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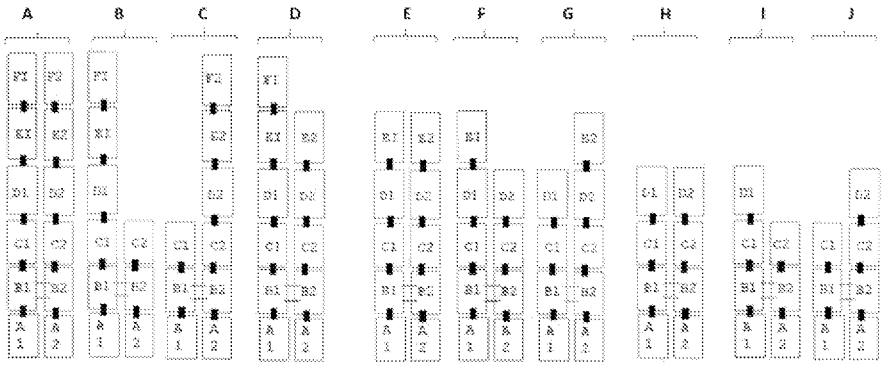
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, 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,

(54) Title: SINGLE-CHAIN AND MULTI-CHAIN SYNTHETIC ANTIGEN RECEPTORS FOR DIVERSE IMMUNE CELLS



A1/A2 = SAR signaling chains comprising Hinge domains (Table 29), transmembrane (Table 28), cytosolic domain (Table 30) and optional co-stimulatory domain (Table 31)
B1/B2 = Ig like linker domain (*e.g.*, IgCL, IgG1-CH1, TCRa-Ig-Like-C1-Domain-6MD *etc.*; See Table 13)
C1/C2 = vL, vH, Va, Vb, Vg, Vd
D1/D2 = AABD
E1/E2 = AABD
F1/F2 = AABD

FIG. 1

(57) **Abstract:** The disclosure provides single-chain and multi-chain synthetic antigen receptors (SARs), methods of making such synthetic antigen receptors, cells expressing said SARs, nucleic acids encoding said SARS, and uses thereof for the treatment of diseases and disorder. The receptors include unispecific, bispecific, multispecific and universal next generation SAR designs. Also presented are novel accessory modules that can be co-expressed with the SARs of the disclosure, cells expressing said accessory modules with and/or without a SAR, and vectors comprising nucleic acids encoding polypeptides for membrane anchored low-affinity variants of cytokines (e.g., IL-2 and/or IL-15), membrane anchored cytokines with epitope tags, and multi-purpose gene switches that serve suicide, survival and marker functions. In one aspect, the disclosure relates to a novel SAR design that confers TCR-like antigen binding specificity to any cell, including the ability to bind to a peptide antigen in association with an MHC or HLA molecule.

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[Continued on next page]

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:

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— *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))*
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/17177

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/16; A61K 38/17; A61K 38/19; A61P 35/00 (2022.01)

CPC - A61K 38/16; A61K 38/17; A61K 38/19; A61P 35/00

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.
X --- Y --- A	WO 2018/102795 A2 (UNIVERSITY OF SOUTHERN CALIFORNIA) 07 June 2018; paragraphs [0010], [0015]-[0016], [0036]-[0037], [0055], [0079], [0085], [0088], [00103], [00119], [00120], [00128], [00141], [00148]-[00149], [00200], [00282], [00309], [00314], [00322], [00325], [00357], [00363], [00367], [00406]-[00407], [00549], [00572]; SEQ ID NO: 2776; SEQ ID NO: 3066; claims 1, 111	1-5, 13-25, 70-72, 80-84, 86-93 --- 73/2, 74/2, 75/1-2, 77/16, 79, 85, 94 --- 76/1-2
Y	US 2003/0181706 A1 (WALKER, MG et al.) 25 September 2003; paragraph [0114]; SEQ ID NO: 13	73/2
Y	US 10,456,420 B2 (NANTKWEST , Inc.) 29 October 2019; column 2, lines 5-10, 50-55; SEQ ID NO: 2	74/2
Y	WO 2013/033626 A2 (TRUSTEES OF DARTMOUTH COLLEGE) 7 March 2013; paragraph [0072]; SEQ ID NO: 22	5/1-2
Y	US 2015/0252080 A1 (UNIVERSITY OF MIAMI) 10 September 2015; paragraph [0098]; SEQ ID NO: 12	77/16
Y	US 2016/0046724 A1 (BROGDON, J et al.) 18 February 2016; paragraphs [0021]; SEQ ID NO: 1	79
Y	US 2018/0355379 A1 (SALK INSTITUTE FOR BIOLOGICAL STUDIES) 13 December 2018; paragraphs [0034], [0120]-[0121], [0148]; SEQ ID No: 15	85

