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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2024 (12.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/254543 A3

(51) International Patent Classification:
C07K 14/47 (2006.01) *A61K 45/06* (2006.01)

(21) International Application Number:
PCT/US2024/033129

(22) International Filing Date:
07 June 2024 (07.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/472,192 09 June 2023 (09.06.2023) US

(71) Applicants: **EMORY UNIVERSITY** [US/US]; Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US). **CHILDREN'S HEALTHCARE OF ATLANTA, INC.** [US/US]; 1575 Northeast Expressway, Atlanta, Georgia 30329 (US).

(72) Inventors: **RAIKAR, Sunil**; c/o Emory University Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US). **DOERING, Christopher**; c/o Emory University Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US). **SPENCER, H. Trent**; c/o Emory University Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US). **KNIGHT, Kristopher**; c/o Emory University Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US). **BROWN, Harrison**; c/o Emory University Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US).

(74) Agent: **MASON, James C.** et al.; Emory University, Office of Technology Transfer, 1599 Clifton Road NE, 4th Floor, Atlanta, Georgia 30322 (US).

(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,

(54) Title: RECOMBINANT ANCESTRAL VARIANT ASPARAGINASES AND USES IN MANAGING CANCER

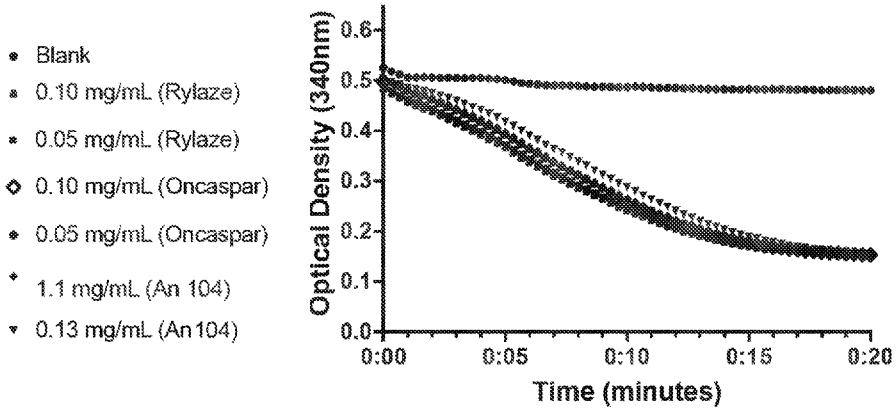

FIG. 2A

(57) Abstract: Disclosed herein are recombinant asparaginases with ancestral variant sequences for uses as therapeutics. In certain embodiments, it is contemplated that the ancestral variant asparaginases have reduced immunogenicity thereby preventing or reducing the risk of host inactivation and/or anaphylaxis. In certain embodiments, this disclosure relates to treating diseases associated with asparagine dependence comprising administering an effective amount of an ancestral variant asparaginase or conjugate disclosed herein to a subject in need thereof.

[Continued on next page]

WO 2024/254543 A3

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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:

24 April 2025 (24.04.2025)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/33129

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 14/47 (2024.01)
ADD. A61K 45/06 (2024.01)

CPC - INV. C07K 14/435, C12N 15/85, A61K 47/60

ADD. C07K 14/47, A61P 35/02

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	EP 1 321 519 A1 (KABUSHIKI KAISHA HAYASHIBARA) 25 June, 2003 (25.06.2003) para [0070], SEQ ID NO: 18 (pp 45-47)	1, 3-11, 21-24
X, P	UniProtKB protein F6QHN0_ORNAN, 30 April 2024 [online]. [Retrieved on 3 October 2024]. Retrieved from the internet <URL: https://rest.uniprot.org/uniprotkb/F6QHN0.txt> entire document GenBank Accession F6QHN0 "ASPG asparaginase [Ornithorhynchus anatinus (platypus)]". 30 April 2024 (30.06.2024), Entire document	1, 3-11, 21-24

☐ 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
02 October 2024 (02.10.2024)	NOV 12 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

International application No.
PCT/US 24/33129

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 24/33129

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:
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.

Group I+: Claims 1-11, 21-24, 25-35 and 45-48, directed to an isolated recombinant asparaginase composition comprising an amino acid sequence that elicits a lower immunogenic response in a patient compared to E. coli L-asparaginase or Erwinia chrysanthemi L-asparaginase. The recombinant asparaginase will be searched to the extent that the asparaginase has 80% or greater sequence identity to SEQ ID NO: 1. The first named invention was determined based on alternative recombinant asparaginase amino acid sequences in claim 1. 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, 3-11 and 21-24, limited to said recombinant asparaginase encompass this first named invention, and thus these claims will be searched without fee to the extent that the recombinant asparaginase encompasses 80% or greater sequence identity to SEQ ID NO: 1. Additional recombinant asparaginases will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected recombinant asparaginases.

---continued on first 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, 3-11 and 21-24, limited to SEQ ID NO: 1

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.

Continuation of Box No. III. Observations where unity of invention is lacking

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 recombinant asparaginase having 80% or greater sequence identity to SEQ ID NO: 54 (claims 25, 27-35 and 45-48). Another exemplary election would be a recombinant asparaginase wherein the asparaginase has 80% or greater sequence identity to SEQ ID NO: 10 (claims 1-11, 21-24).

Group II: Claims 12-20 and 36-44, drawn to a method of treating diseases associated with asparagine dependence (e.g. cancer) comprising administering a recombinant asparaginase to a subject in need thereof, e.g., a recombinant asparaginase that elicits a lower immunogenic response in a patient compared to E. coli L-asparaginase or Erwinia chrysanthemi L-asparaginase.

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:

Special Technical Features

No technical features are shared between the amino acid sequences of the peptides of Group I+ and, accordingly, Group I+ lacks unity a priori.

Group I+ requires an isolated composition comprising a recombinant asparaginase, not required by Group II.

Group II requires a method of treating diseases associated with asparagine dependence, not required by Group I+.

Common Technical Features

The inventions of Groups I+ and II share the technical feature of a recombinant asparaginase that elicits a lower immunogenic response in a patient compared to E. coli L-asparaginase or Erwinia chrysanthemi L-asparaginase. Each invention of Group I+ requires xxx not required by any of the other inventions.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2022/0056430 A1 to Jazz Pharmaceuticals Ireland Ltd., (hereinafter "Jazz Pharma"). Jazz Pharma teaches said recombinant asparaginase (para [0038] "The present invention relates, inter alia, to a modified protein comprising ... an L-asparaginase ..., said modified protein has an L-asparagine depletion activity at least about 20% higher than the unmodified L-asparaginase.", para [0005] "L-asparaginases of bacterial origin have a high immunogenic and antigenic potential and frequently provoke adverse reactions ranging from mild allergic reaction to anaphylactic shock ... E. coli L-asparaginase is particularly immunogenic", para [0041] "the polypeptide or peptide described herein mediates a decreased immunogenicity of said modified protein").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Groups I+ and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.