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(71) Applicant: AUROBAC THERAPEUTICS SAS  
[FR/FR]; 29 avenue Tony Garnier, 69007 LYON (FR).

(72) Inventors: SCHUCH, Raymond; 116 Lake Drive, MOUNTAIN LAKES, New Jersey 07046 (US). VILA FARRES, Xavier; c/o 28 Wells Avenue, 3rd Floor, YONKERS, New York 10701 (US). SWIFT, Steven; c/o 28 Wells Avenue, 3rd Floor, YONKERS, New York 10701 (US).

(74) Agent: SEKHRI, Redha; Cabinet Becker & Associés, 25, rue Louis Le Grand, 75002 Paris (FR).

(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.

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(54) Title: ENGINEERED LYSIN POLYPEPTIDE CONSTRUCTS ACTIVE AGAINST GRAM-NEGATIVE BACTERIA

(57) Abstract: The present disclosure is directed to an engineered lysin comprising: (a) a Gram-negative base lysin; and (b) one or more antimicrobial peptide (AMP) and/or cell penetrating peptide (CPP), and optionally an outer membrane binding motif and at least one linker, or an active fragment thereof, or a variant thereof having at least 80% sequence identity. Also disclosed herein are methods of treating a bacterial infection caused by at least one Gram-negative bacteria and methods of killing at least one Gram-negative bacteria, comprising administering the engineered lysin disclosed herein.



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<b>A. CLASSIFICATION OF SUBJECT MATTER</b>					
INV.	C07K14/47	A61K38/00	A61P31/04	C12N9/36	C12N9/50
ADD.	C12R1/01	C12R1/185	C12R1/22	C12R1/38	C12R1/425

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)  
**C07K A61K A61P C12N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**EPO- Internal**

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<b>WO 2020/046747 A1 (CONTRAFECT CORP [US])</b> 5 March 2020 (2020-03-05) [095], [0106] - [0107]; [0110] - [0112]; claims 1-57 -----	1-5,7,8, 19,21-42
Y	<b>WO 2019/191633 A2 (CONTRAFECT CORP [US])</b> 3 October 2019 (2019-10-03) [084], [095] - [096], [099] - [101]; claims 1-41 ----- -/--	1-5,7,8, 19,21-42

<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.
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\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance "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
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Date of the actual completion of the international search	Date of mailing of the international search report
<b>9 October 2024</b>	<b>16/12/2024</b>

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <p style="text-align: center;"><b>Dumont, Elisabeth</b></p>
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2024/066700

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GHOSE CHANDRABALI ET AL: "Gram-Negative Bacterial Lysins", ANTIBIOTICS, vol. 9, no. 2, 11 February 2020 (2020-02-11), page 74, XP055800842, DOI: 10.3390/antibiotics9020074 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168136/pdf/antibiotics-09-00074.pdf &gt; page 7, par. 4.1</p> <p style="text-align: center;">-----</p>	1-5,7,8, 19,21-42
A	<p>LIM JEONGHYUN ET AL: "Eradication of drug-resistant Acinetobacter baumannii by cell-penetrating peptide fused endolysin", JOURNAL OF MICROBIOLOGY, THE MICROBIOLOGICAL SOCIETY OF KOREA, SEOUL, vol. 60, no. 8, 25 May 2022 (2022-05-25), pages 859-866, XP037921945, DOI: 10.1007/S12275-022-2107-Y abstract</p> <p style="text-align: center;">-----</p>	1-5,7,8, 19,21-42
Y	<p>SCHWUDKE DOMINIK ET AL: "Broad-Host-Range Yersinia Phage PY100: Genome Sequence, Proteome Analysis of Virions, and DNA Packaging Strategy", JOURNAL OF BACTERIOLOGY, vol. 190, no. 1, 1 January 2008 (2008-01-01), pages 332-342, XP093212349, US ISSN: 0021-9193, DOI: 10.1128/JB.01402-07 Retrieved from the Internet: URL:https://journals.asm.org/doi/pdf/10.1128/JB.01402-07&gt; invention 1/SEQ ID NO:2</p> <p style="text-align: center;">-----</p>	1-5,7,8, 19,21-42
A	<p>GERSTMANS H. ET AL: "A VersaTile-driven platform for rapid hit-to-lead development of engineered lysins", SCIENCE ADVANCES, vol. 6, no. 23, 3 June 2020 (2020-06-03), page eaaz1136, XP055779045, US ISSN: 2375-2548, DOI: 10.1126/sciadv.aaz1136 abstract</p> <p style="text-align: center;">-----</p>	1-5,7,8, 19,21-42