☒ 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
10 July 2022 (10.07.2022)	AUG 10 2022
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 Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/17177

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2020/0038441 A1 (NANTKWEST, INC.) 6 February 2020; paragraphs [0135]-[0136]; SEQ ID NO:15	94
A	US 2007/0124833 A1 (ABAD, MS et al.) 31 May 2007; SEQ ID NO: 23,422	76/1-2
A	US 2018/0296699 A1 (SYNO MINICIRCLE BIOTECHNOLOGY CO. LTD.) 18 October 2018; paragraph [0043]; SEQ ID NO: 10	76/1-2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US22/17177

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/US22/17177

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.: 12, 78, 91, 118-172, 174-176, 179-182, 192-205,
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 Supplemental Page-***-

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.:
-***-Please See Supplemental Page-***-

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-***-

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 examined, the appropriate additional examination fees must be paid.

Groups I+, claims 1-11, 13-77, 79-90, 92-117, 173, 177-178, and 183-191, antibody (heterologous antigen binding domain), CD16A (naturally occurring receptor), SEQ ID NO: 3536 (peptide linker), CD19 (target antigen), SEQ ID NO: 2682 (heterologous antigen binding domain sequence), SEQ ID NO: 3743 (naturally occurring receptor sequence), SEQ ID NO: 9669 (hinge, transmembrane, and cytosolic domain sequence), SEQ ID NO: 3914 (membrane associated domain sequence), SEQ ID NO: 3944 (cytosolic domain sequence), SEQ ID NO: 9856 (activation domain sequence), Cytokine (accessory module), SEQ ID NO: 7833 (accessory module sequence) are directed to synthetic antigen receptors and polypeptides, methods, and cells related thereto.

Group II, claims 206-220 is directed to cells expressing a receptor and methods of production and use thereof.

Group III, claims 221-227, is directed to an isolated fusion protein, and methods and compositions related thereto.

Groups IV+, claims 228-229, SEQ ID NO: 1600 (polynucleotide encoding a synthetic immune receptor), SEQ ID NO: 3994 (synthetic immune receptor polypeptide) are directed to polynucleotide sequences encoding polypeptides encompassing a synthetic immune receptor.

Group V, claims 230-245, is directed to a method for generating a synthetic protein.

The receptors, polypeptides, methods, and cells of Claims 1 (in-part), 2-4, 5 (in-part), 13-22, 23 (in-part), 24-25, 70, 71-77 (each in-part), 79-84, 85 (in-part), 86-90, and 92-94 (each in-part) are believed to encompass the first named invention of Groups I+ and are the claims that will be searched to the extent that they comprise a heterologous antigen binding domain encompassing an antibody (first exemplary heterologous antigen binding domain), a naturally occurring receptor encompassing CD16A (first exemplary naturally occurring receptor), a peptide linker encompassing SEQ ID NO: 3536 (first exemplary peptide linker), a target antigen encompassing CD19 (first exemplary target antigen), a heterologous antigen binding domain sequence encompassing SEQ ID NO: 2682 (first exemplary heterologous antigen binding domain sequence), a naturally occurring receptor sequence encompassing SEQ ID NO: 3743 (first exemplary naturally occurring receptor sequence), a hinge, transmembrane, and cytosolic domain sequence encompassing SEQ ID NO: 9669 (first exemplary hinge, transmembrane, and cytosolic domain sequence), a membrane associated domain sequence encompassing SEQ ID NO: 3914 (first exemplary membrane associated domain sequence), a cytosolic domain sequence encompassing SEQ ID NO: 3944 (first exemplary cytosolic domain sequence), an activation domain sequence encompassing SEQ ID NO: 9856 (first exemplary activation domain sequence), an accessory module encompassing a cytokine (first exemplary accessory module), and an accessory module sequence encompassing SEQ ID NO: 7833 (first exemplary accessory module sequence). This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 1, 5, 23, 51, 63, 72-77, 93, and 94 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional species, with specified SEQ ID NO: for each when appropriate, where available as an option within at least one searchable claim, to be searched. Additional species will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected species. Electing certain alternative species, e.g. a different type of antigen binding domain, may also necessitate electing sequences and related options, which the applicant will need to elect in turn, or the first named species of those will be assumed to be elected. 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/examined. Exemplary elections include a single chain variable fragment (heterologous antigen binding domain) and SEQ ID NO: 2924 (heterologous antigen binding domain sequence) or a membrane-anchored cytokine (accessory module) and SEQ ID NO: 7825 (accessory module sequence).

