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(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, 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.

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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

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))

(88) Date of publication of the international search report:
19 October 2017

(54) Title: COMPOSITIONS FOR ENHANCING TARGETED GENE EDITING AND METHODS OF USE THEREOF

(57) Abstract: Compositions and methods for enhancing targeted gene editing and methods of use thereof are disclosed. In the most preferred embodiments, gene editing is carried out utilizing a gene editing composition such as triplex-forming oligonucleotides, CRISPR, zinc finger nucleases, TALENS, or others, in combination with a gene modification potentiating agent such as stem cell factor (SCF), a CHK1 or ATR inhibitor, or a combination thereof. A particular preferred gene editing composition is triplex-forming peptide nucleic acids (PNAs) substituted at the γ position for increased DNA binding affinity. Nanoparticle compositions for intracellular delivery of the gene editing composition are also provided and particular advantageous for use with in vivo applications.



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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:

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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.:

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.

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/00
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

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, BIOSIS, WPI Data, COMPENDEX, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/133803 A1 (HELIX THERAPEUTICS INC [US]; UNIV YALE [US]; DEL CAMPO JACOB [US]; SCH) 27 October 2011 (2011-10-27) cited in the application pages 7-15	1-29
Y	WO 2010/123983 A1 (UNIV YALE [US]; GLAZER PETER M [US]; DEL CAMPO JACOB [US]) 28 October 2010 (2010-10-28) cited in the application the whole document	1-29
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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

Date of the actual completion of the international search

5 September 2017

Date of mailing of the international search report

15/09/2017

Name and mailing address of the ISA/

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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.
Y	N A MCNEER ET AL: "Systemic delivery of triplex-forming PNA and donor DNA by nanoparticles mediates site-specific genome editing of human hematopoietic cells in vivo", GENE THERAPY, vol. 20, no. 6, 18 October 2012 (2012-10-18), pages 658-669, XP055177307, ISSN: 0969-7128, DOI: 10.1038/gt.2012.82 the whole document	1-29
Y	FAYE A. ROGERS ET AL: "Local Delivery of Gene-Modifying Triplex-Forming Molecules to the Epidermis", JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 133, no. 3, 1 March 2013 (2013-03-01), pages 685-691, XP055376908, US ISSN: 0022-202X, DOI: 10.1038/jid.2012.351 the whole document	1-29
Y	HEIKO KUHN ET AL: "Sequence specificity at targeting double-stranded DNA with a gamma-PNA oligomer modified with guanidinium G-clamp nucleobases", ARTIFICIAL DNA: PNA & XNA, vol. 1, no. 1, 1 July 2010 (2010-07-01), pages 45-53, XP055376683, ISSN: 1949-095X, DOI: 10.4161/adna.1.1.12444 the whole document	1-29
Y	RAMAN BAHAL ET AL: "Nanoparticle for delivery of antisense [gamma]PNA oligomers targeting CCR5", ARTIFICIAL DNA: PNA & XNA, vol. 4, no. 2, 1 April 2013 (2013-04-01), pages 49-57, XP055376890, ISSN: 1949-095X, DOI: 10.4161/adna.25628 the whole document	1-29
Y	BAHAL RAMAN ET AL: "Single-Stranded gamma PNAs for In Vivo Site-Specific Genome Editing via Watson-Crick Recognition", CURRENT GENE THERAPY, BENTHAM SCIENCE PUBLISHERS LTD, NL, vol. 14, no. 5, 1 January 2014 (2014-01-01), pages 331-342, XP008184778, ISSN: 1566-5232, DOI: 10.2174/1566523214666140825154158 the whole document	1-29
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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.
Y	GUPTA ANISHA ET AL: "Nanotechnology for delivery of peptide nucleic acids (PNAs)", JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL, vol. 240, 8 January 2016 (2016-01-08), pages 302-311, XP029759367, ISSN: 0168-3659, DOI: 10.1016/J.JCONREL.2016.01.005 page 305 - page 307	1-29
X,P	RAMAN BAHAL ET AL: "In vivo correction of anaemia in [beta]-thalassemic mice by [gamma]PNA-mediated gene editing with nanoparticle delivery", NATURE COMMUNICATIONS, vol. 7, 26 October 2016 (2016-10-26), page 13304, XP055376685, DOI: 10.1038/ncomms13304 the whole document	1-62,66, 67
X	STEVEN LIN ET AL: "Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery", ELIFE, vol. 3, 15 December 2014 (2014-12-15), page e04766, XP055294945, England DOI: 10.7554/eLife.04766 the whole document	67
A	NATALIA RIVERA-TORRES ET AL: "The Position of DNA Cleavage by TALENs and Cell Synchronization Influences the Frequency of Gene Editing Directed by Single-Stranded Oligonucleotides", PLOS ONE, vol. 9, no. 5, 1 May 2014 (2014-05-01), page e96483, XP055394837, DOI: 10.1371/journal.pone.0096483	1-62,66
A	JINGYUE JIA ET AL: "Bacterial Delivery of TALEN Proteins for Human Genome Editing", PLOS ONE, vol. 9, no. 3, 11 March 2014 (2014-03-11), pages e91547-12, XP055394839, DOI: 10.1371/journal.pone.0091547	1-62,66
A	BRYAN STROUSE ET AL: "Combinatorial gene editing in mammalian cells using ssODNs and TALENs", SCIENTIFIC REPORTS, vol. 4, no. 1, 21 January 2014 (2014-01-21), XP055394840, DOI: 10.1038/srep03791	1-62,66
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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	THOMAS GAJ ET AL: "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering", TRENDS IN BIOTECHNOLOGY, 1 May 2013 (2013-05-01), XP055065263, ISSN: 0167-7799, DOI: 10.1016/j.tibtech.2013.04.004 -----	1-62,66

