

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
4 January 2007 (04.01.2007)

PCT

(10) International Publication Number  
**WO 2007/002007 A3**(51) International Patent Classification:  
C12N 9/00 (2006.01)(21) International Application Number:  
PCT/US2006/023866

(22) International Filing Date: 20 June 2006 (20.06.2006)

(25) Filing Language: English

(26) Publication Language: English

## (30) Priority Data:

|            |                            |    |
|------------|----------------------------|----|
| 60/692,978 | 21 June 2005 (21.06.2005)  | US |
| 60/692,965 | 21 June 2005 (21.06.2005)  | US |
| 60/699,556 | 15 July 2005 (15.07.2005)  | US |
| 60/699,555 | 15 July 2005 (15.07.2005)  | US |
| 60/702,006 | 22 July 2005 (22.07.2005)  | US |
| 60/793,522 | 19 April 2006 (19.04.2006) | US |
| 60/793,525 | 19 April 2006 (19.04.2006) | US |

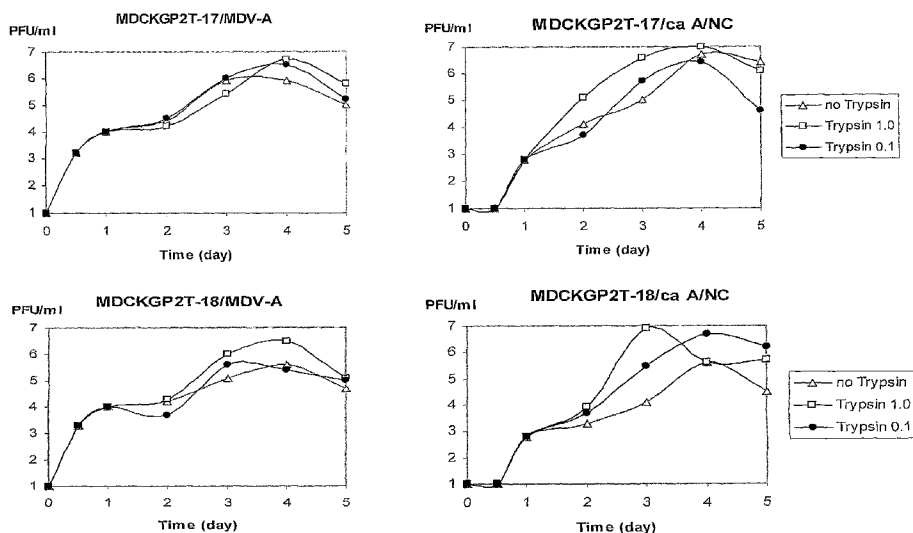
(71) Applicant (for all designated States except US): MED-  
IMMUNE VACCINES, INC. [US/US]; One MedImmune  
Way, Gaithersburg, MD 20878 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): DUKE, Gregory  
[US/US]; 715 Upland Road, Redwood City, CA 94062  
(US). KEMBLE, George [US/US]; 19585 Via GrandeDrive, Saratoga, CA 95070 (US). YOUNG, James  
[US/US]; 9905 River View Court, Potomac, MD 20854  
(US). MO, Chengjun [CN/US]; 102 Lyell Street, Los  
Altos, CA 94022 (US). HAZARI, Nisha [US/US]; 297 N.  
Bernardo Avenue, Mountain View, CA 94043 (US).(74) Agent: KREPPEL, Lisa; MEDIMMUNE VACCINES,  
INC., One MedImmune Way, Gaithersburg, MD 20878  
(US).(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).

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR EXPRESSING A HETEROLOGOUS PROTEASE

**MDCK\_GP2Trypsinogen**(57) Abstract: The present invention provides methods and compositions for expressing a protease or pro-protease in cells that do not naturally express the protease or pro-protease. The invention further provides methods of producing viruses, *e.g.*, influenza viruses, in such cells. The invention also provides methods for increasing the titer of influenza viruses grown in cells that express such a heterologous protease or pro-protease. Additionally, the invention provides a protease from *Streptomyces griseus* useful in the methods and compositions.



**Published:**

— *with international search report*

**Previous Correction:**

see PCT Gazette No. 19/2007 of 10 May 2007

**(88) Date of publication of the international search report:**

21 June 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.*

**(15) Information about Correction:**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/23866

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): C12N 9/00 (2007.01)

USPC: 435/183

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)

USPC: 435/183,212,219,252.3,320.1;536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

<http://scholar.google.com>; Keyword= influenza and protease; serine protease stable expression<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed>; Keyword= influenza and protease; serine protease stable expression

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST: DB=PGPB,USPT,USOC,EPAB,JPAB; terms=serine protease and influenza

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                               | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | US 2005/0019777 A1 (Darrow et al) 27 Jan 2005 (27.01.2005), para [0072]-[0073], [0078], [0086], [0096], [0206]                                                                                                                                                                                                                                                                                                                                                                                   | 1-3, 5-11             |
| X         | US 2003/0171324 A1 (Ramakrishnan) 11 Sep 2003 (11.09.2003), para [0106]-[0107].                                                                                                                                                                                                                                                                                                                                                                                                                  | 1, 4                  |
| X         | US 6,348,327 B1 (Gorman et al) 19 Feb 2002 (19.02.2002), col 2, ln 18; col 14, lns 6-14; col 28, ln 35; col 14, lns 15-48                                                                                                                                                                                                                                                                                                                                                                        | 1, 10                 |
| Y         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 12-18, 21, 22         |
| Y         | Stieneke-Grober et al., Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease, The EMBO Journal 1992, vol. 11, no.7, pages 2407-2414. [online][Retrieved on 2007-01-04]. Retrieved from the internet <URL: <a href="http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=556715&amp;blobtype=pdf">http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=556715&amp;blobtype=pdf</a> >, page 2408, col 1, first paragraph;           | 12-18                 |
| Y         | HORIMOTO et al., Reverse Genetics Provides Direct Evidence for a Correlation of Hemagglutinin Cleavability and Virulence of an Avian Influenza A Virus, JOURNAL OF VIROLOGY, May 1994, Vol. 68, No. 5, pages 3120-3128. [online][Retrieved on 2007-01-04]. Retrieved from the internet <URL: <a href="http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&amp;pubmedid=8151777">http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&amp;pubmedid=8151777</a> >, abstract | 12-18                 |
| Y         | US 5,753,489 A (Kistner et al) 19 May 1998 (19.05.1998), abstract                                                                                                                                                                                                                                                                                                                                                                                                                                | 13, 14                |

☒ Further documents are listed in the continuation of Box C.

\* 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" 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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

06 Jan 2007 (06.01.2007)

Date of mailing of the international search report

28 FEB 2007

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/23866

| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                       |
|-------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                             | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Relevant to claim No. |
| X<br>—<br>Y                                           | Kato et al., <i>Transcriptional Control by A-Factor of Two Trypsin Genes in Streptomyces griseus</i> J Bacteriol. 2005 Jan, vol 187, No. 1, pages 286-295. [online] [Retrieved on 01-05-2007], retrieved from the internet <URL: <a href="http://j.b.asm.org/cgi/reprint/187/1/286">http://j.b.asm.org/cgi/reprint/187/1/286</a> >; nucleotide sequence accession (GenBank): ID: AB182575 GI:49204732; Protein sequence accession: protein_id="BAD24659.1 GI:49204734"; [online], online access at <URL: <a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a> > | 19, 20<br>21, 22      |