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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2017 (28.09.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/165245 A3

(51) International Patent Classification:
C12N 15/10 (2006.01) *C07K 14/725* (2006.01)
C12N 15/867 (2006.01) *C07K 14/705* (2006.01)
C12N 5/0783 (2010.01) *C07K 14/715* (2006.01)
C12N 5/10 (2006.01) *A61K 48/00* (2006.01)

(21) International Application Number:
PCT/US2017/023112

(22) International Filing Date:
19 March 2017 (19.03.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/390,093	19 March 2016 (19.03.2016)	US
62/360,041	08 July 2016 (08.07.2016)	US
62/467,039	03 March 2017 (03.03.2017)	US

(71) Applicants: **F1 ONCOLOGY, INC.** [US/US]; 625 N. Flagler Dr., Suite 625, West Palm Beach, Florida 33401 (US). **F1 ONCOLOGY, SEZC**; BritCay House, 236 Eastern Avenue, Grand Cayman, KY1 (KY).

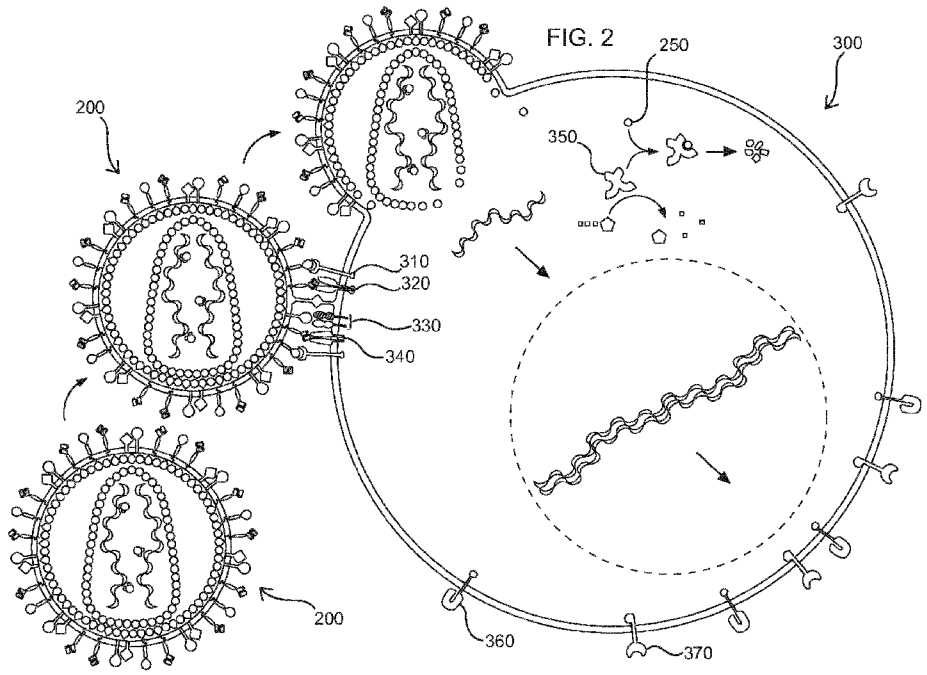
(72) Inventors: **FROST, Gregory Ian**; 505 S Flagler Drive, Suite 900, West Palm Beach, Florida 33401 (US). **ONUFFER, James Joseph**; 1032 Fountain Street, Alameda, California 94501 (US). **GUIBINGA, Ghiabe H.**; 1613 Fort Stockton Drive, San Diego, California 92103 (US). **KUNDU, Anirban**; 229 Boltin Ave. Apt-4, Grand Cayman, KY1 (KY).

(74) Agent: **VACCHIANO, Emanuel**; 8110 Woodside Lane, Burr Ridge, Illinois 60527 (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, DJ, DK, DM, DO,

WO 2017/165245 A3

(54) Title: METHODS AND COMPOSITIONS FOR TRANSDUCING LYMPHOCYTES AND REGULATED EXPANSION THEREOF



(57) Abstract: The present disclosure provides methods for genetically modifying lymphocytes and methods for performing adoptive cellular therapy that include transducing T cells and/or NK cells without prior ex vivo stimulation. The methods typically include engineered signaling polypeptides that can include a lymphoproliferative element, and/or a chimeric antigen receptor (CAR), for example a microenvironment restricted CAR. Additional elements of such engineered signaling polypeptides are provided herein, as well as vectors, such as retroviral vectors, packaging cell lines and methods of making the same. Furthermore, recombinant retroviruses and methods of making the same are provided. Numerous controls are provided, including ribos witches that are controlled, for example in vivo, by nucleoside analogues.

