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(71) Applicant: **MANTRA BIO, INC.** [US/US]; 455 Mission Bay Blvd South, San Francisco, CA 94158 (US).

(72) Inventors: **SZABO, Linda**; Mantra Bio, Inc., 455 Mission Bay Blvd South, San Francisco, CA 94158 (US). **GOTTLIEB, Colin, David**; Mantra Bio, Inc., 455 Mission Bay Blvd South, San Francisco, CA 94158 (US). **PLANEY, Catherine, Rosemary**; 1095 Escalero Ave., Pacifica, CA 94044 (US).

(74) Agent: **ADRIANO, Sarah, B.**; Adriano & Associates, 690 E. Green Street, Suite 300, Pasadena, CA 91101 (US).

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(54) Title: EXTRACELLULAR VESICLES COMPRISING NON-NATURALLY OCCURRING MODULAR RNA HAIRPINS AND USES THEREOF

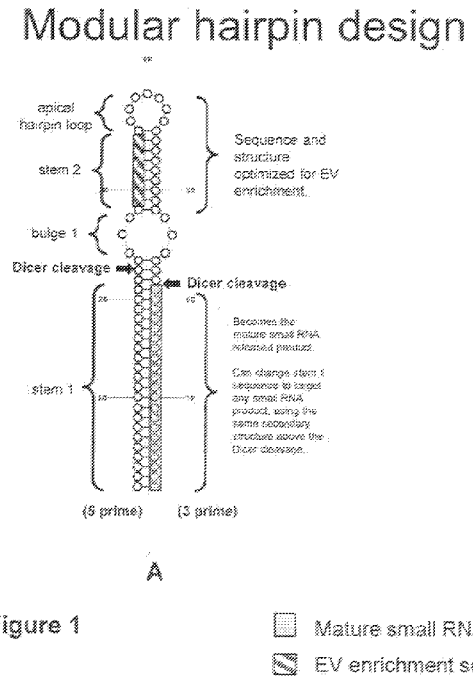


Figure 1

(57) Abstract: The invention provides EVs and/or exosomes that selectively enrich non-naturally occurring RNA hairpins. Following the engineered EVs and/or exosomes uptake by the recipient cell or fusion to the plasma membrane of the recipient cell, the RNA hairpin or its processed product is released to the cytoplasm resulting in altered regulation of target gene expression or level of an RNA target. The invention additionally provides methods of production and treatment thereof.

[Continued on next page]

WO 2023/097268 A3

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Declarations under Rule 4.17:

— *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:
10 August 2023 (10.08.2023)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/80419

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/10, C12N 15/113 (2023.01)
ADD. C12N 15/11 (2023.01)

CPC - INV. C12N 15/1096, C12N 15/113
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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.
Y	US 2020/0208155 A1 (CARIBOU BIOSCIENCES, INC.) 02 July 2020 (02.07.2020) para [0012]; [0024]-[0035]; [0071]; [0087]-[0090]; [0127]; Tables 2, 4	1, 4, 7-21, 31-34
Y	WO 2021/154888 A1 (MANTRA BIO, INC.) 05 August 2021 (05.08.2021) para [21]; [125]-[135]	1, 4, 7-21, 31-34
Y	US 2020/0199597 A1 (VOYAGER THERAPEUTICS, INC.) 25 June 2020 (25.06.2020) abstract; para [0029]; [0070]; [0330]-[0332]	9-12
A	WO 2021/113218 A1 (SHAPE THERAPEUTICS INC.) 10 June 2021 (10.06.2021) full document	1, 4, 7-21, 31-34

☐ 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
15 May 2023 (15.05.2023)	JUN 15 2023
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

International application No.
PCT/US 22/80419

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 22/80419

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.: 5-6, 22-30, 40-45, 47-69, 72-81
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:
Claims 5-6, 24-29, 40-45, 47-69, 72-81 are held unsearchable because these claims recite sequences for which no SEQ ID NOs were provided in response to the ISA/225, mailed 2/2/2023. Claims 22, 23, and 30 are unsearchable as an omnibus type claims not drafted in accordance with PCT Rule 6.2(a). These claims are indefinite as it is unclear what is included or excluded by the claim language "has any of the structures shown in Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7", or "has the sequence as shown in Figure 13".

