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(54) Title: ENGINEERED LYMPHOCYTE COMPOSITIONS, METHODS AND SYSTEMS

(57) Abstract: The present inventions provides systems and methods to manufacture genetically modified lymphocyte cells via the use of linear DNA expression amplicons, and uses of such genetically modified lymphocyte cells to treat disease. The present invention also provides for the composition of genetically modified lymphocyte cells.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/28205

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 48/00; C12N 5/071; A61K 39/00; C12N 15/00; C12N 5/16; C07K 16/00; C12Q 1/68; H04W 8

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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)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/0158749 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 08 June 2017 (08.06.2017), Abstract, para [0012], [0029], [0031], [0033], [0070], [0110], [0111], [0124], [0138], [0140], [0143], [0155], [0164], [0170], [0176], [0406], and [0422]	1-7
Y	MARTELLA et al., Mammalian Synthetic Biology: Time for Big MACs. ACS Synth Biol. 2016, Vol. 5(10), p. 1040-1049. Abstract; pg 1041, col 1, para 1; pg 1042, col 1, para 1 and last para; pg 1043, col 2, and Fig 1; and pg 1046, col 1, para 2	1-7
Y	US 2017/0016025 A1 (CELLECTIS) 19 January 2017 (19.01.2017), Abstract, para [0007], [0011], [0023], [0076], and [0186]	7
Y(an alternative)	US 2016/0348073 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 01 December 2016 (01.12.2016), Abstract, para [0003]-[0005], [0011], [0078], [0081], [0082], [0154], and [0166]	7
A	NOVOSIADLY et al., High-content molecular profiling of T-cell therapy in oncology. Mol Ther Oncolytics. 2016, Vol. 3: 16009. PDF File: pg 1-6. Entire Documentation especially Abstract; pg 1, col 1, para 1; and pg 3, col 1, para 1	1-7
A	CHEN et al., Linear DNAs Concatemerize in Vivo and Result in Sustained Transgene Expression in Mouse Liver. Mol Ther. 2001, Vol. 3(3), p. 403-10. Entire Documentation especially Abstract; pg 404, col 2, para 3; pg 405, Fig 1; and pg 406, Fig 2	1-7

☒ 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
"D" document cited by the applicant in the international application
"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
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/28205

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YONG et al., Expression of a Chimeric Antigen Receptor in Multiple Leukocyte Lineages in Transgenic Mice. PLoS One. 2015, Vol. 10(10):e0140543. PDF File: pg 1-28. Entire Documentation especially Abstract; pg 4, Fig 1; pg 17, last para; and pg 18, up para	1-7
A	WALSENG et al., A TCR-based Chimeric Antigen Receptor. Sci Rep. 2017, Vol. 7(1):10713. PDF File: pg 1-10. Entire Documentation especially Abstract; pg 1, para 2; pg 2, last para; and pg 3, Fig 1	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/28205

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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, Claims 1-7, directed to an engineered lymphocyte cell.

Group II, claims 8-14, directed to a method for the production of engineered lymphocyte cells.

Group III, claims 15-16, directed to a system for the production of transfected lymphocytes.

Group IV, claims 17-20, directed to a method of treating cancer.

**********Continued in the extra 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 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.:
1-7

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.

Continuation of:
Box No III (unity of invention is lacking)

The inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Feature
Groups I-IV are related as a product (Group I), a method of making the product (Group II), a system for the production of the product (Group III), and a method of using the product (Group IV).

Group II includes the special technical feature of amplifying the template via PCR, and verifying the sequence, not required by Groups I and III-IV.

Group III includes the special technical features of a system comprising a cell sorting apparatus, and a culture storage and expansion device, wherein all aspects of the system are integrated by robotic process automation, not required by Groups I-II and IV.

Group IV includes the special technical feature of treating cancer, not required by Groups I-III.

Common Technical Features
The inventions of Groups I-IV share the technical feature of an engineered lymphocyte cell (Groups IV, transfected lymphocytes);
--the inventions of Groups I-II and IV further share the technical features of an engineered lymphocyte cell comprising: a linear DNA expression amplicon, wherein said amplicon includes an expression cassette for a
CAR, TCR and/or antibody (claim 1); and
--the inventions of Groups II and III further share the technical features of PCR and next generation sequencing (NGS).