[Continued on next page]

DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, 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).

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:
02 November 2017 (02.11.2017)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/023112

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/10 C12N15/867 C12N5/0783 C12N5/10 C07K14/725
C07K14/705 C07K14/715 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 C07K 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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2016/033331 A1 (SHORT JAY M [US]; BIOATLA LLC [US]) 3 March 2016 (2016-03-03) cited in the application claims 1-17, 35-43	1,69,70, 72-74
Y	S. KONG ET AL: "Suppression of Human Glioma Xenografts with Second-Generation IL13R-Specific Chimeric Antigen Receptor-Modified T Cells", CLINICAL CANCER RESEARCH, vol. 18, no. 21, 1 November 2012 (2012-11-01), pages 5949-5960, XP055242650, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-0319 the whole document	1,69,70, 72-74

-/--

[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
27 June 2017	27/09/2017

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	Hornig, Horst

3

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/023112

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, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of 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/US2017/023112

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.:

1-74

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.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KRISHNAMURTHY ET AL: "Targeting an ancient retrovirus expressed in melanoma using adoptive T- cell therapy", DISSERTATION (, UNIVERSITY OF TEXAS, US , 24 February 2012 (2012-02-24), pages 1-107, XP008182481, Retrieved from the Internet: URL:http://digitalcommons.library.tmc.edu/utgsbs_dissertations/547/ the whole document	1,69,70, 72-74

Y	WO 2014/186469 A2 (UNIV TEXAS [US]) 20 November 2014 (2014-11-20) the whole document	1,69,70, 72-74

X	WO 2013/127964 A1 (HELMHOLTZ ZENTRUM MUENCHEN [DE]; SIRION BIOTECH GMBH [DE]) 6 September 2013 (2013-09-06)	71-74
Y	the whole document	1,69,70

X	BURNS J C ET AL: "VESICULAR STOMATITIS VIRUS G GLYCOPROTEIN PSEUDOTYPED RETROVIRAL VECTORS: CONCENTRATION TO VERY HIGH TITER AND EFFICIENT GENE TRANSFER INTO MAMMALIAN AND NONMAMMALIAN CELLS", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, NATIONAL ACADEMY OF SCIENCES, US, vol. 90, 1 September 1993 (1993-09-01), pages 8033-8037, XP000674727, ISSN: 0027-8424, DOI: 10.1073/PNAS.90.17.8033	71
Y	the whole document	1,69,70, 72-74

X	SHARMA S ET AL: "Efficient infection of a human T-cell line and of human primary peripheral blood leukocytes with a pseudotyped retrovirus vector", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, NATIONAL ACADEMY OF SCIENCES, US, vol. 93, no. 21, 1 January 1996 (1996-01-01), pages 11842-11847, XP002161681, ISSN: 0027-8424, DOI: 10.1073/PNAS.93.21.11842	71-74
Y	the whole document	1,69,70, 72-74

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TILL BRIAN G ET AL: "CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results.", BLOOD, vol. 119, no. 17, 26 April 2012 (2012-04-26), pages 3940-3950, XP002771432, ISSN: 1528-0020 the whole document	1,69,70, 72-74
Y	CHRISTIAN S. HINRICHS ET AL: "Exploiting the curative potential of adoptive T-cell therapy for cancer", IMMUNOLOGICAL REVIEWS., vol. 257, no. 1, 13 January 2014 (2014-01-13), pages 56-71, XP055249662, US ISSN: 0105-2896, DOI: 10.1111/imr.12132 the whole document	1,69,70
Y	O'CONNOR COLLEEN M ET AL: "Adoptive T-cell therapy improves treatment of canine non-Hodgkin lymphoma post chemotherapy", SCIENTIFIC REPORTS,, vol. 2, 1 February 2012 (2012-02-01), XP002763531, ISSN: 2045-2322 the whole document	1,69,70
A	CHRISTIAN BERENS ET AL: "RNA aptamers as genetic control devices: The potential of riboswitches as synthetic elements for regulating gene expression", BIOTECHNOLOGY JOURNAL, vol. 10, no. 2, 1 February 2015 (2015-02-01), pages 246-257, XP055279945, DE ISSN: 1860-6768, DOI: 10.1002/biot.201300498 the whole document	1-74
A	WO 2008/156987 A2 (UNIV YALE [US]; BREAKER RONALD R [US]; WEINBERG ZASHA; SUDARSAN NARASI) 24 December 2008 (2008-12-24) the whole document	1-74
A	WO 2012/153142 A2 (UNIV MANCHESTER [GB]; DIXON NEIL [GB]; MICKLEFIELD JASON [GB]) 15 November 2012 (2012-11-15) the whole document	1-74