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 2011133803 A1	27-10-2011	US 2011262406 A1 WO 2011133803 A1	27-10-2011 27-10-2011
WO 2010123983 A1	28-10-2010	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: 1-36, 66

A triplex-forming composition comprising a Hoogsteen-binding PNA segment and a Watson-Crick-binding PNA segment, wherein one or more of the PNA monomers are gamma-PNA monomers, capable of binding or hybridizing to a target region in a cell's genome to induce strand invasion, displacement and triple helix formation among the two PNA segments and the polypurine stretch of the cell's genome.

2. claims: 60-62(completely); 37-59, 67(partially)

A method of modifying the genome of a cell, comprising contacting the cell with:
 - a c-Kit ligand;
 and
 - a "gene editing technology" selected from triplex forming molecules, pseudocomplementary oligonucleotides, a CRISPR system, zinc-finger nucleases, TALENs, small fragment homologous replacement, and intron-encoded meganucleases; insofar as this subject-matter is not already encompassed by invention 1.

3. claims: 63(completely); 37-59, 67(partially)

A method of modifying the genome of a cell, comprising contacting the cell with:
 - an ATR-Chk1 cell cycle checkpoint pathway inhibitor;
 and
 - a "gene editing technology" selected from triplex forming molecules, pseudocomplementary oligonucleotides, a CRISPR system, zinc-finger nucleases, TALENs, small fragment homologous replacement, and intron-encoded meganucleases; insofar as this subject-matter is not already encompassed by inventions 1-2.

4. claims: 64(completely); 37-59, 67(partially)

A method of modifying the genome of a cell, comprising contacting the cell with:
 - a DNA polymerase alpha inhibitor;
 and
 - a "gene editing technology" selected from triplex forming molecules, pseudocomplementary oligonucleotides, a CRISPR system, zinc-finger nucleases, TALENs, small fragment homologous replacement, and intron-encoded meganucleases; insofar as this subject-matter is not already encompassed by inventions 1-3.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5. claims: 65(completely); 37-59, 67(partially)

A method of modifying the genome of a cell, comprising contacting the cell with:

- a HSP90 inhibitor;

and

- a "gene editing technology" selected from triplex forming molecules, pseudocomplementary oligonucleotides, a CRISPR system, zinc-finger nucleases, TALENs, small fragment homologous replacement, and intron-encoded meganucleases; insofar as this subject-matter is not already encompassed by inventions 1-4.