However, these shared technical features do not represent a contribution over prior art as being obvious over US 2017/0158749 A1 to BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM (hereinafter 'UNIV_TX'), in view of an article entitled 'Mammalian Synthetic Biology: Time for Big MACs' by MARTELLA et al. (hereinafter 'Martella'; ACS Synth Biol. 2016, Vol. 5(10), p. 1040-1049), and further in view of an article entitled 'High-content molecular profiling of T-cell therapy in oncology' NOVOSIADLY et al. (hereinafter 'Novosiadly'; Mol Ther Oncolytics. 2016, Vol. 3: 16009. PDF File: pg 1-6) as follows:

UNIV_TX discloses an engineered lymphocyte cell (para [0029] - 'T cells can be engineered', wherein 'T cells' are 'lymphocytes', see the claim 2 that follows; para [0012] - 'CAR T cells may comprise random or engineered point mutations in the CAR'; Abstract - 'Chimeric antigen receptors (CARs) and CAR-expressing T cells') comprising:
--a DNA expression sequence, wherein said sequence includes an expression cassette for a CAR, TCR and/or antibody (para [0124] - 'expression cassette including DNA sequences that encode a CAR ... allow for delivery of the DNA encoding a CAR to immune such as T cells'; para [0138] - 'transfecting and re-directing T cells ..."Naked DNA" means DNA encoding a chimeric T-cell receptor (cTCR) contained in an expression cassette or vector in proper orientation for expression'; para [0031] - 'CAR including the scFv sequence of monoclonal antibody'; Abstract - 'Chimeric antigen receptors (CARs) and CAR-expressing T cells').
UNIV_TX further discloses methods of amplification of Chimeric antigen receptor (CAR), a cloned vector and plasmid (para [0170] - 'Cetuximab-derived CAR was cloned into an intermediate vector... and amplified on large-scale as described in generation...was cloned ... place Cetux-CAR under control of a T7 promoter'; Abstract - 'Chimeric antigen receptors (CARs) and CAR-expressing T cells'; para [0164] - 'large scale amplification and purification of plasmid ... were performed'; para [0140] - 'T-cell propagation (survival) ... assessed by: (i) q-PCR using primers specific for the CAR'), which produces amplicons.
UNIV_TX does not specifically teach wherein the 'DNA expression sequence' is 'a linear DNA expression amplicon'. Martella discloses a method of engineering mammalian cells (Abstract - 'engineering and enhancement of mammalian cells'), the method comprising using a linear DNA as an expression vector for transgene delivery (Abstract - 'Mammalian artificial chromosomes (MACs) are functional, add-on chromosomal elements,...deployed as useful gene expression vectors ... MACs are maintained as a single-copy and can accommodate multiple gene expression cassettes of, in theory, unlimited DNA size', wherein 'Mammalian artificial chromosomes (MACs) ...as useful gene expression vectors...unlimited DNA size' comprising 'a linear DNA as an expression vector'; pg 1042, col 2, para 1 - 'linear MAC comprises both centromere and telomeric ends...MACs can hold large amount of DNA with theoretically no upper size limit to the DNA cloned'; pg 1043, col 2 - 'MACs have been built starting from human chromosome elements therefore they are named as human artificial chromosomes (HACs)'; pg 1043, Fig 1) for increasing effective transgene delivery with reduced limitations of traditional vectors (Abstract - 'large DNA constructs-potentially up to chromosome scale-and the effective delivery of these large DNA payloads to the host cell...Mammalian artificial chromosomes (MACs) are functional... deployed as useful gene expression vectors ... MACs are maintained as a single-copy and can accommodate multiple gene expression cassettes of, in theory, unlimited DNA size'; Abstract, Fig; pg 1041, col 1, para 2 - 'advantages of MACs used as a platform for loading and delivery of large synthetic genetic constructs into mammalian cells'; pg 1042, col 2, para 1 - 'linear MAC comprises both centromere and telomeric ends. ... artificial chromosome is therefore stably maintained as a single copy vector without integration into the host chromosomes, allowing quantitative control on number of transgene copies... MACs can hold large amount of DNA with theoretically no upper size limit to the DNA cloned').
Although Martella does not expressively teach wherein the 'linear DNA expression vector' is 'a linear DNA expression amplicon', one of the ordinary skill in the art at the time the invention was made would have known to obtain 'a linear DNA expression vector' that is 'a linear DNA expression amplicon' for obtaining enough amount of the linear DNA expression vector comprising transgenes for engineering selected host cells, based on the combination of Martella and UNIV_TX as well as the common practice in the art, in order to combine methods, knowledge, and common practice available in the art for facilitating obtaining an engineered lymphocyte with desired features with an expected success without undue experimentation.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of UNIV_TX and Martella, to obtain an engineered lymphocyte cell comprising: a DNA expression sequences, wherein said sequences includes an expression cassette for a CAR, TCR and/or antibody, based on the teaching of UNIV_TX, and further wherein the 'DNA expression sequence' is 'a linear DNA expression amplicon', based on the combination of Martella and UNIV_TX as well as the common practice in the art, in order to combine methods, knowledge, and common practice available in the art for facilitating obtaining an engineered lymphocyte with desired features with an expected success without undue experimentation.
**********Continued in the next extra sheet**********

