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94303 (US).
- (81) **Designated States** (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
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GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
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claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))

- (88) **Date of publication of the international search report:**
5 June 2014

(54) **Title:** METHODS AND COMPOSITIONS FOR DELIVERY OF BIOLOGICS

(57) **Abstract:** Nucleases and methods of using these nucleases for genetic alteration of red blood cells (RBCs), for example for providing for a protein lacking in a monogenic disorder or a biologic for the treatment of exposure to a toxin using genetically altered RBCs.



INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/18 C12N15/85
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 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 practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, 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	<p>RAMSUBIR S ET AL: "In vivo delivery of human acid ceramidase via cord blood transplantation and direct injection of lentivirus as novel treatment approaches for Farber disease", MOLECULAR GENETICS AND METABOLISM, vol. 95, no. 3, 1 November 2008 (2008-11-01), pages 133-141, XP025546364, ACADEMIC PRESS, SAN DIEGO, CA, US ISSN: 1096-7192, DOI: 10.1016/J.YMGME.2008.08.003 [retrieved on 2008-09-20] abstract page 134, left-hand column, paragraph 3 page 138, left-hand column, paragraph 2 - right-hand column, paragraph 2 page 138, right-hand column, paragraph 3 figures 4-5</p> <p style="text-align: center;">-/--</p>	1-13

☒ 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 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

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Date of the actual completion of the international search

9 April 2014

Date of mailing of the international search report

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Name and mailing address of the ISA/

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Surdej, Patrick

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

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>table 1 page 134, left-hand column, paragraph 4 -----</p> <p>D. WANG ET AL: "Reprogramming erythroid cells for lysosomal enzyme production leads to visceral and CNS cross-correction in mice with Hurler syndrome", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 106, no. 47, 24 November 2009 (2009-11-24), pages 19958-19963, XP055088499, ISSN: 0027-8424, DOI: 10.1073/pnas.0908528106 abstract page 19958, left-hand column, last paragraph - right-hand column, paragraph 1 page 19958, right-hand column, last paragraph - page 19959, left-hand column, paragraph 1 page 19959, left-hand column, last paragraph - right-hand column, paragraph 1 page 19961, left-hand column, paragraph 3 - right-hand column, paragraph 1 page 19961, right-hand column, last paragraph - page 19962, left-hand column, paragraph 2 page 19963, right-hand column, paragraph 1 figure 4</p>	1-13
X	<p>-----</p> <p>WO 88/08450 A1 (FINLAYSON BIRDWELL [US]; PECK AMMON BROUGHTON [US]) 3 November 1988 (1988-11-03) page 1, paragraph 1 page 3, line 4 - line 14 page 8, line 2 - line 28 page 23, line 22 - page 24, line 4 page 26, line 31 - page 29, line 18 page 32, line 10 - line 20 page 38, line 18 - line 28 claims 1, 2</p>	1-13
X	<p>-----</p> <p>US 2006/253913 A1 (HUANG YUE-JIN [CA] ET AL) 9 November 2006 (2006-11-09) paragraphs [0002], [0006], [0119] claims 1, 13, 24, 37 -----</p> <p style="text-align: center;">-/--</p>	1-13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VLADIMIR R MUZYKANTOV: "Drug delivery by red blood cells: vascular carriers designed by mother nature", EXPERT OPINION ON DRUG DELIVERY, vol. 7, no. 4, 1 April 2010 (2010-04-01), pages 403-427, XP055088510, ISSN: 1742-5247, DOI: 10.1517/17425241003610633 abstract page 407, paragraph 4 - page 408, paragraph 1 page 410, paragraph 2 - page 411 -----	1-13
Y	WENFANG (SPRING) TAN ET AL: "Chapter Two - Precision Editing of Large Animal Genomes", ADVANCES IN GENETICS, vol. 80, 1 January 2012 (2012-01-01), pages 37-97, XP009170794, ACADEMIC PRESS ISSN: 0065-2660, DOI: 10.1016/B978-0-12-404742-6.00002-8 abstract page 61, last paragraph - page 64, paragraph 1 page 64, paragraph 2 - page 66, paragraph 1 -----	1-13
Y	EIRINI P PAPAPETROU ET AL: "Genomic safe harbors permit high [beta]-globin transgene expression in thalassemia induced pluripotent stem cells", NATURE BIOTECHNOLOGY, vol. 29, no. 1, 1 January 2011 (2011-01-01), pages 73-78, XP055089159, ISSN: 1087-0156, DOI: 10.1038/nbt.1717 abstract page 75, right-hand column, last paragraph - page 76, right-hand column, paragraph 1 figure 3 ----- -/--	1-13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>YIXUAN WANG ET AL: "Genetic correction of [beta]-thalassemia patient-specific iPS cells and its use in improving hemoglobin production in irradiated SCID mice", CELL RESEARCH, vol. 22, no. 4, 7 February 2012 (2012-02-07), pages 637-648, XP055089172, ISSN: 1001-0602, DOI: 10.1038/cr.2012.23 abstract page 638, left-hand column, paragraph 2 page 639, left-hand column, last paragraph - right-hand column, paragraph 1 page 643, right-hand column, paragraph 2 - page 644, left-hand column page 644, right-hand column, paragraph 2 figures 6-7</p>	1-13
X	<p>-----</p> <p>XIAO YAN XHOU ET AL: "MOUSE MODEL FOR THE LYSOSOMAL DISORDER GALACTOSIALIDOSIS AND CORRECTION OF THE PHENOTYPE WITH OVEREXPRESSING ERYTHROID PRECURSORCELLS", GENES AND DEVELOPMENT, vol. 9, no. 21, 1 November 1995 (1995-11-01), pages 2623-2634, XP000564401, COLD SPRING HARBOR LABORATORY PRESS, PLAINVIEW, NY, US ISSN: 0890-9369 abstract page 2624, right-hand column, paragraph 1 page 2631, left-hand column</p>	1-11,14
X	<p>-----</p> <p>T. LEIMIG ET AL: "Functional amelioration of murine galactosialidosis by genetically modified bone marrow hematopoietic progenitor cells", BLOOD, vol. 99, no. 9, 1 May 2002 (2002-05-01), pages 3169-3178, XP055088501, ISSN: 0006-4971, DOI: 10.1182/blood.V99.9.3169 abstract page 3171, right-hand column, last paragraph - page 3172, right-hand column, paragraph 1</p> <p>-----</p> <p>-/--</p>	1-11,14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

