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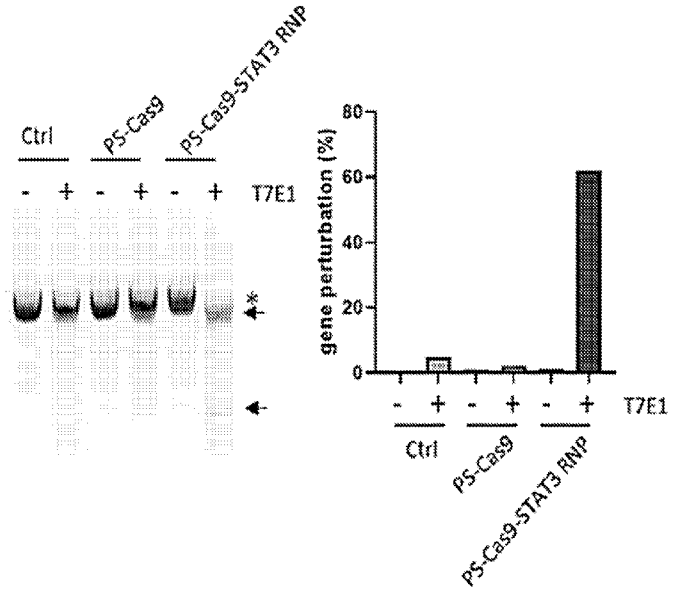
WO 2021/237160 A3

(54) Title: PHOSPHOROTHIOATE NUCLEIC ACID CONJUGATES INCLUDING DNA EDITING ENZYMES

FIG. 17B



(57) Abstract: Provided herein are, *inter alia,* complexes useful for editing (e.g., repairing, modifying) DNA in a cell *in vitro* and *in vivo*. The complexes provided herein include a DNA editing agent bound to a phosphorothioate nucleic acid through a chemical linker. The chemical linker (e.g., disulfide linker) may be a linker that dissociates once the complex has entered the inside of the cell, thereby releasing the DNA editing agent and allowing the DNA editing agent to access and edit a cellular target sequence. The complexes provided herein exhibit high transfection efficiency and editing efficacy and therefore provide for useful therapeutic and diagnostic tools.

[Continued on next page]

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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— *with sequence listing part of description (Rule 5.2(a))*

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/33760

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/90; C12N 9/22; C12P 19/34 (2021.01)

CPC - C12N 15/907; C12N 9/22; C12P 19/34; A61P 35/00; C12N 2310/20; C12N 2310/315

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.
X --- Y --- A	US 2016/0362667 A1 (CARIBOU BIOSCIENCES, INC.) 15 December 2016; paragraphs [0012], [0018], [0021], [0022], [0026], [0044], [0064], [0066], [0068], [0130], [0139], [0142], [0143], [0144], [0145], [0218], [0228]; fig. 10	1-2, 6-15, 19, 69/1, 70/1, 71/70/1 --- 3-5, 16-18, 20, 72/70/1, 73/70/1, 74/70/1 --- 21-43, 69/22, 70/22, 71/70/22, 72/70/22, 73/70/22, 74/70/22
Y	(KUHN, J et al.) Delivery of Cas9/sgRNA Ribonucleoprotein Complexes via Hydroxystearyl Oligoamino Amides. Bioconjugate Chemistry. 18 March 2020, Epub 3 February 2020, Vol. 31, No. 3; pages 729-742; page 736, 2nd column, 2nd paragraph; DOI: 10.1021/acs.bioconjchem.9b00853	3
Y --- A	WO 2009/143345 A2 (UNIVERSITY OF MASSACHUSETTS) 26 November 2009; page 2, lines 15-27; page 3, lines 6-17; page 5, lines 11-13; page 37, lines 7-8	4-5 --- 22-43, 69/22, 70/22, 71/70/22, 72/70/22, 73/70/22, 74/70/22
Y	(VORVIS, C et al.) Transcriptomic and CRISPR/Cas9 technologies reveal FOXA2 as a tumor suppressor gene in pancreatic cancer. American Journal of Physiology. Gastrointestinal and Liver Physiology. 1 June 2016, Epub 5 May 2016, Vol. 310, No. 11; pages G1124-G1137; page G1132, 2nd column, 1st paragraph; fig. 6A; DOI: 10.1152/ajpgi.00035.2016	18, 74/70/1

