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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 December 2021 (30.12.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/263167 A3

(51) International Patent Classification:
C07K 14/54 (2006.01) *A61K 38/20* (2006.01)

(21) International Application Number:
PCT/US2021/039191

(22) International Filing Date:
25 June 2021 (25.06.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/045,041 26 June 2020 (26.06.2020) US
63/199,218 14 December 2020 (14.12.2020) 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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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).

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

(88) Date of publication of the international search report:
21 April 2022 (21.04.2022)

(54) Title: IL-10 MUTEINS AND FUSION PROTEINS THEREOF

(57) Abstract: The present disclosure relates, in general, to muteins of IL-10 that are stable as monomers, antigen binding proteins that bind to TREM-1, and antigen binding proteins comprising IL-10 muteins and antigen binding moieties, e.g., anti-TREM-1 antibodies, and compositions thereof. The disclosure also provides methods of treating inflammatory disease, such as inflammatory bowel disease or ulcerative colitis, using the compositions.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/54 A61K38/20
ADD.

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

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, Sequence Search, BIOSIS, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2016/068583 A1 (VAN VLASSELAER PETER [US] ET AL) 10 March 2016 (2016-03-10) claims 1, 104, 108-109 see also the whole document and in particular paragraphs [0113], [0347], Table 1 and Fig.5. ----- -/--	1,2,4, 35-40 3

[x] Further documents are listed in the continuation of Box C.

[x] 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

"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
11 February 2022	25/02/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ury, Alain

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZDANOV ALEXANDER: "Structural Features of the Interleukin-10 Family of Cytokines", CURRENT PHARMACEUTICAL DESIGN, vol. 10, no. 31, 1 December 2004 (2004-12-01), pages 3873-3884, XP55851067, NL ISSN: 1381-6128, DOI: 10.2174/1381612043382602 Retrieved from the Internet: URL:https://mcl1.ncifcrf.gov/wlo_pubs/zdanov_04.pdf> see the whole document in particular Fig.1 and page 3878, left column.	1-4, 35-40
A	DATABASE UniProt [Online] 20 February 2014 (2014-02-20), "RecName: Full=Cytokine synthesis inhibitory factor {ECO:0000256|ARBA:ARBA00014947}; AltName: Full=Interleukin-10 {ECO:0000256|ARBA:ARBA00016754};", XP002804479, retrieved from EBI accession no. UNIPROT:A0A6I9IZI6 Database accession no. A0A6I9IZI6 the whole document	1-4, 35-40
A	VAN DER LINDE K ET AL: "A functional interleukin-10 mutation in Dutch patients with Crohn's disease", DIGESTIVE AND LIVER DISEASE, W.B. SAUNDERS, GB, vol. 37, no. 5, 1 May 2005 (2005-05-01), pages 330-335, XP004849697, ISSN: 1590-8658, DOI: 10.1016/J.DLD.2004.12.009 the whole document	1-4, 35-40
A	US 2015/218244 A1 (EMRICH THOMAS [DE] ET AL) 6 August 2015 (2015-08-06) paragraphs [0014], [0015], [0084], [0085]	1-4, 35-40
A	US 2008/260820 A1 (BORRELLY GILLES [FR] ET AL) 23 October 2008 (2008-10-23) sequence 826	1-4, 35-40

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE Geneseq [Online] 24 December 2008 (2008-12-24), "Human interleukin-10 mutein, SEQ ID 826.", XP002804480, retrieved from EBI accession no. GS_PROT:ATS35421 Database accession no. ATS35421 the whole document	

A	DE 198 51 675 A1 (BAYER AG [DE]) 11 May 2000 (2000-05-11) paragraph [0006]	1-4, 35-40

A	US 2016/235816 A1 (CHAVEZ RAYMOND A [US] ET AL) 18 August 2016 (2016-08-18) paragraphs [0016], [0054]	1-4, 35-40