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>WO 2010/125471 A2 (SAN RAFFAELE CENTRO FOND [IT]; FOND TELETHON [IT]; BIFFI ALESSANDRA [I]) 4 November 2010 (2010-11-04) page 3, line 9 - line 13 page 14, last paragraph page 34, line 13 - line 18 page 48 - page 70 page 58, left-hand column - page 59 example 2 figures 22-24 claims 1, 11, 12, 18, 19</p>	1-11,14
X	<p>US 2011/294114 A1 (VAN DER LOO JOHANNES CHRISTIAAN MARIA [US] ET AL) 1 December 2011 (2011-12-01) paragraphs [0003], [0008] - [0009], [0026] - [0028], [0030], [0223] - [0227] examples 56-62</p>	1-11,14
X	<p>JOHN A BARRANGER ET AL: "Gene Transfer Approaches to the Lysosomal Storage Disorders", NEUROCHEMICAL RESEARCH, vol. 24, no. 4, 1 April 1999 (1999-04-01), pages 601-615, XP019289468, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NE ISSN: 1573-6903 abstract page 601 page 602, left-hand column, paragraph 3 page 602, left-hand column, last paragraph - right-hand column, paragraph 1 page 603, right-hand column, paragraph 3 - paragraph 5</p>	1-11,14
X	<p>KIM EUN YOUNG ET AL: "Long-term expression of the human glucocerebrosidase gene in vivo after transplantation of bone-marrow-derived cells transformed with a lentivirus vector", JOURNAL OF GENE MEDICINE, vol. 7, no. 7, July 2005 (2005-07), pages 878-887, XP055103722, ISSN: 1099-498X abstract page 886, left-hand column, paragraph 3</p>	1-11,14