Continuation of:
The previous extra sheet - Box No III (unity of invention is lacking)

Furthermore, UNIV_TX further discloses methods of amplification of Chimeric antigen receptor (CAR), a cloned vector and plasmid using methods comprising PCR (para [0170] - 'Cetuximab-derived CAR was cloned into an intermediate vector... and amplified on large-scale as described in generation...was cloned ... place Cetux-CAR under control of a T7 promoter'; Abstract - 'Chimeric antigen receptors (CARs) and CAR-expressing T cells'; para [0164] - 'large scale amplification and purification of plasmid ... were performed'; para [0140] - 'T-cell propagation (survival) ... assessed by: (i) q-PCR using primers specific for the CAR'), as well as sequencing a gene of interest in T cells (lymphocyte cells) (para [0229] - 'High-Throughput Gene Expression and CDR3 Sequencing'; para [0252] - 'High throughput sequencing of CDR3 regions ... in the TCR.beta. chain in T cells').

The said combination does not specifically teach next generation sequencing (NGS). Novosiadly discloses engineered T cell populations can be successfully monitored by next-generation sequencing (NGS) (pg 3, col 1, para 1 - 'Antigen-specific T-cell populations can be successfully monitored by next-generation sequencing (NGS) and its modification,... an approach that allows for accurate quantification based on unique nucleotide sequences of genetically rearranged TCR...amplifies the hypervariable complementarity-determining region 3 (CDR3) regions of the TCR and employs high-throughput sequencing to characterize thousands of TCR CDR3 chains simultaneously'; pg 1, col 1, para 1 - 'adoptive transfer of ex-vivo expanded T cells, either nonmodified or following genetic engineering to express chimeric antigen receptor (CAR) or T-cell receptor (TCR)').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of UNIV_TX and Martella with that of Novosiadly, to obtain engineered lymphocyte cell comprising: a linear DNA expression amplicon, wherein said amplicon includes an expression cassette for a CAR, TCR and/or antibody, based on the combination of UNIV_TX and Martella, and further wherein the engineered lymphocyte cell is monitored using the methods of PCR and next generation sequencing (NGS), based on the combination of Novosiadly, Martella, and UNIV_TX, in order to combine methods and knowledge available in the art for facilitating obtaining an engineered lymphocyte with desired features with an expected success without undue experimentation.

Without a shared special technical feature, the inventions lack unity with one another.

Groups I-IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note:

I) Claim 1 contains the first appeared abbreviations "CAR, TCR" each without a full name introduced.

II) Claim 8 contains the first appeared abbreviation "NGS" without a full name introduced.

III) Claim 16 is objected to as lacking a proper antecedent basis for the "system" limitation. For the purposes of this ISR, it is assumed that claim 16 depends from claim 15.