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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))*

Published:

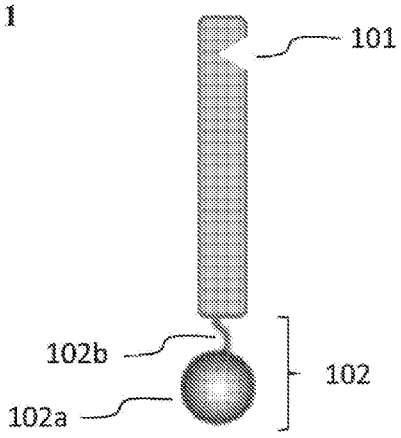
— *with international search report (Art. 21(3))*

— *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:
24 August 2017

(54) Title: CHIMERIC PROTEINS AND METHODS OF REGULATING GENE EXPRESSION

FIGURE 1



(57) Abstract: The present disclosure provides systems, compositions and methods for regulating expression of a target polynucleotide in a cell. The systems, compositions and methods comprise a chimeric receptor polypeptide, a chimeric adaptor polypeptide, at least one actuator moiety and a cleavage moiety.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/12885

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C12N 15/11, 15/90, 9/22 (2017.01)
CPC - C12N 9/22, 15/11, 15/63, 15/111, 15/902, 15/1137

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 2015/0232881 A1 (GLUCKSMANN, A et al.) August 20, 2015; paragraphs [1060], [1073], [1256], [1257], [1289], [1312], [1590], [1674]; claim 16	46-63
Y	WO 2014/018423 A2 (THE BROAD INSTITUTE, INC., et al.) January 30, 2014; paragraphs [00124], [00175], [00213]; claim 1	46-63
Y	WO 2015/139139 A1 (UNIVERSITÉ LAVAL) September 24, 2015; paragraph [00107]	56
Y	US 2004/0197346 A1 (O'HARE, PFJ et al.) October 7, 2004; abstract	57
A	WO 2015/150771 A1 (UCL BUSINESS PLC.) October 8, 2015; abstract; page 6, fourth paragraph; page 13, fourth paragraph; page 15, fourth paragraph; page 21, third paragraph	1-7, 8/6-7, 9-35, 36/34-35, 37-47, 64-70, 71/69-70, 72-84
A	US 2014/0068797 A1 (DOUDNA, JA et al.) March 6, 2014; paragraph [0130]	1-7, 8/6-7, 9-35, 36/34-35, 37-45, 64-70, 71/69-70, 72-84
A	WO 2014/196932 A1 (AGENCY FOR SCIENCE, TECHNOLOGY AND RESEARCH) December 11, 2014; paragraphs [00010], [00033]; claim 5	1-7, 8/6-7, 9-35, 36/34-35, 37-47, 64-70, 71/69-70, 72-84
A	Kopan et al., 'The Canonical Notch Signaling Pathway: Unfolding The Activation Mechanism. Cell, 2009, Vol. 137, No.2, pp216-233; page 216, second column, first paragraph	64-70, 71/69-70, 72-84

☒ 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	Date of mailing of the international search report
08 June 2017 (08.06.2017)	29 JUN 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/12885

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UniProtKB Q01705 (NOTC1_09 December 2015; [retrieved 08 June 2017]. Retrieved from the Internet: <URL: http://www.uniprot.org/uniprot/Q01705#sequences>; pp 1-29; page 21.	64-70, 71/69-70, 72-84

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/12885

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:

-***-Please See Supplemental Page-***-

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, 8/6-7, 9-35, 36/34-35, 37-70, 71/69-70, 72-84

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
Information on patent family members

International application No.
PCT/US17/12885

-***-Continued from Box No. 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 1-84 are directed toward a system and methods for regulating expression of a target polynucleotide in a cell, comprising a chimeric receptor polypeptide comprising a gene modulating polypeptide and a chimeric adaptor polypeptide comprising a cleavage moiety.

Group II, Claims 85-101 are directed toward a system for regulating expression of a target polynucleotide, comprising a chimeric receptor polypeptide comprising a cleavage moiety; and a chimeric adaptor polypeptide comprising a gene modulating polypeptide and a receptor binding moiety.

Group III: Claims 102-114 are directed toward a chimeric receptor polypeptide comprising: (a) an extracellular antigen interacting domain which binds an antigen.

The inventions listed as Groups I-III 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: the special technical features of Group I include a chimeric adaptor polypeptide comprising a cleavage moiety, not present in any other Group; the special technical features of Group II include a chimeric receptor protein comprising a cleavage moiety, not present in any other Group; the special technical features of Group III include an extracellular antigen interacting domain, not present in any other Group.

