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(54) Title: GENETICALLY ENGINEERED CELLS AND METHODS OF MAKING THE SAME

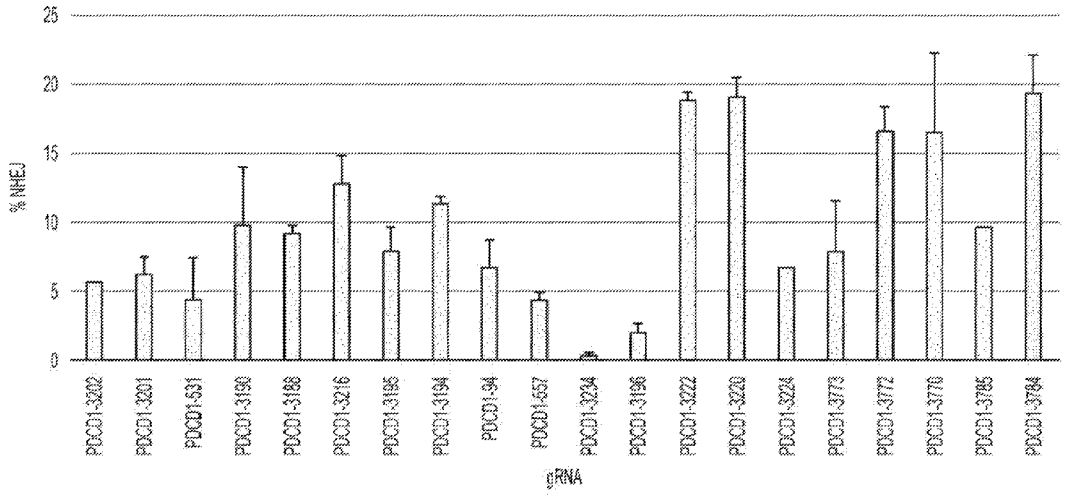

FIG. 15

(57) Abstract: Provided are CRIS PR/CAS -related methods, compositions and components for editing a target nucleic acid sequence, or modulating expression of a target nucleic acid sequence, and applications thereof in connection with cancer immunotherapy comprising adoptive transfer of engineered T cells or T cell precursors.

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[Continued on next page]

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Declarations under Rule 4.17:

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

Published:

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— *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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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/031464

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/725 C12N9/22 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)
C07K 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,P	LEVI J. RUPP ET AL: "CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells", SCIENTIFIC REPORTS, vol. 7, no. 1, 7 April 2017 (2017-04-07), XP055387393, DOI: 10.1038/s41598-017-00462-8 Discussion and material and methods ----- -/--	162-231, 381,384, 385

[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
12 September 2017	17/11/2017

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/031464

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.:

162-231(completely); 381, 384, 385(partially)

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: 162-231(completely); 381, 384, 385(partially)

A method of altering a T cell comprising contacting the T cell with one or more Cas9 molecule/gRNA molecule complexes, wherein the gRNA molecule(s) in the one or more Cas9 molecule/gRNA molecule complexes comprise a targeting domain which is complementary with a target domain from the PDCD1 gene, the corresponding T cell and methods of treatment related to said T cell.

2-63. claims: 1-161(partially)

Said invention relates to A composition, comprising (a) an engineered immune cell comprising a recombinant receptor that specifically binds to one of the antigens selected from Her2, Ll-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, FBP, fetal acethycholine receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, Ll-cell adhesion molecule (CD171), MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, MAGE A3, CE7, Wilms Tumor 1 (WT-1), cyclin A1 (CCNA1), BCMA and interleukin 12, repectively ; and (b) an agent capable of inducing a genetic disruption of a PDCD7 gene encoding a PD-1 polypeptide, wherein said agent is capable of inducing said genetic disruption in, and/or preventing or reducing PD-1 expression in, at least 70 %, at least 75 %, at least 80 %, or at least or greater than 90 % of the cells in the composition and/or at least 70 %, at least 75 %, at least 80 %, or at least or greater than 90 % of the cells in the composition that express the recombinant receptor.

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64. claims: 1-161(partially)

Said invention relates to A composition, comprising (a) an engineered immune cell comprising a recombinant receptor that specifically binds to one of the antigens selected from Her2, Ll-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHa2, ErbB2, ErbB3, ErbB4, FBP, fetal acethycholine receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, Lewis Y, Ll-cell adhesion molecule (CD171), MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, estrogen receptor,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

progesterone receptor, ephrinB2, CD123, CS-1, c-Met, GD-2, MAGE A3, CE7, Wilms Tumor 1 (WT-1), cyclin A1 (CCNA1), BCMA and interleukin 12, repectively ; and (b) an agent capable of inducing a genetic disruption of a PDCD7 gene encoding a PD-1 polypeptide, wherein said agent is capable of inducing said genetic disruption in, and/or preventing or reducing PD-1 expression in, at least 70 %, at least 75 %, at least 80 %, or at least or greater than 90 % of the cells in the composition and/or at least 70 %, at least 75 %, at least 80 %, or at least or greater than 90 % of the cells in the composition that express the recombinant receptor.

