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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))

(54) Title: A PLATFORM FOR T LYMPHOCYTE GENOME ENGINEERING AND IN VIVO HIGH-THROUGHPUT SCREENING THEREOF

(57) Abstract: The present invention includes compositions and methods for T cell genome editing and screening in vivo. In certain aspects, the invention includes an sgRNA library for genome-scale mutagenesis.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/27967

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/11, 15/63 (2018.01)
CPC - C12N 15/111, 15/1093, 2310/20, 2330/31, 15/63

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 --- A	US 2016/0168594 A1 (BROAD INSTITUTE, INC.) 16 June 2016 (16.06.2016). Especially para [0165], [0384], sheet 93 fig 56A.	1, 3/1 ----- 2, 3/2, 5/(1,2)
Y --- A	WO 2017/081097 A1 (IFOM FONDAZIONE ISTITUTO FIRCA DI ONCOLOGIA MOLECOLARE) 18 May 2017 (18.05.2017). Especially pg 4 ln 5, pg 7 ln 10.	1, 3/1 ----- 2, 3/2, 5/(1,2)
Y --- A	US 2009/0187997 A1 (STERN et al.) 23 July 2009 (23.07.2009). Especially para [0161], [0262], [0264]	1, 3/1 ----- 2, 3/2, 5/(1,2)
Y --- A	US 2005/0214823 A1 (BLUME et al.) 29 September 2005 (29.09.2005). Especially SEQ ID NO: 474247	3/1 ----- 5/1
A	US 2016/0311908 A1 (MERRIMACK PHARMACEUTICALS, INC.) 27 October 2016 (27.10.2016). Especially SEQ ID NO: 32	5/(1,2)

☐ 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

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/27967

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.: 18
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:
---Go to Extra Sheet for continuation-----

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.:
1-6, 30, 31, (42-45)(in part) limited to Thy1.1 cassette, SEQ ID NO 1 and SEQ ID NO: 129,213 (claims 1, 2, 3, 5)

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/US 18/27967

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. ☒ forming part of the international application as filed:

☒ 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:

GenCore ver 6.4.1 SEQ ID NOs: 1-3, 129213, 129214, 129215

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/27967

continuation of Box III: Observations where Unity of Invention is lacking

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 examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-6, 30, 31, (42-45)(in part) drawn to a vector comprising a 5' LTR (long terminal repeat) and a 3' LTR.

The vector construct will be searched to the extent that the vector comprises the first named cassette, Thy1.1, nucleic acid sequence, SEQ ID NO: 129,213 (claim 2) and the first named sgRNA expression cassette, SEQ ID NO: 1 (claims 3, 5). It is believed that claims 1, 2, 3, 5, read on this first named invention and thus these claims will be searched without fee to the extent that they Thy1.1 cassette, nucleic acid SEQ ID NO: 129,213 and sgRNA expression cassette SEQ ID NO: 1. Additional cassettes, nucleic acid sequences, and sgRNA expression cassettes will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected cassettes, nucleotide sequences, and/or sgRNA expression cassettes. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: the vector comprises cassette mCherry, nucleotide sequence SEQ ID NO: 129,126 and sgRNA expression cassette SEQ ID NO: 129,222 (claims 30, 31, (43,45)(in part)).

Group II+: Claims 7-11, 24-29, drawn to a sgRNA library composition or kit comprising a plurality of vectors, wherein each vector comprises an expression cassette for an sgRNA.

Group II+ will be searched upon payment of additional fee(s). The library or kit composition may be searched, for example, to the extent that the plurality of expression cassettes comprises SEQ ID NOs: 1,2 for an additional fee and election as such. It is believed that claims 7,8, 11(in part), 24-26 read on this exemplary invention. Additional sgRNA libraries or kits will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be library or kit searched/examined. An exemplary election would be library or kit comprises sgRNA expression cassettes SEQ ID NOs: 129,222 and 129,223 (claims 9, 10, 11(in part), 27-29).

Group III: Claims 12-17, 19-23, drawn to performing genome editing and performing screening of a T cell.

Group IV: Claims 32-41, (42-45)(in part), drawn to a vector comprising a 5' ITR (inverted terminal repeat) and a 3' ITR.

The inventions listed as Groups I+, II+, III, 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:

Technical Features:

Group I+ inventions have the special technical feature of a vector composition comprising a vector comprising a 5' LTR and a 3' UTR, not required by Groups II+, III, IV

Group II+ inventions have the special technical feature of a composition of expression cassettes with specific nucleotide sequences, not required by Groups I+, III, IV

Group III has the special technical feature of performing genome editing and performing screening of a T cell in vitro or in vivo, not required by Groups I+, II+, IV.

Group IV has the special technical feature of a vector comprising a 5' ITR and a 3' ITR, not required by Groups I+, II+, III:

Group IV has special technical feature of a SB100x cassette [a Sleeping Beauty transposase cassette], not required by Groups I+, II+, III.

No technical features are shared between the vector nucleic acid sequences and sgRNA cassette sequences of Group I+ and Group II+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+, II+, III, IV were considered to share the technical features of:

1. Groups I+, II+, III, IV share the common technical feature of an expression vector with a sgRNA insert comprising a sgRNA library or kit.
2. Group I+ inventions share the technical feature of claims 1 and 30.
3. Group II+ inventions share the technical feature of claims 7, 9, 24 and 27.

these shared technical features are previously disclosed by US 2014/0357530 A1 to Broad Institute, Inc. (hereinafter "Broad '530"), in view of US 2016/0168594 A1 to Broad Institute, Inc. (hereinafter "Broad '594").

