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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 January 2022 (20.01.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/015880 A3

(51) International Patent Classification:
C12N 15/62 (2006.01) *A61K 48/00* (2006.01)
A61K 39/39 (2006.01) *A61K 39/385* (2006.01)
A61K 39/00 (2006.01)

(21) International Application Number:
PCT/US2021/041675

(22) International Filing Date:
14 July 2021 (14.07.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/051,472 14 July 2020 (14.07.2020) US
63/110,929 06 November 2020 (06.11.2020) US
63/120,677 02 December 2020 (02.12.2020) US

(71) Applicant: **CUE BIOPHARMA, INC.** [US/US]; 21 Erie Street, Cambridge, Massachusetts 02139 (US).

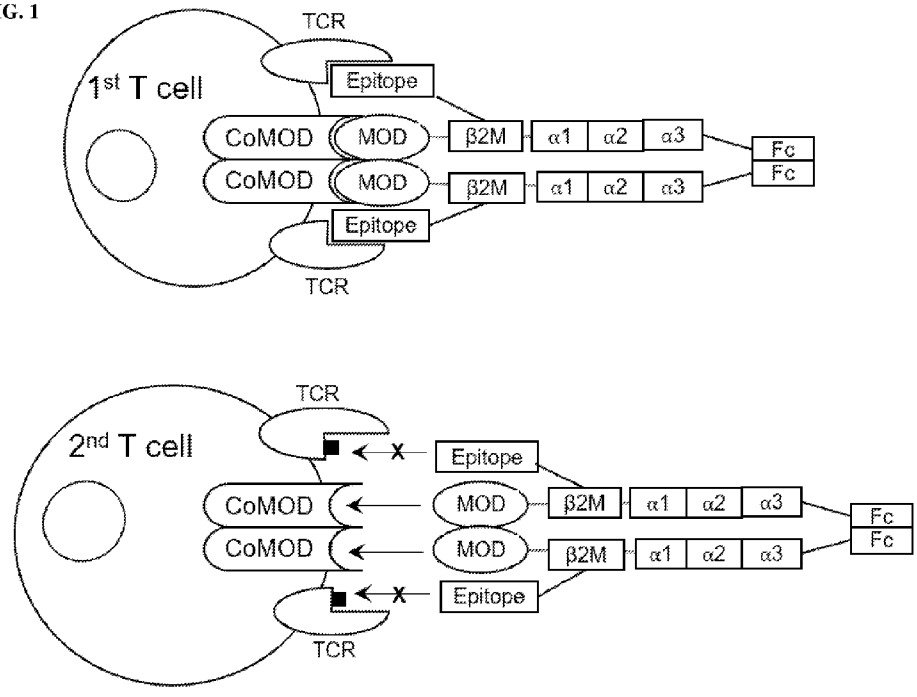
(72) Inventors: **SEIDEL III, Ronald D.**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **CHAPARRO, Rodolfo J.**; 244 Pearl St., Cambridge, Massachusetts 02139 (US). **ROSS, John F.**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **LOW, Chee Meng**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **SURI, Anish**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **MONIZ, Raymond J.**; c/o Cue Biopharma, Inc., 21 Erie Street, Cambridge, Massachusetts 02139 (US). **SAGGU, Gurpanna**; 45 Winter Street, Westwood, Massachusetts 02090 (US).

(74) Agent: **RICIGLIANO, Joseph W.**; Hoffmann & Baron LLP, 6900 Jericho Turnpike, Syosset, New York 11791 (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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

(54) Title: T-CELL MODULATORY POLYPEPTIDES WITH CONJUGATION SITES AND METHODS OF USE THEREOF

FIG. 1



(57) Abstract: The present disclosure provides T cell modulatory polypeptides (T-Cell-MPs) comprising a chemical conjugation site and at least one immunomodulatory polypeptide sequence that may be selected to exhibit reduced binding affinity to its cognate co-immunomodulatory polypeptide. The unconjugated T-Cell-MPs may be conjugated to a molecule displaying an epitope to form a T-Cell-MP-epitope conjugate. The T-Cell-epitope conjugates are useful for modulating the activity (e.g., increasing proliferation or cytotoxic activity) of T cells specific to the conjugate epitope, and accordingly for use as therapeutics.