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GROHER F ET AL: "Synthetic riboswitches - A tool comes of age", BIOCHIMICA ET BIOPHYSICA ACTA. GENE REGULATORY MECHANISMS, ELSEVIER, AMSTERDAM, NL, vol. 1839, no. 10, Sp. Iss. SI, 1 October 2014 (2014-10-01), pages 964-973, XP002759901, ISSN: 1874-9399, DOI: 10.1016/J.BBAGRM.2014.05.005 [retrieved on 2014-05-17] the whole document	1-74
A	WELZ R ET AL: "Ligand binding and gene control characteristics of tandem riboswitches in Bacillus anthracis", RNA, vol. 13, no. 4, 16 February 2007 (2007-02-16), pages 573-582, XP002615439, COLD SPRING HARBOR LABORATORY PRESS, US ISSN: 1355-8382, DOI: 10.1261/RNA.407707 [retrieved on 2007-02-16] the whole document	1-74
A	DESAI S K ET AL: "Genetic screens and selections for small molecules based on a synthetic riboswitch that activates protein translation", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, US, vol. 126, 1 January 2004 (2004-01-01), pages 13, 247-13, 254, XP002982412, ISSN: 0002-7863, DOI: 10.1021/JA048634J the whole document	1-74
A	VAN-THUAN NGUYEN ET AL: "Multiple GO-SELEX for efficient screening of flexible aptamers", CHEMICAL COMMUNICATIONS - CHEMCOM., vol. 50, no. 72, 23 July 2014 (2014-07-23) , page 10513, XP055319646, ISSN: 1359-7345, DOI: 10.1039/C4CC03953J the whole document	1-74
A	PIKOVSKAYA OLGA ET AL: "Structural principles of nucleoside selectivity in a 2'-deoxyguanosine riboswitch.", NATURE CHEMICAL BIOLOGY, vol. 7, no. 10, 14 August 2011 (2011-08-14), pages 748-755, XP002771433, ISSN: 1552-4469 the whole document	1-74

-/--

3

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LEVAY AGATA ET AL: "Identifying high-affinity aptamer ligands with defined cross-reactivity using high-throughput guided systematic evolution of ligands by exponential enrichment.", NUCLEIC ACIDS RESEARCH, vol. 43, no. 12, E82, 13 July 2015 (2015-07-13), pages 1-10, XP002771434, ISSN: 1362-4962 cited in the application the whole document	1-74
A,P	WO 2017/034615 A1 (BIOATLA LLC [US]) 2 March 2017 (2017-03-02) the whole document	1-74
A,P	WO 2017/011804 A1 (JUNO THERAPEUTICS INC [US]) 19 January 2017 (2017-01-19) the whole document	1-74

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/023112

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016033331 A1	03-03-2016	AU 2015308818 A1	09-03-2017
		CA 2959141 A1	03-03-2016
		CN 107074975 A	18-08-2017
		EP 3186284 A1	05-07-2017
		KR 20170042325 A	18-04-2017
		US 2016207989 A1	21-07-2016
		US 2017260261 A1	14-09-2017
		WO 2016033331 A1	03-03-2016
		WO 2017034615 A1	02-03-2017
WO 2014186469 A2	20-11-2014	AU 2014265487 A1	26-11-2015
		CA 2911961 A1	20-11-2014
		CN 105408473 A	16-03-2016
		EP 2997134 A2	23-03-2016
		HK 1222674 A1	07-07-2017
		JP 2016525881 A	01-09-2016
		KR 20160007653 A	20-01-2016
		US 2016158285 A1	09-06-2016
		US 2017224798 A1	10-08-2017
		WO 2014186469 A2	20-11-2014
WO 2013127964 A1	06-09-2013	EP 2820138 A1	07-01-2015
		JP 2015511483 A	20-04-2015
		US 2015064788 A1	05-03-2015
		WO 2013127964 A1	06-09-2013
WO 2008156987 A2	24-12-2008	AU 2008266684 A1	24-12-2008
		CA 2688575 A1	24-12-2008
		CN 101849020 A	29-09-2010
		EP 2164996 A2	24-03-2010
		EP 2426218 A1	07-03-2012
		EP 2426219 A1	07-03-2012
		JP 2010528617 A	26-08-2010
		KR 20100017905 A	16-02-2010
		SG 174103 A1	29-09-2011
		SG 174104 A1	29-09-2011
		US 2010286082 A1	11-11-2010
		WO 2008156987 A2	24-12-2008
WO 2012153142 A2	15-11-2012	EP 2707487 A2	19-03-2014
		US 2014213779 A1	31-07-2014
		WO 2012153142 A2	15-11-2012
WO 2017034615 A1	02-03-2017	AU 2015308818 A1	09-03-2017
		CA 2959141 A1	03-03-2016
		CN 107074975 A	18-08-2017
		EP 3186284 A1	05-07-2017
		KR 20170042325 A	18-04-2017
		US 2016207989 A1	21-07-2016
		US 2017260261 A1	14-09-2017
		WO 2016033331 A1	03-03-2016
		WO 2017034615 A1	02-03-2017
WO 2017011804 A1	19-01-2017	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-74