☒ 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/US21/33760

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	WO 2019/135816 A2 (THE BROAD INSTITUTE, INC., et al.) 11 July 2019; paragraphs [0011], [0018], [0030], [0061], [0062], [0247], [0383], [0385]	16-17, 72/70/1, 73/70/1 --- 21-43, 69/22, 70/22, 71/70/22, 72/70/22, 73/70/22, 74/70/22
Y	(D'AMICO, S et al.) STAT3 is a master regulator of epithelial identity and KRAS-driven tumorigenesis. Genes and Development. 1 September 2018, Epub 22 August 2018, Vol. 32, No.17-18; pages 1175-1187; page 1177, 2nd column, 1st paragraph; page 1184, 2nd column, 2nd paragraph; DOI: 10.1101/gad.311852.118	20
A	(BOULTON, TG et al.) STAT3 activation by cytokines utilizing gp130 and related transducers involves a secondary modification requiring an H7-sensitive kinase. Proceedings of the National Academy of Sciences of the USA. 18 July 1995, Vol. 92, No. 15; pages 6915-6922; pages 6920-6922; DOI: 10.1073/pnas.92.15.6915	21
A	(CALDENHOVEN, E et al.) STAT3 beta, a splice variant of transcription factor STAT3, is a dominant negative regulator of transcription. The Journal of Biological Chemistry. 31 May 1996, Vol. 271, No. 22; pages 13221-13230; pages 13228-13230; fig. 1A; DOI: 10.1074/jbc.271.22.13221	21
A	US 2016/0317671 A1 (CITY OF HOPE) 3 November 2016; abstract; paragraph [0048]	22-43, 69/22, 70/22, 71/70/22, 72/70/22, 73/70/22, 74/70/22
A	(BASILA, M et al.) Minimal 2'-O-methyl phosphorothioate linkage modification pattern of synthetic guide RNAs for increased stability and efficient CRISPR-Cas9 gene editing avoiding cellular toxicity. PLoS One. 27 November 2017, Vol. 12, No. 11; pages 1-19; entire document; DOI: 10.1371/journal.pone.0188593	1-43, 69-74

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/33760

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/33760

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.:
Group I, Claims 1-43, and 69-74 (each in-part)

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/US21/33760

-***-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 1-43, and 69-74 (each in-part) are directed toward complexes for delivering gene editing agents to cells, compositions comprising the complexes, and methods associated therewith.

Group II, Claims 44-68, 69-74 (each in-part) are directed toward complexes comprising targeting agents, compositions comprising the complexes, and methods associated therewith.

The inventions listed as Groups I and II 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 first gene editing agent and a second gene editing agent, each linked to corresponding phosphorothioate nucleic acids, wherein the nucleic acids linked to each gene editing agent are complementary to each other, not present in Group II; the special technical features of Group II include a gene editing agent linked to a phosphorothioate nucleic acid; and a targeting agent linked to a second phosphorothioate nucleic acid, not present in Group I.

Groups I and II share the technical features including: a complex for delivering a gene editing agent to a cell, said complex comprising: (i) a double-stranded phosphorothioate oligonucleotide; (ii) a gene editing agent covalently bound to a first phosphorothioate nucleic acid through a first chemical linker; and an agent covalently bound to a second phosphorothioate nucleic acid through a second chemical linker; wherein at least a portion of said first phosphorothioate nucleic acid and a portion of said second phosphorothioate nucleic acid are complementary to each other; and wherein at least a portion of said first phosphorothioate nucleic acid is hybridized to at least a portion of said second phosphorothioate nucleic acid thereby forming said double-stranded phosphorothioate oligonucleotide; a pharmaceutical composition comprising the complex and a pharmaceutically acceptable excipient; a method of delivering a gene editing agent to a cell, said method comprising contacting a cell with the complex, thereby delivering said gene editing agent to said cell; and one or more sequences selected from the group consisting of SEQ ID NO: 1, and SEQ ID NOs: 30-40.