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[Continued on next page]

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:
03 March 2022 (03.03.2022)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/41675

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/62, A61K 39/39, A61K 39/00, A61K 48/00, A61K 39/385 (2021.01)

CPC - C07K 14/70539, C12N 5/0636, C07K 2319/00, C07K 2319/31, C07K 2319/40

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 2019/0062400 A1 (CUE BIOPHARMA, INC.) 28 February 2019 (28.02.2019) Abstract; Claim 1; Claim 4; Claim 12; Claim 14; Claim 18; Claim 23; para [0005]; para [0019]; para [0071]; SEQ ID 242	1-10, 13
Y	WO 2019/051091 A1 (CUE BIOPHARMA, INC.) 14 March 2019 (14.03.2019) Abstract; Claim 11; para [0084]; para [00359]; para [0364]	1-10, 13

☐ 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
14 November 2021	JAN 20 2022

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/41675

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:

      ☒ 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 13*ter*.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 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.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.
PCT/US 21/41675

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:
----Please see continuation in 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.:
1-10, 13, limited as noted on 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.

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

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 1-23, directed to an unconjugated T cell modulatory polypeptide (T-Cell-MP). The T cell modulatory polypeptide will be searched to the extent that the T cell modulatory polypeptide encompasses a single unconjugated T-Cell-MP comprising a b2M polypeptide sequence wherein the b2M polypeptide sequence has at least 100% sequence identity to at least 90 contiguous aa of the mature human b2M polypeptidc NP_004039.1, SEQ ID NO:61, a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide bond, wherein the MHC-H polypeptide sequence comprises an amino acid sequence having at least 95% sequence identity to at least 200 contiguous aa of: HLA-A*0101 (SEQ ID NO:24), and a single MOD polypeptide sequence wherein the MOD polypeptide is anti-CD28 (note, these are the first claimed sequences for the inventive T cell modulatory polypeptide embodiment). It is believed that claims 1-10, 13 encompass this first named invention, and thus these claims will be searched without fee to the extent that the T cell modulatory polypeptide encompasses a single unconjugated T-Cell-MP comprising a b2M polypeptide sequence wherein the b2M polypeptide sequence has at least 100% sequence identity to at least 90 contiguous aas the mature human b2M polypeptidc NP_004039.1, SEQ ID NO:61, a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide bond wherein the MHC-H polypeptide sequence comprises an amino acid sequence having at least 95% sequence identity to at least 200 contiguous aas of: HLA-A*0101 (SEQ ID NO:24), and a single MOD polypeptide sequence wherein the MOD polypeptide is selected independently from the group consisting of anti-CD28. Additional T cell modulatory polypeptide(s) comprising additional MHC-H sequence(s), MOD sequence(s), duplexed or higher order T-Cell-MP(s) and/or conjugate(s) with epitope(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected T cell modulatory polypeptide(s) comprising additional MHC-H sequence(s), MOD sequence(s), duplexed or higher order T-Cell-MP(s) and/or conjugate(s) with epitope(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 an unconjugated T cell modulatory polypeptide comprising a single T-Cell-MP comprising a b2M polypeptide sequence wherein the b2M polypeptide sequence has at least 100% sequence identity to at least 90 contiguous aa of the mature human b2M polypeptidc NP_004039.1, SEQ ID NO:61, a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide bond, wherein the MHC-H polypeptide sequence comprises an amino acid sequence having at least 95% sequence identity to at least 200 contiguous aa of: HLA-A*0201 (SEQ ID NO:27), and a single MOD polypeptide sequence wherein the MOD polypeptide is anti-CD28 (claims 1-10, 13).

Group II, Claims 24-25, directed to a method of treating disease.

The inventions listed as Groups 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 amino acid sequence. Each amino acid sequence comprises a unique peptide, and is considered a distinct technical feature.
Additionally,
Group I+ has the special technical feature of an unconjugated T cell modulatory polypeptide, that is not required by Group II.
Group II has the special technical feature of a method of treating disease comprising administering to a subject in need thereof an effective amount of a T-Cell-MP-epitope conjugate, that is not required by Group I+.