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRITTI ANGELA: "Gene therapy for lysosomal storage disorders.", EXPERT OPINION ON BIOLOGICAL THERAPY, vol. 11, no. 9, September 2011 (2011-09), pages 1153-1167, XP009176355, ISSN: 1744-7682 the whole document	1-11,14
X	----- VAN TIL NIEK P ET AL: "Lentiviral gene therapy of murine hematopoietic stem cells ameliorates the Pompe disease phenotype", BLOOD, vol. 115, no. 26, July 2010 (2010-07), pages 5329-5337, XP055103823, abstract page 5329, right-hand column, last paragraph page 5331, right-hand column, last paragraph - page 5333, left-hand column, paragraph 1 page 5333, left-hand column, paragraph 4 - page 5335, right-hand column, paragraph 1 figure 1	1-11,14
X	----- GENTNER BERNHARD ET AL: "Identification of hematopoietic stem cell-specific miRNAs enables gene therapy of globoid cell leukodystrophy.", SCIENCE TRANSLATIONAL MEDICINE, vol. 2, no. 58, 58RA84, 17 November 2010 (2010-11-17), pages 66-76, XP009176430, ISSN: 1946-6242 abstract page 66, right-hand column, paragraph 3 page 71, right-hand column, paragraph 2 - page 74, left-hand column, paragraph 5 page 75, left-hand column, paragraph 2 - paragraph 3 figure 6 ----- -/--	1-11,14

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/050107

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>HOFLING A ALEX ET AL: "Human CD34+ hematopoietic progenitor cell-directed lentiviral-mediated gene therapy in a xenotransplantation model of lysosomal storage disease.", MOLECULAR THERAPY : THE JOURNAL OF THE AMERICAN SOCIETY OF GENE THERAPY, vol. 9, no. 6, June 2004 (2004-06), pages 856-865, XP055103845, ISSN: 1525-0016 abstract page 858, left-hand column, paragraph 2 page 860, right-hand column, last paragraph - page 862, right-hand column, paragraph 1 page 863, right-hand column, paragraph 2 -----</p>	1-11,14
X	<p>BIFFI A ET AL: "Metachromatic leukodystrophy: an overview of current and prospective treatments.", BONE MARROW TRANSPLANTATION, vol. 42 Suppl 2, October 2008 (2008-10), pages S2-S6, XP055103876, ISSN: 1476-5365 abstract page s4, right-hand column, last paragraph - page s5, left-hand column, paragraph 3 -----</p>	1-11,14
X,P	<p>BIFFI ALESSANDRA: "Genetically-modified hematopoietic stem cells and their progeny for widespread and efficient protein delivery to diseased sites: the case of lysosomal storage disorders", CURRENT GENE THERAPY, vol. 12, no. 5, 1 October 2012 (2012-10-01), pages 38-388, XP009176356, NL ISSN: 1875-5631 the whole document -----</p>	1-11,14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/050107

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 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 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. ☐ 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 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.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ US2013/ 050107

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 12, 13(completely); 1-11(partially)

An isolated genetically modified red blood cell (RBC) or RBC precursor cell comprising an integrated transgene that expresses a protein, wherein the protein degrades or detoxifies one or more toxins

2. claims: 14(completely); 1-11(partially)

An isolated genetically modified red blood cell (RBC) or RBC precursor cell comprising an integrated transgene that expresses a protein, wherein the protein is lacking in a lysosomal storage disease

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/050107

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8808450	A1	03-11-1988	NONE
US 2006253913	A1	09-11-2006	NONE
WO 2010125471	A2	04-11-2010	
		AU 2010243276	A1 17-11-2011
		CA 2759438	A1 04-11-2010
		CN 102596255	A 18-07-2012
		EA 201171335	A1 30-05-2012
		EP 2424571	A2 07-03-2012
		JP 2012525141	A 22-10-2012
		KR 20120038403	A 23-04-2012
		SG 175839	A1 29-12-2011
		US 2012128643	A1 24-05-2012
		WO 2010125471	A2 04-11-2010
US 2011294114	A1	01-12-2011	
		US 2011294114	A1 01-12-2011
		US 2013302898	A1 14-11-2013