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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))

(54) Title: METHODS AND COMPOSITIONS FOR IMPROVED HOMOLOGY DIRECTED REPAIR

(57) Abstract: The disclosure features methods of increasing repair of a DNA double stranded break (DSB) in a target gene by the homology-directed repair (HDR) pathway. The disclosure also features compositions for use in the methods.



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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/11 C12N15/113 C12N15/10 C12N9/22 A61K31/7088
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, WPI Data, BIOSIS, Sequence Search, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/172775 A1 (CHILDREN'S MEDICAL CENTER CORP [US]) 5 October 2017 (2017-10-05) paragraphs [0160], [0189]; claims 1,2,5,6,26-28,33-38,45-47,49,50 -----	1-155
X	WO 2017/165826 A1 (EDITAS MEDICINE INC [US]) 28 September 2017 (2017-09-28) page 169; claims 1,7,49,50,64,104 ----- -/-	1-155



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

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INTERNATIONAL SEARCH REPORT

International application No

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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.
A	STEPHAN RIESENBERG ET AL: "Targeting repair pathways with small molecules increases precise genome editing in pluripotent stem cells", NATURE COMMUNICATIONS, vol. 9, no. 1, 4 June 2018 (2018-06-04), XP055483367, DOI: 10.1038/s41467-018-04609-7 page 3, left-hand column page 5, right-hand column - page 6, left-hand column -& Stephan Riesenber ET AL: "SUPPLEMENTARY INFORMATION Targeting repair pathways with small molecules increases precise genome editing in pluripotent stem cells Supplementary Figures and Tables", Nature Communications, 4 June 2018 (2018-06-04), XP055734254, Retrieved from the Internet: URL:https://www.nature.com/articles/s41467-018-04609-7#Sec23 [retrieved on 2020-09-28] figures S5, S6 -----	1-155
A	ROBERT F ET AL: "Pharmacological inhibition of DNA-PK stimulates Cas9-mediated genome editing", GENOME MEDICINE,, vol. 7, no. 1, 27 August 2015 (2015-08-27), pages 1-11, XP002769169, ISSN: 1756-994X, DOI: 10.1186/S13073-015-0215-6 figures 1-3 -----	1-155
Y	WO 2017/132746 A1 (GOVERNING COUNCIL UNIV TORONTO [CA]; SINAI HEALTH SYSTEM [CA]) 10 August 2017 (2017-08-10) claims 1,16-23; sequences 2,3,4,9 -----	1-155
Y	WO 2016/073990 A2 (EDITAS MEDICINE INC [US]) 12 May 2016 (2016-05-12) page 30 - page 31; claim 1 pages 413,421 ----- -/--	1-155

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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.
A	MARELLA D CANNY ET AL: "Inhibition of 53BP1 favors homology-dependent DNA repair and increases CRISPR-Cas9 genome-editing efficiency", NATURE BIOTECHNOLOGY, vol. 36, no. 1, 27 November 2017 (2017-11-27), pages 95-102, XP055487471, us ISSN: 1087-0156, DOI: 10.1038/nbt.4021 figures 3,4 -----	1-155
A	PAULSEN BRUNA S ET AL: "Ectopic expression of RAD52 and dn53BP1 improves homology-directed repair during CRISPR-Cas9 genome editing", NATURE BIOMEDICAL ENGINEERING, NATURE PUBLISHING GROUP UK, LONDON, vol. 1, no. 11, 9 October 2017 (2017-10-09), pages 878-888, XP036428862, DOI: 10.1038/S41551-017-0145-2 figures 3,4,6 -----	1-155
A	ANASTASIA LOMOVA ET AL: "Improving Gene Editing Outcomes in Human Hematopoietic Stem and Progenitor Cells by Temporal Control of DNA Repair : Improving Gene Editing Outcomes in Human HSPCs", STEM CELLS (MIAMISBURG), vol. 37, no. 2, 29 October 2018 (2018-10-29), pages 284-294, XP055732479, ISSN: 1066-5099, DOI: 10.1002/stem.2935 figure 1 -----	1-155
A	SCHIROLI GIULIA ET AL: "Precise Gene Editing Preserves Hematopoietic Stem Cell Function following Transient p53-Mediated DNA Damage Response", CELL STEM CELL, vol. 24, no. 4, 21 March 2019 (2019-03-21), page 551, XP085654766, ISSN: 1934-5909, DOI: 10.1016/J.STEM.2019.02.019 figure 6 ----- -/--	1-55

INTERNATIONAL SEARCH REPORT

International application No

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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.
A	DANNER ERIC ET AL: "Control of gene editing by manipulation of DNA repair mechanisms", MAMMALIAN GENOME, SPRINGER NEW YORK LLC, US, vol. 28, no. 7, 3 April 2017 (2017-04-03), pages 262-274, XP036304552, ISSN: 0938-8990, DOI: 10.1007/S00335-017-9688-5 [retrieved on 2017-04-03] page 268; table 1 -----	1-155

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017172775 A1	05-10-2017	US 2020149038 A1 WO 2017172775 A1	14-05-2020 05-10-2017
WO 2017165826 A1	28-09-2017	EP 3433363 A1 US 2019345490 A1 WO 2017165826 A1	30-01-2019 14-11-2019 28-09-2017
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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.:

1-155

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: 1-155

A method for increasing homology directed repair (HDR) of a double-strand break (DSB) in a target gene in a cell, the method comprising contacting a cell with a 53BP1 inhibitor, wherein the cell is a quiescent human cell that is induced to divide, and wherein the DSB is mediated by a site-directed nuclease, thereby increasing HDR of the DSB in the target gene in the cell.

2. claims: 156-228

A method for increasing HDR of a DSB in a target gene in a population of human CD34+ cells, the method comprising contacting the population of human CD34+ cells with an effective amount of an inhibitor of the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) DNA-PKcs inhibitor, and wherein the DSB is mediated by a site-directed nuclease, thereby increasing HDR of the DSB in the target gene in the population of human CD34+ cells.
