(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 May 2024 (16.05.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/102811 A3

(51) International Patent Classification:
A61P 9/00 (2006.01) *C12N 15/11* (2006.01)
C07K 14/47 (2006.01) *C12N 15/864* (2006.01)

(21) International Application Number:
PCT/US2023/079074

(22) International Filing Date:
08 November 2023 (08.11.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/423,716 08 November 2022 (08.11.2022) US

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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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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).

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))*
— *of inventorship (Rule 4.17(iv))*

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:
18 July 2024 (18.07.2024)

(54) Title: MUSCLE-SPECIFIC BASE EDITORS FOR CORRECTION OF MUTATIONS CAUSING DILATED CARDIOMYOPATHY

(57) Abstract: Provided herein are compositions and methods for treating or preventing dilated cardiomyopathy (DCM) in a subject in need thereof, e.g., through correcting one or more point mutations at the RBM20 locus using CRISPR-associated base editing.

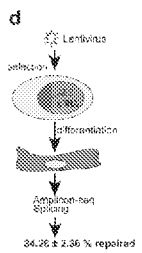


Fig. 2

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[Continued on next page]

(15) Information about Correction:
see Notice of 13 June 2024 (13.06.2024)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/079074

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61P 9/00*** (2024.01); ***C07K 14/47*** (2024.01); ***C12N 15/11*** (2024.01); ***C12N 15/864*** (2024.01)

CPC: ***C12N 15/111; C07K 14/47; C12N 15/86; A61P 9/00;*** *C12N 2320/30; C12N 2750/14143*

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	WO 2020/236936 A1 (BEAM THERAPEUTICS INC.) 26 November 2020 (26.11.2020) entire document	1-37
A	WO 2016/112351 A1 (BIO-RAD LABORATORIES INC. et al.) 14 July 2016 (14.07.2016) entire document	1-37
A	US 2021/0395822 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY et al.) 23 December 2021 (23.12.2021) entire document	1-37

☐ 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
04 April 2024 (04.04.2024)	**24 May 2024 (24.05.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/079074

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.
   b. ☑ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.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/US2023/079074

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 examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-37 are drawn to methods for correcting a point mutation at the RBM20 locus in a cell.

Group II+: claims 38-41 are drawn to sgRNAs that specifically target the RBM20 gene, iPSCs, cardiomyocytes, and pharmaceutical compositions.

The first invention of Group I+ is restricted to an sgRNA sequence selected to be SEQ ID NO: 1 and methods comprising the same. The 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. Specifically, the first named invention was selected based on the first listed sgRNA sequence species presented in the claims (see claim 7). It is believed that claims 1-37 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NO: 1.

The first invention of Group II+ is restricted to an sgRNA sequence selected to be SEQ ID NO: 1 and sgRNAs, iPSCs, cardiomyocytes, and pharmaceutical compositions comprising the same.

Applicant is invited to elect additional sgRNA sequences and their respective, corresponding, SEQ ID NOs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be an sgRNA sequence selected to be SEQ ID NO: 2 and methods comprising the same. Additional an sgRNA sequence selected to be SEQ ID NO: 1 and methods comprising the same and their respective, corresponding, SEQ ID NOs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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/examined.

The inventions listed in 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 Groups I+ and II+ formulas do not share a significant structural element responsible for sgRNAs requiring the selection of alternative sgRNA sequences where "wherein the sgRNA comprises a sequence having about 80% or greater identity to any one of SEQ ID NOs: 1-9" and "An sgRNA that specifically targets the RBM20 gene comprising a sequence having about 80% or greater identity to any one of SEQ ID NOs: 1-9."

The special technical features of Group I+, methods for correcting a point mutation at the RBM20 locus in a cell, are not present in Group II+; the special technical features of Group II+, sgRNAs that specifically target the RBM20 gene, iPSCs, cardiomyocytes, and pharmaceutical compositions, are not present in Group I+.

Additionally, even if Groups I+ and II+ were considered to share the technical features of a method for correcting a point mutation at the RBM20 locus in a cell, the method comprising: introducing into the cell (i) a single guide RNA (sgRNA) targeting a sequence comprising the point mutation and (ii) a base editor (BE), wherein the sgRNA binds to the base editor and directs it to the target sequence, whereupon the base editor corrects the point mutation at the RBM20 locus in the cell; an sgRNA that specifically targets the RBM20 gene comprising a sequence; an iPSC comprising the sgRNA and a base editor (BE) comprising an RNA-guided catalytically impaired nuclease fused to a nucleobase deaminase enzyme; a cardiomyocyte derived from the iPSC; and a pharmaceutical composition comprising a plurality of iPSCs, or a plurality of cardiomyocytes. However, these shared technical features do not represent a contribution over the prior art.

WO 2020/236936 A1 to Beam Therapeutics Inc. (hereinafter, "Beam") teaches a method for correcting a point mutation (methods for treating Rett Syndrome using an adenosine base editor to precisely correct a single nucleotide polymorphism to correct a deleterious mutation; Para. [0006]), the method comprising: introducing into the cell (i) a single guide RNA (sgRNA) targeting a sequence comprising the point mutation

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

(administering to a subject…a guide polynucleotide…a single guide RNA; Para. [0007]) and (ii) a base editor (BE) (administering to a subject an adenosine base editor; Para. [0007]), wherein the sgRNA binds to the base editor and directs it to the target sequence (the adenosine base editor is in complex with a sgRNA comprising a nucleic acid sequence complementary to the MECP2 gene or regulatory element thereof comprising the single nucleotide polymorphism associated with RETT; Para. [0007]), whereupon the base editor corrects the point mutation (the nucleobase alteration changes the single nucleotide polymorphism associated with RETT to a wild type nucleobase; Para. [0007]); an sgRNA that specifically targets a gene comprising a sequence (a sgRNA comprising a nucleic acid sequence complementary to the MECP2 gene or regulatory element thereof comprising the single nucleotide polymorphism associated with RETT; Para. [0007]); a base editor (BE) comprising an RNA-guided catalytically impaired nuclease fused to a nucleobase deaminase enzyme (an adenosine base editor…wherein the adenosine base editor comprises a programmable DNA binding domain and an adenosine deaminase domain…the polynucleotide programmable DNA binding domain is a nuclease inactive variant; Paras. [0007], [0009]); a pharmaceutical composition (a pharmaceutical composition; Para. [0021]).

US 2021/0395822 A1 to The Board of Trustees of the Leland Stanford Junior University et al. (hereinafter, "Stanford") teaches the RBM20 locus in a cell (IPSCs comprising at least one RBM20 allele encoding a P633L mutation; abstract), the RBM20 gene (IPSCs comprising at least one RBM20 allele encoding a P633L mutation; abstract); an iPSC comprising the sgRNA (as shown in figure 1D, transfect cells with Cas9-GFP and sgRNA…human iPSCs were transfected; Paras. [0143], [0144]); a cardiomyocyte derived from the iPSC (the iPSCs were differentiated into cardiomyocytes; Para. [0145]); and a plurality of iPSCs, or a plurality of cardiomyocytes (the iPSCs were differentiated into cardiomyocytes; Para. [0145]).

The inventions listed in Groups I+ and II+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

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.: **1-37**

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.