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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(in))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

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(54) Title: ENGINEERED RESPIRATORY SYNCYTIAL VIRUSES WITH CONTROL OF CELL-TO-CELL VIRUS TRANSMISSION FOR ENHANCED SAFETY OF LIVE VIRUS VACCINES

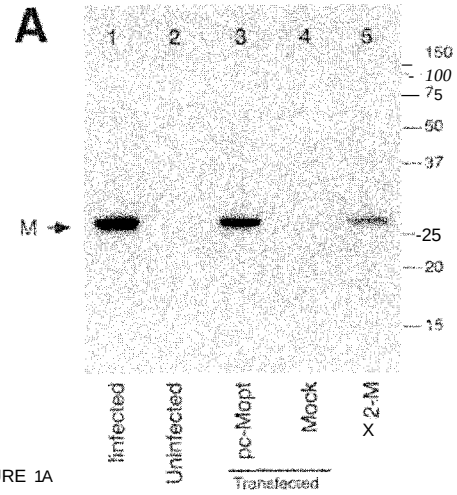

FIGURE 1A

(57) Abstract: Highly antigenic yet safe vaccines against diseases caused by *Paramyxoviridae* viruses such as respiratory syncytial virus (RSV) are provided. The vaccines comprise attenuated *Paramyxoviridae* viruses with high antigenicity but which display impaired cell-to-cell transmission as a result of genetic manipulation of the gene encoding the matrix (M) protein. In the viruses, the M protein is absent or mutated to a less active form. Screening or assay systems and methods for evaluating the infectivity of mutant M proteins anf for identifying suitable M candidates for live-attenuated vaccine virus and VLP production, are also provided.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/040539

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/00 C12Q1/02 G01N33/68
ADD.

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

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)

EPO-Internal , WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 200642 Thomson Scientific, London, GB; AN 2006-406442 XP002690880, & JP 2006 121948 A (KY0RITSU SH0JI KK) 18 May 2006 (2006-05-18) abstract --- -/- ·	1,3,7

[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 application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich 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
24 January 2013	16/05/2013

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, Fax: (+31-70) 340-3016	Sitch , David

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2012/040539

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/04Q539

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:

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 addi tional 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 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 report covers only those claims for which fees were paid, specifically claims Nos. :

4. [X] 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.:

1-9

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

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

1. claims: 1-9

   A matrix protein manipulated (especially deleted) live, attenuated virus, and the use thereof.

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2. claims: 10-29

   A viral replication assessement system comprising a) host cell comprising nucleic acids encoding mutant M protein + control element (= system), and b) an -null virus;
   A method to assess viral replication comprising measuring viral progeny by providing a) of the above system, b) infecting host cell with M null virus, and measuring titre of viral progeny;
   A method to identify M protein mutations that alter infectious virus production comprising providing a) of the above system, b) infecting host cells with M null virus, and measuring viral progeny titre, comparing such to a control titre using system with wild type virus, wherein a difference in titre signifies that the M protein in question is one which alters infectious virus production;
   A method to make a virus having a decreased capacity for virus production by providing a) of the above system, b) infecting host eell] with M null virus, measuring viral progeny titre, comparing such to a control titre using system with wild type virus, wherein if titre is less than that obtained with control virus thereby identifying the M protein in question as one which results in decreased infectious virus production, and then engineering a virus to express said identified M protein.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INOUE M ET AL: "A new Sendai virus vector defi cient in the matri x gene does not form virus parti cles and shows extensi ve cel l-to-cel l spreadi ng" , JOURNAL OF VI ROLOGY, THE AMERICAN SOCI ETY FOR MICROBIOLOGY, US, vol . 77, no. 11, 1 January 2003 (2003-01-01) , pages 6419-6429 , XP002288853 , ISSN: 0022-538X, D0I : 10. 1128/JVI .77.11.6419-6429 .2003 page 6419 abstract	1-3 ,7,8
X	ARMEANU S0RIN ET AL: "Severe impai rment of dendri tic cel l allostimul atory acti vity by Sendai virus vectors is overcome by matri x protei n gene del eti on" , JOURNAL OF IMMUNOLOGY, vol . 175, no. 8, October 2005 (2005-10) , pages 4971-4980, XP002690881 , ISSN: 0022-1767 page 4971 abstract	1-3 ,7,8
X	QINGZH0NG YU ET AL: "Del eti on of the M2-2 gene from avi an metapneumovi rus subgroup C impai rs virus repl i cati on and immunogeni city in Turkeys" , VI RUS GENES, KLUWER ACADEMIC PUBLISHERS, B0, vol . 42, no. 3, 2 February 2011 (2011-02-02) , pages 339-346, XP019914894, ISSN: 1572-994X, D0I : 10. 1007/S11262-011-0577-9 page 339 - page 340, l eft-hand col umn , paragraph 2	1-3 ,7,8
X	WANG C-T ET AL: "CONDITIONAL INFECTI VITY OF A HUMAN IMMUNODEFICI ENCY VI RUS MATRIX DOMAIN DELETION MUTANT", JOURNAL OF VI ROLOGY, THE AMERICAN SOCI ETY FOR MICROBIOLOGY, US, vol . 67, no. 12, 1 December 1993 (1993-12-01) , pages 7067-7076, XP002921434, ISSN: 0022-538X page 7067 - page 7068, paragraph 1	1,3,7
X	WO 02/44334 A2 (AVI RON INC [US]) 6 June 2002 (2002-06-06) page 23, l ine 10 - l ine 29	1-9

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/040539

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	MITRA RUCHIRA ET AL: "The Human Respiratory Syncytial Virus Matrix Protein Is Required for Maturation of Viral Filaments" , JOURNAL OF VIROLOGY, vol . 86, no. 8, April 2012 (2012-04) , pages 4432-4443 , XP002690882 , page 4432 abstract ----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/040539

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