Groups I-III share the technical features including: a chimeric receptor polypeptide comprising: an antigen interacting domain which binds an antigen; an intracellular gene modulation domain comprising protein; a peptide cleavage domain; wherein upon binding of the antigen interacting domain to the antigen; the receptor polypeptide is modified, wherein receptor modification comprises a conformational change or chemical modification; and wherein cleavage of the peptide cleavage domain occurs. Groups I and III share the technical features including: a chimeric receptor polypeptide comprising an intracellular gene modulation domain; and a relation to SEQ ID NO: 39. Groups I and II share the technical features including: a system for regulating expression of a target polynucleotide; a chimeric adaptor polypeptide; a protease or cleavage moiety; and wherein the gene modulating polypeptide comprises an actuator moiety linked to a peptide cleavage domain, wherein upon cleavage of the peptide cleavage domain or site, the actuator moiety is activated to complex with a target polynucleotide.

However, these shared technical features are previously disclosed by WO 2015/150771 A1 to UCL Business Plc. (hereinafter 'UCL') in view of the article 'The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism' by Kopan et al. (hereinafter 'Kopan') in light of evidence provided by UniProtKB Accession Q01705 (hereinafter 'Q01705').

UCL discloses a chimeric receptor polypeptide (a chimeric receptor polypeptide; abstract) comprising: an antigen interacting domain which binds an antigen (comprising: an antigen interacting domain which binds an antigen; abstract); an intracellular gene modulation domain comprising protein (an intracellular signaling component comprising a signaling component and a dimerization binding domain (an intracellular gene modulation domain comprising protein); abstract); wherein upon binding of the antigen interacting domain to the antigen; the receptor polypeptide is modified, wherein receptor modification comprises a conformational change or chemical modification (wherein binding of the antigen to the antigen binding domain results in signaling (wherein upon binding of the antigen interacting domain to the antigen; the receptor polypeptide is modified, wherein receptor modification comprises a conformational change or chemical modification); page 6, fourth paragraph); a chimeric receptor polypeptide comprising an intracellular gene modulation domain (a chimeric receptor polypeptide comprising an intracellular first dimerization domain that binds to a dimerizing signaling domain (gene modulation domain); abstract; page 13, fourth paragraph; page 15, fourth paragraph); a system for regulating expression of a target polynucleotide (a system for regulating expression of a target polynucleotide; abstract; page 13, fourth paragraph; page 15, fourth paragraph); a chimeric adaptor polypeptide (a polypeptide comprising a second dimerization binding domain (a chimeric adaptor polypeptide); abstract). UCL further discloses wherein the dimerization domains bind to an inducer of dimerization (wherein the dimerization domains bind to an inducer of dimerization; abstract).

UCL does not disclose a peptide cleavage domain; a protease or cleavage moiety; wherein cleavage of the peptide cleavage domain occurs; a chimeric receptor polypeptide comprising an intracellular gene modulation domain; and a relation to SEQ ID NO: 39; and wherein the gene modulating polypeptide comprises an actuator moiety linked to a peptide cleavage domain, wherein upon cleavage of the peptide cleavage domain or site, the actuator moiety is activated to complex with a target polynucleotide.

Kopan discloses wherein Notch signaling occurs by release of an intracellular notch domain from a membrane tether by proteolysis following receptor activation (wherein Notch signaling occurs by release of an intracellular notch domain from a membrane tether by proteolysis following receptor activation; page 216, second column, first paragraph; wherein the intracellular domain of the Notch1 receptor comprises SEQ ID NO: 39 - wherein residues 1427-1752 of the Notch1 receptor sequence are 100% identical to Applicants' SEQ ID NO: 39); and translocation of the intracellular domain to the nucleus to associate with a DNA binding protein and activate target genes (translocation of the intracellular domain to the nucleus to associate with a DNA binding protein and activate target genes; page 216, second column, first paragraph).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of UCL to have used a chimeric adaptor protein, as disclosed by UCL, comprising a protease effective to cleave a gene modulating polypeptide comprising an activator domain, such as the intracellular domain of Notch1, as disclosed by Kopan, in order to effect target gene expression modulation, as disclosed by Kopan, only in the presence of a dimerization inducer, as disclosed by UCL, in order to more effectively control the regulation of gene expression in a subject administered the construct, or cells comprising the construct.

Since none of the special technical features of the Groups I-III inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the UCL and Kopan references, unity of invention is lacking.