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

(88) Date of publication of the international search report:
15 May 2025 (15.05.2025)

(54) Title: METHODS AND COMPOSITIONS FOR TREATING ALPHA-1 ANTITRYPSIN DEFICIENCY

(57) Abstract: Compositions and methods for editing deleterious mutations associated with Alpha-1 Antitrypsin Deficiency (A1AD). In particular embodiments, the invention provides methods for treating A1AD using a modified adenosine base editor with improved on-target editing and decreased off-target editing to correct mutations associated with A1AD.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/10 (2024.01)
ADD. A61K 38/46, A61K 48/00, C12N 15/11 (2024.01)

CPC - INV. C12N 15/102
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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.
A	US 2023/0159956 A1 (BEAM THERAPEUTICS INC.) 25 May 2023 (25.05.2023) para [0014]; SEQ ID NO: 1	1-2
A	US 2023/0116627 A1 (OHIO STATE INNOVATION FOUNDATION) 13 April 2023 (13.04.2023) para [0106]; SEQ ID NO: 16	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
"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
30 August 2024	NOV 13 2024
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

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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 fi led.

   b. ☐ furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/34189

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.: 4-27, 31-37, 39-94
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:
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 searched, the appropriate additional search fees must be paid.

----- See Extra Pages -----

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.:
1-2 limited to SEQ ID NO: 554 M1135L

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.

--continued from: Box No. III Observations where unity of invention is lacking--

Group I+, Claims 1-3, 95-98, directed to a base editor. The base editor will be searched to the extent that the base editor encompasses wherein the polynucleotide programmable DNA binding domain variant comprises a single alteration selected from the group consisting of M1135L, of an amino acid sequence that is at least 100% identical to SEQ ID NO: 554. The first named invention was determined based on the first claimed single alteration and sequence (claim 1) for the inventive base editor embodiment. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-2 encompass this first named invention, and thus these claims will be searched without fee to the extent that the base editor encompasses wherein the polynucleotide programmable DNA binding domain variant comprises a single alteration selected from the group consisting of M1135L, of an amino acid sequence that is at least 100% identical to SEQ ID NO: 554. Additional base editor polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected base editor polypeptide(s). Applicants must further indicate, if applicable, the claims which encompass 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. An exemplary election would be a base editor comprising wherein the polynucleotide programmable DNA binding domain variant comprises a single alteration selected from the group consisting of E1250K, of an amino acid sequence that is at least 100% identical to SEQ ID NO: 554 (claims 1-2).

Group II+, claims 28-30, directed to a guide polynucleotide, or a polynucleotide encoding the guide polynucleotide. Group II+ will be searched upon payment of additional fees. The guide polynucleotide may be searched, for example, to encompass wherein the guide polynucleotide comprises a nucleotide sequence selected from the group consisting of 5'-ACCAUCGACAAGAAAGGGACUGA-3' (SEQ ID NO: 466) for an additional fee and election as such. It is believed that claim 28 reads on this exemplary invention. Additional guide polynucleotide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected guide polynucleotide(s). 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. Another exemplary election would be a guide polynucleotide wherein the guide polynucleotide comprises a nucleotide sequence selected from the group consisting of 5'-CCAUCGACAAGAAAGGGACUGA -3' (SEQ ID NO: 559) (claim 28).

Group III, claim 38, directed to a method of editing an alpha-1 antitrypsin polynucleotide.

The inventions listed as Groups I+, II+ and III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Groups I+ and II+ each include the special technical feature of a unique amino acid and/or nucleic acid sequence. Each guide nucleic acid sequence targets a unique sequence, and is considered a distinct technical feature. Each amino acid sequence comprises a unique base editor peptide, and is considered a distinct technical feature.
Additionally,
Group I+ has the special technical feature of a base editor, that is not required by Groups II+ and III.
Group II+ has the special technical feature of a guide polynucleotide, or a polynucleotide encoding the guide polynucleotide, that is not required by Groups I+ and III.
Group III has the special technical feature of a method of editing an alpha-1 antitrypsin polynucleotide, that is not required by Groups I+ and II+.

Common technical features

No technical features are shared between the base editor amino acid and/or guide nucleic acid sequences in each of Groups I+ and II+ and, accordingly, these groups lack unity a priori.

Additionally, no technical features are shared between Groups I+, II+ and III.

Additionally, even if Groups I+ and III were considered to share the technical features of including:
a base editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain; and
even if Groups II+ and III were considered to share the technical features of including:
a guide polynucleotide,
these shared technical features are previously disclosed by prior art, as discussed below.

Group I+ inventions additionally share the technical features of including:
a base editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain, wherein the polynucleotide programmable DNA binding domain variant comprises an alteration of an amino acid sequence, or a fragment thereof lacking an N- terminal methionine;

a TadA variant;

a Cas9 variant.

----- See next Extra Page -----

--continued from previous page, Box No. III Observations where unity of invention is lacking--

However, these shared technical features are previously disclosed by US 2023/0159956 A1 to Beam Therapeutics, Inc. (hereinafter 'Beam').

Beam teaches a base editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and an adenosine deaminase domain (para [0043] - "wherein the base editor comprises a polynucleotide programmable DNA binding domain and an adenosine deaminase domain"), wherein the polynucleotide programmable DNA binding domain variant comprises an alteration of an amino acid sequence, or a fragment thereof lacking an N terminal methionine (Claim 10 - "The method of claim 8, wherein the modified SpCas9 comprises the amino acid substitution D1332A and one or more of D1135M, S1137Q, G1218K, E1219F, D1332A, R1335E, and T1337R, or corresponding amino acid substitutions thereof; or
wherein the modified SpCas9 comprises amino acid substitutions D1135M, S1137Q, G1218K, E1219F, A1322R, D1332A, R1335E, and T1337R, or corresponding amino acid substitutions thereof.").

Beam further teaches a guide polynucleotide, a TadA variant; and a Cas9 variant (Claim 19 - "The method of claim 18, wherein the TadA deaminase is TadA*7.10."; Claim 20 - "The method of claim 1, wherein the one or more guide RNAs comprises a CRISPR RNA (crRNA) and a trans-encoded small RNA (tracrRNA), wherein the crRNA comprises a nucleic acid sequence complementary to a SERPINA1 nucleic acid sequence comprising the SNP associated with A1AD; or wherein the base editor is in complex with a single guide RNA (sgRNA) comprising a nucleic acid sequence complementary to an SERPINA1 nucleic acid sequence comprising the SNP associated with A1AD."; Claim 10).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+, II+ and III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

RE: item 4: claims 4-27, 31-37, 39-94 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Note, Claim 98 is objected to for lack of antecedent basis. As drafted, claim 98 depends from claim 97 which fails to recite a TadA variant. For the purposes of this application, claim 98 is construed as though drawn to a Cas9 variant from claim 97.