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A	LAURA KELLY ERDMAN: "Host Inflammatory Pathways in Malaria Infection: Potential Therapeutic Targets and Biomarkers of Disease Severity", GRADUATE DEPARTMENT OF INSTITUTE OF MEDICAL SCIENCE, 1 November 2011 (2011-11-01), XP055164149, the whole document	11-32, 34-40

A	BOSCO MARIA CARLA ET AL: "Therapeutic Potential of Targeting TREM-1 in Inflammatory Diseases and Cancer", CURRENT PHARMACEUTICAL DESIGN, BENTHAM SCIENCE PUBLISHERS LTD, NL , vol. 22, no. 41 1 January 2016 (2016-01-01), pages 6209-6233, XP009517926, ISSN: 1873-4286, DOI: 10.2174/1381612822666160826110539 Retrieved from the Internet: URL:http://eurekaselect.com/openurl/content.php?spage=6209&genre=article&volume=22&issue=41&issn=1381-6128 the whole document	11-32, 34-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/039191

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. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
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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.:

11-32, 34-40(all partially)

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.:

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: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helical loop AB.

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2. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helical loop CD.

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3. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helical loop DE.

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4. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix A.

---

5. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix B.

---

6. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix C.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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7. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix D.

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8. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix E.

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9. claims: 1-4, 35-40(all partially)

A human interleukin-10 (IL-10) mutein comprising an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 2, wherein said IL-10 mutein has at least one mutation selected from a mutation in helix F.

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10. claims: 5-9(completely); 35-40(partially)

An isolated antigen binding protein, wherein the antigen binding protein: a. is an antibody or antibody fragment; b. binds to human TREM-1 having the amino acid sequence set forth in SEQ ID NO: 20; c. comprises a light chain variable domain, and d. comprises a heavy chain variable domain.

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11. claims: 10(completely); 12-32, 34-40(partially)

An antigen binding protein comprising an antigen-binding moiety and one or two IL-10 moieties wherein :a. the antigen-binding moiety is an antibody or antibody fragment, b. each IL-10 moiety is independently monovalent or bivalent, c. each IL-10 moiety is independently selected from one or more human IL-10 muteins having sequences that are 90% identical to SEQ ID NO: 2, and d. at least one IL-10 moiety is covalently bound to the antigen-binding moiety.

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12. claims: 11(completely); 12-32, 34-40(partially)

An antigen binding protein comprising: (a) a polypeptide sequence having the formula A-L-M or M-L-A, wherein i) A is an immunoglobulin heavy chain of an IgG antibody that binds

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

to a TREM-1 protein set out in SEQ ID NO: 20, ii) L is a linker peptide comprising from 4 to 20 amino acids and iii) M is a mutein of IL-10 having at least 90% sequence identity to wt IL-10 set out in SEQ ID NO: 2; and (b) an immunoglobulin light chain of an IgG antibody that binds TREM-1 protein set out in SEQ ID NO: 20, wherein the immunoglobulin heavy chain of (a) and the immunoglobulin light chain of (b) form an IgG antibody moiety that binds TREM-1, wherein the protein comprises one or two molecules of the polypeptide of (a) and one or two molecules of the light chain of (b), optionally wherein only 1 polypeptide of (a) comprises an M moiety.

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13. claims: 33(completely); 34-40(partially)

An antigen binding protein comprising an antigen-binding moiety and one or two IL-10 moieties wherein:a. the antigen-binding moiety is an antibody or antibody fragment;b. each IL-10 moiety is independently monovalent or bivalent;c. each IL-10 moiety is independently selected from one or more human IL-10 muteins having sequences that are 90% identical to SEQ ID NO: 2;d. at least one IL-10 moiety is covalently bound to the antigen-binding moiety, and/ore. the antigen-binding moiety competes for binding to a human TREM-1 protein with the antigen binding moiety of any one of claims 24 to 27.

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14. claims: 41-44

An isolated antigen binding protein, wherein the antigen binding protein: a. is an antibody or antibody fragment; b. binds to human TREM-1 having the amino acid sequence set forth in SEQ ID NO: 20; c. comprises a light chain variable domain as defined in the claims and d. comprises a heavy chain variable domain as defined in the claims.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/039191

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