However, these shared technical features are previously disclosed by WO 2019/135816 A2 to The Broad Institute, et al. (hereinafter 'Broad'), in view of US 2020/0140864 A1 to Alnylam Pharmaceuticals, Inc. (hereinafter 'Alnylam'), and further in view of WO 2018/049226 A1 to Bluebird Bio, Inc. (hereinafter 'Bluebird').

Broad discloses a complex for delivering a gene editing agent to a cell (a complex for delivering a CRISPR/Cas protein (gene editing agent) to a cell; paragraphs [0011], [0061]), said complex comprising: (i) a double-stranded (said complex comprising: (i) a double-stranded; paragraphs [0061],[0455]) phosphorothioate oligonucleotide (phosphorothioate oligonucleotide; paragraph [0247]); (ii) a gene editing agent covalently bound to a first nucleic acid through a first chemical linker (a CRISPR/Cas protein (gene editing agent) covalently bound to a first nucleic acid through a first chemical linker; paragraphs [0016]); a pharmaceutical composition comprising the complex and a pharmaceutically acceptable excipient (a pharmaceutical composition comprising the complex and a pharmaceutically acceptable excipient; paragraphs [0061], [0635]); a method of delivering a gene editing agent to a cell, said method comprising contacting a cell with the complex, thereby delivering said gene editing agent to said cell (a method of delivering a gene editing agent to a cell, said method comprising contacting a cell with the complex, thereby delivering said gene editing agent to said cell; abstract, paragraphs [0018], [0061]).

Broad does not disclose a gene editing agent covalently bound to a first phosphorothioate nucleic acid through a first chemical linker; and an agent covalently bound to a second phosphorothioate nucleic acid through a second chemical linker; wherein at least a portion of said first phosphorothioate nucleic acid and a portion of said second phosphorothioate nucleic acid are complementary to each other; and wherein at least a portion of said first phosphorothioate nucleic acid is hybridized to at least a portion of said second; and one or more sequences selected from the group consisting of SEQ ID NO: 1, and SEQ ID NOs: 30-40phosphorothioate nucleic acid thereby forming said double-stranded phosphorothioate oligonucleotide

Alnylam discloses an agent covalently bound to a second phosphorothioate nucleic acid through a second chemical linker (an agent covalently bound to a second phosphorothioate nucleic acid through a second chemical linker; paragraphs [0014], [0019]).

Bluebird discloses wherein a homing nuclease cleaves a sequence comprising SEQ ID NO: 31 (wherein a homing nuclease cleaves a sequence comprising SEQ ID NO: 37 (SEQ ID NO: 31); page 64, lines 23-27; wherein nt 12-36 of SEQ ID NO: 37 are 100% identical to Applicant's SEQ ID NO: 31).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Broad to have provided a linked adaptor, as disclosed by Broad, comprising a phosphorothioate nucleic acid, as disclosed by Broad, in order to reduce the susceptibility of the adaptor to nuclease digestion. It further would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Broad to have provided a nucleic acid, such as a phosphorothioate nucleic acid, as disclosed by Broad, linked to a nucleic acid editing enzyme, as disclosed by Broad, wherein the linked nucleic acid hybridized with a second nucleic acid to form a double-stranded structure, as disclosed by Alnylam, comprising an agent, such as a targeting ligand, as disclosed by Alnylam (paragraph [0014]), in order to enable specific delivery of the editing enzyme complex to a cell expressing a receptor for the ligand. It would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Broad to have provided a guide nucleic acid targeting a sequence comprising SEQ ID NO: 31, based on the disclosure of Bluebird, in order to enable targeted editing, or cleavage and repair of the site.

Since none of the special technical features of the Groups I and II 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 Broad, Alnylam, and Bluebird references, unity of invention is lacking.