Common technical features

No technical features are shared between the T cell modulatory polypeptide amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II were considered to share the technical features of including:
an unconjugated T cell modulatory polypeptide (T-Cell-MP), the polypeptide comprising:
(i) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L1 linkers;
(ii) an optional L2 linker polypeptide sequence joining the one or more MOD polypeptide sequences to a b2M polypeptide sequence;
(iii) the b2M polypeptide sequence;
(iv) an L3 linker polypeptide sequence from 10-50 aa in length;

-----please see continuation on next sheet ----------

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

-------continued from previous sheet ----------

(v) a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions at positions 84 and 139 that form an intrachain disulfide bond;
(vi) an optional L4 linker polypeptide sequence;
(vii) a scaffold polypeptide sequence;
(viii) an optional L5 linker polypeptide sequence; and
(ix) optionally one or more MOD polypeptide sequences, or two or more MOD polypeptide sequences in tandem, wherein when there are two or more MOD polypeptide sequences they are optionally joined to each other by independently selected L6 linkers;
wherein the unconjugated T-Cell-MP comprises at least one MOD polypeptide sequence as part of element (i) or (ix)); and
wherein at least one of the b2M polypeptide sequence, the L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site for epitope conjugation,
these shared technical features are made obvious by US 2019/0062400 A1 to Cue Biopharma, Inc., (hereinafter 'Cue').

Cue teaches an unconjugated T cell modulatory polypeptide (T-Cell-MP) (Abstract - 'The present disclosure provides T-cell modulatory multimeric polypeptides, and compositions comprising same, where the T-cell modulatory multimeric polypeptides comprise a variant immunomodulatory polypeptide of the present disclosure.'), the polypeptide comprising: (i) a MOD polypeptide sequence (Claim 1 - 'A variant CD80 immunomodulatory polypeptide'); (iii) the b2M polypeptide sequence (Claim 12 - 'The multimeric polypeptide of any one of claims 4-11, wherein the first MHC polypeptide is a beta2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.');
(iv) an L3 linker polypeptide sequence from 10-50 aa in length (Claim 23 - 'The multimeric polypeptide of claim 22, wherein the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues.');
(v) a class I MHC-H polypeptide sequence, wherein the MHC-H polypeptide comprises cysteine substitutions that form a disulfide bond (Claim 12 - 'The multimeric polypeptide of any one of claims 4-11, wherein the first MHC polypeptide is a beta2-microglobulin polypeptide; and wherein the second MHC polypeptide is an MHC class I heavy chain polypeptide.'; Claim 14 - 'The multimeric polypeptide of claim 11, wherein the MHC class I heavy chain polypeptide is an HLA-A, an HLA-B, or an HLA-C heavy chain.'; para [0005] - 'In some cases, the covalent linkage is via a disulfide bond. In some cases, the first MHC polypeptide or a linker between the epitope and the first MHC polypeptide comprises an amino acid substitution to provide a first Cys residue, and the second MHC polypeptide comprises an amino acid substitution to provide a second Cys residue, and wherein the disulfide linkage is between the first and the second Cys residues');
(vii) a scaffold polypeptide sequence (Claim 18 - 'The multimeric polypeptide of any one of claims 4-10 and 12-17, wherein multimeric polypeptide comprises an Fc polypeptide, and wherein the Ig Fc polypeptide is an IgG1 Fc polypeptide, an IgG2 Fc polypeptide, an IgG3 Fc polypeptide, an IgG4 Fc polypeptide, an IgA Fc polypeptide, or an IgM Fc polypeptide.');
wherein the unconjugated T-Cell-MP comprises at least one MOD polypeptide sequence as part of element (i) or (ix)) (Claim 1). Cue does not expressly teach wherein at least one of the b2M polypeptide sequence, the L3 linker polypeptide sequence, and/or the MHC-H polypeptide sequence comprises a chemical conjugation site for epitope conjugation. However, since Cue teaches a fusion protein with an epitope (Claim 9 - 'The multimeric polypeptide of claim 4, wherein the multimeric polypeptide comprises: a) a first polypeptide comprising, in order from N-terminus to C-terminus: i) an epitope; and ii) a first MHC polypeptide'), it would have been obvious to one of ordinary skill in the art that the epitope could also be linked via chemical conjugation at a preselected or artificially created site, to provide flexibility in the location of the epitope, according to art known methods. Cue does not expressly teach intrachain disulfide linkage at positions 84 and 139 that form an intrachain disulfide bond. However, one of ordinary skill could have considered amino acid substitutions to form inter- or intrachain disulfide linkages to alter the conformation of the polypeptide, during the course of routine experimentation.

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.