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65. claims: 232-297, 382, 383, 386(completely); 384, 385(partially)

A Cas9 molecule/gRNA molecule complex, wherein the gRNA molecule comprises a targeting domain which is complementary with a target domain from the PDCD1 gene, and the gRNA molecule is modified at its 5' end and/or comprises a 3' polyA tail. Said invention also relates to a composition containing at least two Cas9 molecule/gRNA complexes, each complex comprising a gRNA molecule comprising a targeting domain that is complementary to a target domain from a PDCD 1 gene. Further said invention relates to a Tcell comprising such molecules as well as treatment methods related to said molecules.

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66. claims: 298-325

A gRNA molecule that comprises a targeting domain which is complementary with a target domain from the PDCD7 gene, wherein the gRNA molecule is modified at its 5' end and/or comprises a 3' polyA tail.

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67. claims: 326-380(completely); 381(partially)

A method of making a cell for implantation, comprising contacting the cell with one or more Cas9 molecule/gRNA molecule complexes, wherein the gRNA molecule(s) in the one or more Cas9 molecule/gRNA molecule complexes comprise a targeting domain which is complementary with a target domain from the PDCD1 gene.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHU SU ET AL: "CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients", SCIENTIFIC REPORTS, vol. 6, 28 January 2016 (2016-01-28), page 20070, XP055267477, DOI: 10.1038/srep20070 p.2, paragraphs 2-3 ;aterial and methods Figure 3	162-167, 188-196
A	WO 2014/186585 A2 (SANGAMO BIOSCIENCES INC [US]) 20 November 2014 (2014-11-20) the whole document	162-231
A	WO 2015/121454 A1 (CELLECTIS [FR]) 20 August 2015 (2015-08-20) the whole document	162-231
X,P	JIANGTAO REN ET AL: "Multiplex Genome Editing to Generate Universal CAR T Cells Resistant to PD1 Inhibition", CLINICAL CANCER RESEARCH, vol. 23, no. 9, 4 November 2016 (2016-11-04), pages 2255-2266, XP055387986, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-16-1300 Discussion	162-231
X	WO 2014/191128 A1 (CELLECTIS [FR]) 4 December 2014 (2014-12-04) claims	162-187, 197-209, 381,384, 385
A	CAROLINA BERGER S ET AL: "Safety of targeting ROR1 for cancer immunotherapy with chimeric antigen receptor-modified T cells in a primate model", JOURNAL FOR IMMUNOTHERAPY OF CANCER, BIOMED CENTRAL LTD, LONDON, UK, vol. 2, no. Suppl 3, 6 November 2014 (2014-11-06), page P3, XP021202590, ISSN: 2051-1426, DOI: 10.1186/2051-1426-2-S3-P3 the whole document	162-231

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KATHRIN SCHUMANN ET AL: "Generation of knock-in primary human T cells using Cas9 ribonucleoproteins", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, vol. 112, no. 33, 27 July 2015 (2015-07-27), pages 10437-10442, XP055277999, US ISSN: 0027-8424, DOI: 10.1073/pnas.1512503112 introduction Discussion -----	162
X	WO 2015/161276 A2 (EDITAS MEDICINE INC [US]) 22 October 2015 (2015-10-22) cited in the application claims sequence 508 table 31 example 7 claims -----	162-231
A	WO 2014/059173 A2 (GREGORY P D) 17 April 2014 (2014-04-17) the whole document -----	162-231

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/031464

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014186585 A2	20-11-2014	AU 2014265331 A1	12-11-2015
		CA 2910489 A1	20-11-2014
		CN 105683376 A	15-06-2016
		EP 2997146 A2	23-03-2016
		HK 1223401 A1	28-07-2017
		JP 2016521975 A	28-07-2016
		US 2015056705 A1	26-02-2015
		US 2016090607 A1	31-03-2016
		WO 2014186585 A2	20-11-2014
WO 2015121454 A1	20-08-2015	AU 2015216875 A1	28-07-2016
		CA 2937711 A1	22-07-2016
		CN 106029875 A	12-10-2016
		EP 3105317 A1	21-12-2016
		JP 2017506636 A	09-03-2017
		KR 20160138404 A	05-12-2016
		WO 2015121454 A1	20-08-2015
WO 2014191128 A1	04-12-2014	AU 2014273490 A1	17-12-2015
		CA 2913830 A1	04-12-2014
		EP 3004337 A1	13-04-2016
		JP 2016525888 A	01-09-2016
		US 2016272999 A1	22-09-2016
		WO 2014191128 A1	04-12-2014
WO 2015161276 A2	22-10-2015	AU 2015247323 A1	10-11-2016
		CA 2945335 A1	22-10-2015
		EP 3132030 A2	22-02-2017
		JP 2017513485 A	01-06-2017
		KR 20170009865 A	25-01-2017
		SG 11201608701Q A	29-11-2016
		US 2017175128 A1	22-06-2017
		WO 2015161276 A2	22-10-2015
WO 2014059173 A2	17-04-2014	AU 2013329186 A1	30-04-2015
		CA 2886684 A1	17-04-2014
		EP 2906684 A2	19-08-2015
		HK 1213016 A1	24-06-2016
		JP 2015531242 A	02-11-2015
		US 2014120622 A1	01-05-2014
		US 2017183420 A1	29-06-2017
		WO 2014059173 A2	17-04-2014