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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 October 2016 (13.10.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/164609 A3

(51) International Patent Classification:
C12N 7/00 (2006.01) *C12N 5/10* (2006.01)
C12N 15/864 (2006.01) *A61K 48/00* (2006.01)

(21) International Application Number:
PCT/US2016/026486

(22) International Filing Date:
7 April 2016 (07.04.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/144,862 8 April 2015 (08.04.2015) US
62/220,067 17 September 2015 (17.09.2015) US

(71) Applicant: **GENZYME CORPORATION** [US/US]; 500 Kendall Street, Cambridge, MA 02142 (US).

(72) Inventors: **KYOSTIO-MOORE, Sirkka**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-505A, Bridgewater, NJ 08807 (US). **SOUZA, David**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-505A, Bridgewater, NJ 08807 (US). **VINCENT, Karen**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-505A, Bridgewater, NJ 08807 (US).

(74) Agents: **DONAHUE, Brian A.** et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (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, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, 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, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

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))*

(88) Date of publication of the international search report:
15 December 2016

(54) Title: PRODUCTION OF OVERSIZED ADENO-ASSOCIATED VECTORS

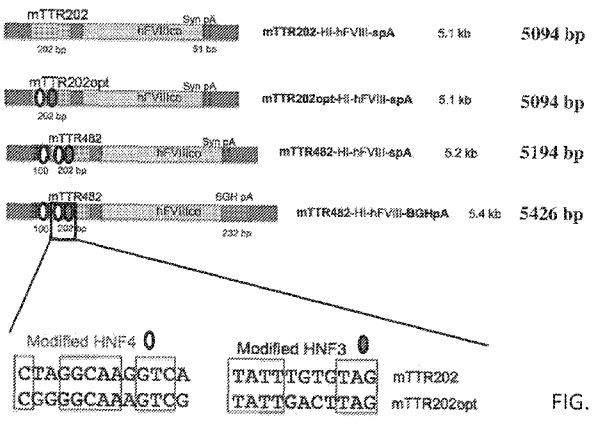

FIG. 1A

(57) Abstract: Provided herein are methods for producing an adeno-associated virus (AAV) particle containing an oversized recombinant AAV genome (e.g., greater than 4.7 kb). In some aspects, the invention provides AAV particles and AAV vectors comprising oversized rAAV genomes. Producer cell lines to produce AAV particles comprising oversized genomes are also provided.

WO 2016/164609 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/026486

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/00 C12N15/864 C12N5/10 A61K48/00
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, Sequence Search, 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	ZHENHUA YUAN ET AL: "A Versatile Adeno-Associated Virus Vector Producer Cell Line Method for Scalable Vector Production of Different Serotypes", HUMAN GENE THERAPY, vol. 22, no. 5, 1 May 2011 (2011-05-01), pages 613-624, XP055312398, US ISSN: 1043-0342, DOI: 10.1089/hum.2010.241 see the section "Creating the 293-based cell line packaging a large-sized gene"; page 621, right-hand column, paragraph 2; figures 1,6; tables 1,2 ----- -/--	1-14, 18-46, 50-84, 88-117, 121-145, 149-159, 168,171, 174

[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" 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
20 October 2016	07/11/2016

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	Brenz Verca, Stefano

1

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.
X	R. SARKAR: "Total correction of hemophilia A mice with canine FVIII using an AAV 8 serotype", BLOOD, vol. 103, no. 4, 23 October 2003 (2003-10-23), pages 1253-1260, XP055312225, US ISSN: 0006-4971, DOI: 10.1182/blood-2003-08-2954 see sections "Single-chain construct" and "Production of AAV vector"; figure 1A -----	36-44, 46, 48-50, 52-58, 60,106, 109-115, 117, 119-129, 131-133, 135-145, 147-149, 151-157, 159,168, 169
X	HUI LU ET AL: "Complete Correction of Hemophilia A with Adeno-Associated Viral Vectors Containing a Full-Size Expression Cassette", HUMAN GENE THERAPY, vol. 19, no. 6, 1 June 2008 (2008-06-01), pages 648-654, XP055311436, US ISSN: 1043-0342, DOI: 10.1089/hum.2007.0182 cited in the application see the whole document, in particular the abstract and the sections "AAV vector plasmid construction" , "AAV vector preparation" and "Construction and packaging of AAV-FVIII vector with large-size genome"; figure 1 -----	36-58, 60,106, 109-129, 131-133, 135-157, 159,160, 162,164, 165, 167-170, 174
X	ZHIJIAN WU ET AL: "Effect of Genome Size on AAV Vector Packaging", MOLECULAR THERAPY, vol. 18, no. 1, 1 January 2010 (2010-01-01), pages 80-86, XP055235504, GB ISSN: 1525-0016, DOI: 10.1038/mt.2009.255 cited in the application the whole document -----	36-38, 40-43, 45,46, 50-58, 60,106, 109, 111-114, 116,117, 121-129, 131-133, 135-137, 139-142, 144,145, 149-157

