585, XP004340379 ISSN: 0264-410X cited in the application the whole document</p>	1,25,26
A	<p>STUKOVA MA, SEREINIG S, ZABOLOTNYH NV, FERKO B, KITTEL C, ROMANOVA J, VINOGRADOVA TI, KATINGER H, KISELEV OI, EGOROV A: "Vaccine potential of influenza vectors expressing Mycobacterium tuberculosis ESAT-6 protein." TUBERCULOSIS, vol. 86, no. 3-4, May 2006 (2006-05), pages 236-246, XP002470606 (Edinb). the whole document</p>	1,10,12, 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/058154

Box No. 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:

see FURTHER INFORMATION sheet PCT/ISA/210

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 No. 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 allsearchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers 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.:

see additional sheet

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: -

Present claim 1 relates to a compound which has a given desired property or effect, namely a replication deficient influenza virus having a complete lack of its functional RNA binding and a complete lack of its effector function. However, the description provide support and disclosure in the sense of Article 6 and 5 PCT only for limited examples having the said property or effect and there is no common general knowledge of this kind available to the person skilled in the art. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20).

The search of independent claim 1 and dependent/ related claims 2-34 was consequently restricted to the specifically disclosed replication deficient influenza viruses having the desired property or effect, see examples 1 to 13, and to the broad concept of having deletions in RNA binding domain and effector domain resulting in lack of said corresponding functions.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2)PCT declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-34

a replication deficient influenza virus characterized in that it comprises

a) a modified NS segment coding for a NS1 protein comprising at least one amino acid modification within position 1 to 73 resulting in complete lack of its functional RNA binding domain and at least one amino acid modification within position 74 and the carboxy-terminal aminoacid residue resulting in complete lack of its effector domain and
b) a heterologous sequence between a functional splice donor site and functional splice acceptor site inserted in the NS segment; a combination of at least two such replication deficient influenza viruses comprising at least one biologically active molecule or derivative or fragment thereof and at least one antigenic structure; vaccine comprising such a replication deficient influenza virus, use of such a replication deficient influenza virus for the preparation of a medicament for therapeutic treatment in patients; vector comprising a nucleotide sequence coding for such a replication deficient influenza virus, methods for producing such replication deficient influenza viruses.

2. claims: 35-39

a fusion protein comprising between 10 to 30 amino acid of the N-terminus of an NS1 protein, a heterologous sequence and a signal peptide fused to the C-terminus of said NS1 peptide

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2008/058154

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