3. ☒ Claims Nos.: 70-71
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.

--continued on extra 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 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, 4, 7-21, 31-34, limited as noted in extra sheet

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 1-4, 7-23, 30-34, directed to an isolated extracellular vesicle. The vesicle will be searched to the extent that the vesicle encompasses a non-naturally occurring RNA hairpin which comprises:
a. a hairpin loop comprising the sequence 5' -UCAGAAAAA- 3',
b. two stems, and
c. one bulge
wherein a bulge is located between each pair or stems and a stem separates the bulge from the hairpin loop and a stem, 1st stem, having a length of any of about 19-45 base pairs is located at the 5' end or the RNA hairpin, further comprising 2 UMPs at 3' end, wherein the RNA hairpin has the structure of SEQ 2 (Fig. 3) (note, these are the first claimed components, sequence and structure for the inventive non-naturally occurring RNA hairpin comprising vesicle). The first named invention was determined based on the first claimed components (two stems and one bulge, claim 1), hairpin loop sequence (claim 11) and structure (Fig. 3, claim 22) for the inventive vesicle 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, 4, 7-23, 30-34 encompass this first named invention, and thus these claims will be searched without fee to the extent that the vesicle encompasses a non-naturally occurring RNA hairpin which comprises:
a. a hairpin loop comprising the sequence 5' -UCAGAAAAA- 3',
b. two stems, and
c. one bulge
wherein a bulge is located between each pair or stems and a stem separates the bulge from the hairpin loop and a stem, 1st stem, having a length of any of about 19-45 base pairs is located at the 5' end or the RNA hairpin, further comprising 2 UMPs at 3' end, wherein the RNA hairpin has the structure of SEQ 2 (Fig. 3). Additional vesicle(s) comprising additional RNA hairpin(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected vesicle(s) comprising additional RNA hairpin(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 vesicle comprising a non-naturally occurring RNA hairpin which comprises:
a. a hairpin loop comprising the sequence 5' -UCAGAAAAA- 3',
b. two stems, and
c. one bulge
wherein a bulge is located between each pair or stems and a stem separates the bulge from the hairpin loop and a stem, 1st stem, having a length of any of about 19-45 base pairs is located at the 5' end or the RNA hairpin, further comprising 2 UMPs at 3' end, wherein the RNA hairpin has the structure of SEQ 6 (Fig. 3) (claims 1, 4, 7-23, 30-34).

Group II, claims 35-39, 46, directed to a method for selective enrichment of a non-naturally occurring RNA hairpin in an extracellular vesicle.

The inventions listed as Group I+ and II 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 Group I+ each include the special technical feature of a unique nucleic acid sequence. Each nucleic acid sequence comprises a unique RNA hairpin, and is considered a distinct technical feature.
Additionally,
Group I+ has the special technical feature of an isolated extracellular vesicle comprising a non-naturally occurring RNA hairpin, that is not required by Group II.
Group II has the special technical feature of a method for selective enrichment of a non-naturally occurring RNA hairpin in an extracellular vesicle, that is not required by Group I+.

Common technical features

No technical features are shared between the RNA hairpin nucleic acid sequences in each of Group I+ inventions and, accordingly, these inventions lack unity a priori.

Additionally, even if Groups I+ and II were considered to share the technical features of including:
an isolated extracellular vesicle comprising a non-naturally occurring RNA hairpin,
these shared technical features are previously disclosed by prior art, as discussed below.

Group I+ inventions additionally share the technical features of including:
an isolated extracellular vesicle comprising: a non-naturally occurring RNA hairpin which comprises:
a. a hairpin loop,
b. two or more stems, and
c. one or more bulges
wherein a bulge is located between each pair or stems and a stem separates the bulge from the hairpin loop and a stem, 1st stem, having a length of any of about 19-45 base pairs is located at the 5' end or the RNA hairpin.