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/066700

## 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

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## 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:

**see additional 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 an 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.:  
**1-5, 7, 8, 19, 21-42 (all partially)**

### 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.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

## 1. claims: 1-5, 7, 8, 19, 21-42(all partially)

Subject-matter relating to an engineered lysin comprising 1) GN685 (SEQ ID NO: 2) and one or more antimicrobial peptides (AMP) selected from claim 1 (b)(1).

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## 2-18. claims: 1-42(partially)

Subject-matter relating to an engineered lysin comprising respectively one of 2) GN3(R100Q, R116Q) (SEQ ID NO: 177), 3) GN515(R100D, R116H) (SEQ ID NO: 15), 4) GN3 (SEQ ID NO: 3), 5) GN3(R100Q, R116H) (SEQ ID NO: 14), 6) GN515 (SEQ ID NO:6), 7) GN568 (SEQ ID NO: 1), 8) GN428 (SEQ ID NO: 4), 9) GN588 (SEQ ID NO: 5), 10) GN569 (SEQ ID NO: 7), 11) GN37 (SEQ ID NO: 8), 12) QPI14001 (SEQ ID NO: 9), 13) GN917 (SEQ ID NO: 10), 14) CAH0532154 (SEQ ID NO: 11), 15) GN577 (SEQ ID NO: 12), 16) GN867 (SEQ ID NO: 13), 17) GN4 (SEQ ID NO: 175), or 18) GN4 (K100D, R116H) (SEQ ID NO: 176) and one or more antimicrobial peptides (AMP) selected from claim 1 (b)(1), wherein each "GN" lysin represents a separate invention. The numbering of the respective inventions has been introduced before each GN lysin for assistance of the applicant.

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## 19. claims: 1-5, 7, 8, 19, 21-42(all partially)

Subject-matter relating to an engineered lysin comprising 19) GN685 (SEQ ID NO: 2) and a cell penetrating peptide (CPP) selected from claim 1 (b)(2).

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## 20-36. claims: 1-42(partially)

Subject-matter relating to an engineered lysin comprising respectively one of 20) GN3(R100Q, R116Q) (SEQ ID NO: 177), 21) GN515(R100D, R116H) (SEQ ID NO: 15), 22) GN3 (SEQ ID NO: 3), 23) GN3(R100Q, R116H) (SEQ ID NO: 14), 24) GN515 (SEQ ID NO:6), 25) GN568 (SEQ ID NO: 1), 26) GN428 (SEQ ID NO: 4), 27) GN588 (SEQ ID NO: 5), 28) GN569 (SEQ ID NO: 7), 29) GN37 (SEQ ID NO: 8), 30) QPI14001 (SEQ ID NO: 9), 31) GN917 (SEQ ID NO: 10), 32) CAH0532154 (SEQ ID NO: 11), 33) GN577 (SEQ ID NO: 12), 34) GN867 (SEQ ID NO: 13), 35) GN4 (SEQ ID NO: 175), or 36) GN4 (K100D, R116H) (SEQ ID NO: 176) and a cell penetrating peptide (CPP) selected from claim 1 (b)(2), wherein each "GN" lysin represents a separate invention. The numbering of the respective inventions has been introduced before each GN lysin for assistance of the applicant.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/066700

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020046747 A1	05-03-2020	AU 2019327378 A1	29-10-2020
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