-/--

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WILLIAM E. GROSE ET AL: "Homologous Recombination Mediates Functional Recovery of Dysferlin Deficiency following AAV5 Gene Transfer", PLOS ONE, vol. 7, no. 6, 15 June 2012 (2012-06-15), page e39233, XP055074203, DOI: 10.1371/journal.pone.0039233 cited in the application see sections "rAAV5.dysferlin gene transfer vector" and Dysferlin gene construction"; figure 1	36-38, 40-45, 48-55, 58,106, 109-117, 119-126, 129,132, 133, 135-137, 139-144, 147-154, 157,168, 172
X	ZIYING YAN ET AL: "Optimization of Recombinant Adeno-Associated Virus-Mediated Expression for Large Transgenes, Using a Synthetic Promoter and Tandem Array Enhancers", HUMAN GENE THERAPY, vol. 26, no. 6, 11 March 2015 (2015-03-11) , pages 334-346, XP055308423, US ISSN: 1043-0342, DOI: 10.1089/hum.2015.001 see section "production of rAAV vectors", "Effective packaging of a functional ferret CFTR minigene into rAAV", items "AV.tg83-fCFTRdeltaR" and "AV.tg83-fCFTRdeltaR(HA)"; figures 3A, 4A	36-38, 40-45, 48-55, 58,106, 109-117, 119-126, 129,132, 133, 135-137, 139-144, 147-154, 157,168, 173
X	WO 2015/038625 A1 (BIOMARIN PHARM INC [US]) 19 March 2015 (2015-03-19) paragraphs [0008], [00145], [00146], [00147], [00173]; claim 6; figures 1-3; examples 1,2,5,6	1-14, 18-46, 50-69, 106-117, 121-145, 149-159, 168,169, 174

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/064277 A1 (UNIV BRUXELLES [BE]) 1 May 2014 (2014-05-01) see Item "AAV9ss-SerpEnh-TTRm-MVM-hFVIIIcopt-SV40pA "; claims 35,37; examples 3,6	36,106, 109-119, 121, 123-126, 129,132, 133, 135-147, 149, 151-154, 157,168, 169
A	WO 01/27303 A1 (UNIV NORTH CAROLINA [US]; WALSH CHRISTOPHER E [US]; CHAO HENGJUN [US];) 19 April 2001 (2001-04-19) figure 2; examples 1, 16	1-174
A	US 2007/042462 A1 (HILDINGER MARKUS [US]) 22 February 2007 (2007-02-22) examples 6-9	1-174
A	US 2004/092008 A1 (SNYDER RICHARD O [US] ET AL) 13 May 2004 (2004-05-13) figures 1-4; examples 1-5	1-174
A	JOHN MARTIN ET AL: "Generation and Characterization of Adeno-Associated Virus Producer Cell Lines for Research and Preclinical Vector Production", HUMAN GENE THERAPY METHODS, vol. 24, no. 4, 1 August 2013 (2013-08-01) , pages 253-269, XP055312388, ISSN: 1946-6536, DOI: 10.1089/hgtb.2013.046 the whole document	1-174
X,P	SIRKKA KYOSTIO-MOORE ET AL: "The impact of minimally oversized adeno-associated viral vectors encoding human factor VIII on vector potency in vivo", MOLECULAR THERAPY - METHODS & CLINICAL DEVELOPMENT, vol. 3, 1 January 2016 (2016-01-01), page 16006, XP055311906, DOI: 10.1038/mtm.2016.6 the whole document	36-69, 106, 109-167

-/--

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/026486

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	NAMBIAR BINDU ET AL: "Evaluation of Producer Cell Line Platform for Production of Oversized AAV-FVIII Vectors", MOLECULAR THERAPY, vol. 24, no. Suppl. 1, May 2016 (2016-05), page S40, XP002763219, & 19TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-GENE-AND-CELL-THERAPY (ASGCT); WASHINGTON, DC, USA; MAY 04 -07, 2016 abstract -----	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/026486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015038625 A1	19-03-2015	AU 2014318890 A1	25-02-2016
		CA 2921232 A1	19-03-2015
		CN 105636981 A	01-06-2016
		EP 3044231 A1	20-07-2016
		KR 20160049015 A	04-05-2016
		SG 11201601932U A	28-04-2016
		TW 201546285 A	16-12-2015
		US 2015071883 A1	12-03-2015
		WO 2015038625 A1	19-03-2015
WO 2014064277 A1	01-05-2014	AU 2013336601 A1	28-05-2015
		CA 2888931 A1	01-05-2014
		JP 2016500519 A	14-01-2016
		WO 2014064277 A1	01-05-2014
WO 0127303 A1	19-04-2001	AU 7879000 A	23-04-2001
		CA 2387484 A1	19-04-2001
		EP 1224312 A1	24-07-2002
		JP 2003511082 A	25-03-2003
		US 2002131956 A1	19-09-2002
		US 2004062752 A1	01-04-2004
		WO 0127303 A1	19-04-2001
US 2007042462 A1	22-02-2007	NONE	
US 2004092008 A1	13-05-2004	NONE	