However, these shared technical features are obvious over US 2020/0208155 A1 to Caribou Biosciences, Inc. (hereinafter 'Caribou'), in view of WO 2021/154888 A1 to Mantra Bio, Inc. (hereinafter 'Mantra').

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Caribou teaches a non-naturally occurring RNA hairpin (para [0033] - "The component polynucleotides of dfs-NATNA compositions of the present invention can comprise additional elements and sequences. In some embodiments, the dfs1-PN further comprises a nexus 3' linker nucleotide sequence 5' to the 3' hairpin"; para [0034] - "The polynucleotide components of a dfs-NATNA composition (e.g., dfs1-PN, dfs2-PN, or dfs1-PN and dfs2-PN) can comprise DNA, RNA, or DNA and RNA") which comprises:
a. a hairpin loop (para [0127] - "comprising, in a 5' to 3' direction, a first stem element nucleotide sequence I, a nexus nucleotide sequence, and a 3' hairpin element"),
b. two or more stems, and
c. one or more bulges (para [0127] - "comprising, in a 5' to 3' direction, a nucleic acid target binding sequence and a first stem element nucleotide sequence II. The first stem element nucleotide sequence I and the first stem element nucleotide sequence II form a first stem-loop element by base-pair hydrogen bonding between the first stem element nucleotide sequence I and the first stem element nucleotide sequence II. In preferred embodiments, the first stem-loop element comprises a lower stem element 3' of the nucleic acid targeting sequence and 5' of the nexus nucleotide sequence, the lower stem element adjacent a bulge element, the bugle element adjacent an upper stem element, and the upper stem element adjacent a loop element"),
wherein a bulge is located between each pair of stems and a stem separates the bulge from the hairpin loop (para [0127] - "In preferred embodiments, the first stem-loop element comprises a lower stem element 3' of the nucleic acid targeting sequence and 5' of the nexus nucleotide sequence, the lower stem element adjacent a bulge element, the bugle element adjacent an upper stem element, and the upper stem element adjacent a loop element") and a stem, having a length of any of about 19-45 base pairs is located at the 5' end of the RNA hairpin (para [0029] - "The lower stem element nucleotide sequence I and the lower stem element nucleotide sequence II form a stem element comprising at least 2 pairs of hydrogen-bonded nucleotides"), but does not explicitly teach an isolated extracellular vesicle comprising a non-naturally occurring RNA hairpin.

Mantra teaches extracellular vesicles comprising an RNA payload (para [133] - "The modified vesicle system described herein, for example modified vesicles such as exosomes, can be used to deliver payloads to target cells"; para [135] - "A payload can be, for example, a small molecule, polypeptide, nucleic acid, lipid, carbohydrate, ligand, receptor, reporter, drug, or combination of the foregoing (e.g., two or more drugs, or one or more drugs combined with a lipid, etc.). Examples of payloads, include, for example therapeutic biologies (e.g., antibodies, recombinant proteins, or monoclonal antibodies), RNA (siRNA, shRNA, miRNA, antisense RNA, mRNA, noncoding RNA, tRNA, rRNA, other RNAs)"). It would have been obvious to one of ordinary skill in the art to apply the non-naturally occurring RNA taught by Caribou to the extracellular vesicle taught by Mantra in order to deliver a hairpin RNA to a target cell.

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

Continuation of item 4 above: Claims 70-71 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).
Claims 5-6, 24-29, 40- 45, 47-69, 72-81 are held unsearchable because these claims recite sequences for which no SEQ ID NOs were provided in response to the ISA/225, mailed 2/2/2023.
Claims 22, 23, and 30 are unsearchable as an omnibus type claims not drafted in accordance with PCT Rule 6.2(a). These claims are indefinite as it is unclear what is included or excluded by the claim language "has any of the structures shown in Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7", or "has the sequence as shown in Figure 13".