----continued on next sheet----

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As to common technical feature #1, Broad '530 teaches a vector with a sgRNA insert comprising a sgRNA library or kit (claim 1; "1. A genome wide library comprising a plurality of CRISPR-Cas system guide RNAs comprising guide sequences that are capable of targeting a plurality of target sequences in a plurality of genomic loci in a population of eukaryotic cells"; Para [0139]; "FIG. 52 shows a list of U6 reverse primer sequences used to generate U6-guide RNA expression cassettes. Each primer needs to be paired with the U6 forward primer "gcactgagggcctattcccatgattc" to generate amplicons containing U6 and the desired guide RNA").

As to shared technical feature #3, claims 7 and 9, Broad '530 teaches an sgRNA library comprising a plurality of vectors, wherein each vector comprises an expression cassette for an sgRNA comprising a nucleotide sequence (claim 1; "1. A genome wide library comprising a plurality of CRISPR-Cas system guide RNAs comprising guide sequences that are capable of targeting a plurality of target sequences in a plurality of genomic loci in a population of eukaryotic cells"; Para [0139]; "FIG. 52 shows a list of U6 reverse primer sequences used to generate U6-guide RNA expression cassettes. Each primer needs to be paired with the U6 forward primer "gcactgagggcctattcccatgattc" to generate amplicons containing U6 and the desired guide RNA"); para [0261]; "Aspects of the invention relate to bicistronic vectors for chimeric RNA and Cas9. Bicistronic expression vectors for chimeric RNA and Cas9 are preferred. In general, and particularly in this embodiment Cas9 is preferably driven by the CBh promoter. The chimeric RNA may preferably be driven by a U6 promoter").

As to shared technical feature #3, claims 24 and 27, Broad '530 teaches an sgRNA library comprising a plurality of vectors, wherein each vector comprises an expression cassette for an sgRNA comprising a nucleotide sequence (claim 1; para [0139], [0261]). Broad '530 further teaches kits with instructional material for their use (para [0325]; In one aspect, the invention provides kits containing any one or more of the elements disclosed in the above methods and compositions? In some embodiments, the kit includes instructions").

As to shared technical feature #2, claim 1, concerning a vector comprising a 5' long terminal repeat (LTR) sequence, a U6 promoter sequence, a BsmB1 restriction site, an EFS sequence, an sgRNA expression cassette, a Thy1.1 cassette, a 3' LTR sequence and an ampicillin resistance gene sequence (AmpR), Broad '594 teaches a vector comprising a 5' long terminal repeat (LTR) sequence, a U6 promoter sequence, an EFS sequence, an sgRNA expression cassette, a 3' LTR sequence (para [0165]; FIG. 56A-56E shows stable modification of hematopoietic stem cells by a lentiviral CRISPR-Cas delivery system A) Depiction of a lentiviral vector for bi-cistronic expression of the sgRNA from a U6 promoter (U6) and Cas9 from a short EF1a promoter (EFS) with a fluorescent protein marker (eGFP) from a picorna virus derived 2A auto-cleavage site (P2A)"; see sheet 93 fig 56A; Para [0384]; "Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors"). Broad does not specifically teach a BsmB1 restriction site, a Thy1.1 cassette, or an ampicillin resistance marker (AmpR). However, inclusion of a cloning site comprising a restriction site, for example BsmB1, were well known in the art to enable modular specific sgRNAs to be inserted into a vector were well-known in the art, for instance, in constructing a sgRNA library. Further, inclusion of a Thy1.1 cassette could have substituted for the fluorescent protein in the construct taught by Broad '594, because it would have enabled expression of a specific cell surface marker for easy isolation of cells, for instance by FACS analysis, as was common practice in the art. Finally, use of an antibiotic resistance marker, such as AmpR, as a cell selection marker was routine in the art for selecting cells that had been successfully transformed or transfected.

As to shared technical feature #2, claim 30, concerning a vector comprising a 5' long terminal repeat (LTR) sequence, a U6 promoter sequence, a BsmB1 restriction site, an EFS sequence, an sgRNA expression cassette, an mCherry sequence, a 2A peptide, a cOVA sequence, a 3' LTR sequence and an ampicillin resistance gene sequence (AmpR), Broad teaches a vector comprising a 5' long terminal repeat (LTR) sequence, a U6 promoter sequence, an EFS sequence, an sgRNA expression cassette, a 2A peptide, and a 3' LTR sequence (para [0165]; see sheet 93 fig 56A; para [0384]. Broad does not specifically teach a BsmB1 restriction site, a mCherry sequence, a cOVA sequence, or an ampicillin resistance marker (AmpR). However, inclusion of a cloning site comprising a restriction site, for example BsmB1, were well known in the art to enable modular specific sgRNAs to be inserted into a vector were well-known in the art, for instance, in constructing a sgRNA library. Further, inclusion of a mCherry could have substituted for the eGFR fluorescent protein reporter in the construct taught by Broad '594, because it would have served as an alternative reporter group and was common practice in the art. In addition, use of an antibiotic resistance marker, such as AmpR, as a cell selection marker was routine in the art for selecting cells that had been successfully transformed or transfected. Concerning inclusion of a cOVA sequence in the vector, use of an ovalbumin antigenic marker in antigen presenting cells (APCs) was well known in the art as a positive control in T cell systems, for stimulation of T cells specific for the OVA peptide and enabling a means of T cell activation.

As the shared technical features were known in the art at the time of the invention, they cannot be considered common shared technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+, II+, III, IV lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning item 4: Claim 18 is a multiple dependent claim and is not drafted according to the second and third sentences of PCT Rule 6.4(a).