A method for genetically modifying and expanding lymphocytes of a subject; A method for performing adoptive cell therapy on a subject, by facilitating transducing resting lymphocytes of a subject; A method for transducing resting T cells and/or resting NK cells from isolated blood;

---

2. claims: 75-118

A recombinant retrovirus;

---

3. claims: 119-144

An isolated polynucleotide for regulating expression of a target polynucleotide, comprising: a polynucleotide encoding a target polynucleotide operably linked to a promoter and a riboswitch, wherein the riboswitch comprises: a.) an aptamer domain capable of binding a nucleoside analogue antiviral drug and having reduced binding to guanine or 2'-deoxyguanosine relative to the nucleoside analogue antiviral drug; and b.) a function switching domain capable of regulating expression of the target polynucleotide, wherein binding of the nucleoside analogue by the aptamer domain induces or suppresses the expression regulating activity of the function switching domain, thereby regulating expression of the target polynucleotide;

---

4. claims: 145-173

A method for selecting a microenvironment restricted antigen-specific targeting region, comprising panning a polypeptide display library by: a. subjecting polypeptides of the polypeptide display library to a binding assay under a normal physiological condition and a binding assay under an aberrant condition; and b. selecting a polypeptide which exhibits an increase in binding activity at the aberrant condition compared to the physiological condition, thereby selecting the microenvironment restricted antigen specific targeting region, by d) contacting the polypeptide library under aberrant conditions with a target antigen bound to a solid support, wherein clones expressing polypeptides that bind the target antigen remain bound to the solid support through the target antigen; e) incubating the solid supports with bound polypeptides under physiological conditions; and f) collecting clones that elute from the solid support under the physiological conditions, thereby isolating the microenvironment restricted antigen-specific targeting region;

---

5. claims: 174-190

A chimeric antigen receptor for binding a target antigen comprisng a) a microenvironment restricted antigen-specific targeting region that exhibits an increase in binding to the target antigen; b) a transmembrane domain; and c) an intracellular activating domain.

---

6. claims: 191-255

A retroviral packaging system, comprising: a mammalian cell comprising: a) a first transactivator expressed from a constitutive promoter and capable of binding a first ligand and a first inducible promoter for affecting expression of a nucleic acid sequence operably linked thereto in the presence versus absence of the first ligand; b) a second transactivator capable of binding a second ligand and a second inducible promoter, and affecting expression of a nucleic acid sequence operably linked thereto in the presence versus absence of the second ligand; and c) a packagable RNA genome for a retroviral particle, wherein the first transactivator regulates expression of the second transactivator and a retroviral REV protein, wherein the second transactivator regulates expression of a gag polypeptide, a pol polypeptide, and one or more pseudotyping elements capable of binding to a target cell and facilitating membrane fusion thereto, and wherein the retroviral proteins are derived from a retrovirus; a method for making a retrovirus by using said retroviral packaging system;

---

7. claims: 256-282

A method for genetically modifying and expanding lymphocytes of a subject, using a recombinant retrovirus capable of binding to CD3 and CD28, and comprising a constitutively active IL-7 receptor mutant; wherein expression of the IL-7 receptor mutant is regulated by a riboswitch that binds a nucleoside analog antiviral drug, wherein binding of the nucleoside analog antiviral drug to the riboswitch increases expression of the IL-7 receptor mutant; and C. a polypeptide capable of binding to CD3 and a polypeptide capable of binding to CD28; said recombinant retrovirus per se; Said genetically modified lymphocyte, wherein expression of said first engineered signaling polypeptide and/or said second engineered signaling polypeptide is regulated by a riboswitch that binds a nucleoside analog antiviral drug, wherein binding of the nucleoside analog antiviral drug to the riboswitch increases expression of the IL-7 receptor mutant.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210