The polynucleotides and polypeptides of Claims 228-229 (each in-part) are believed to encompass the first named invention of Groups IV+ and are the claims that can be searched to the extent that they comprise a polynucleotide encoding a synthetic immune receptor encompassing SEQ ID NO: 1600 (first exemplary polynucleotide encoding a synthetic immune receptor) and a synthetic immune receptor polypeptide encompassing SEQ ID NO: 3994 (first exemplary synthetic immune receptor polypeptide). If additional fees are paid without electing a different polynucleotide and polypeptide, this first invention will be searched.

Applicant is invited to elect additional polynucleotide and polypeptide sequences, with specified SEQ ID NO: for each, to be searched. Additional sequences can be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected sequences. Applicants must further indicate, if applicable, the claims which encompass the first named invention of Groups IV+, 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) can result in only the first claimed invention of groups IV+ to be searched/examined. An exemplary election would be SEQ ID NO: 1601 (polynucleotide encoding a synthetic immune receptor) and SEQ ID NO: 3995 (synthetic immune receptor polypeptide).

The inventions listed as Groups I+ 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: the special technical features of Groups I+ include synthetic antigen receptors comprising multiple modules and heterologous antigen binding domains, not present in Groups II-III, IV+, and V; the special technical features of Group II include a cell that is not a T cell with target recognition properties and function of a T cell, not present in Groups I+, III, IV+, and V; the special technical features of Group III include a fusion protein between a Type II transmembrane protein and a Type I transmembrane protein, not present in Groups I+, II, IV+, and V; the special technical features of Group IV+ include a recombinant polynucleotide encoding a synthetic immune receptor, not present in Groups I+, II-III, and V; and the special technical features of Group V include a method for generating a synthetic protein comprising two or more chains, not present in Groups I+, II-III, and VI+.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from previous Supplemental Box-***-

Groups I+, II-III, IV+, and V share the technical features including: membrane-anchored or transmembrane proteins.

Groups I+, III, IV+, and V share the additional technical features including: chimeric proteins comprising extracellular domains, linkers, transmembrane domains, and cytosolic domains.

Groups I+, II-III, and IV+ share the additional technical features including: chimeric receptors with antigen-binding properties.

Groups II-III share the additional technical features including: cells expressing chimeric receptors.

However, these shared technical features are previously disclosed by WO 2018/102795 A2 to University Of Southern California (hereinafter "USC").

USC discloses membrane-anchored or transmembrane proteins (a synthetic immune receptor that is directed by a leader sequence to the cell membrane as a transmembrane protein; paragraph [00154]); chimeric proteins comprising extracellular domains (an antigen-binding domain of a SIR is extracellular; paragraph [00154]), linkers (SIRs may comprise linkers between domains; paragraph [00155]), transmembrane domains (the SIR includes a transmembrane domain; paragraph [00200]), and cytosolic domains (the SIR includes a cytosolic domain; paragraph [00200]); chimeric receptors with antigen-binding properties (an antigen-binding domain of a SIR; paragraph [00154]); and cells expressing chimeric receptors (SIRs expressed in a cell; paragraph [00153]).

No technical features are shared between the polypeptide and polynucleotide sequences of Groups I+ and V+ and, accordingly, these groups lack unity a priori.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the USC reference, unity of invention is lacking.

-***-Continued From Box III: Item IV-***-

Groups I+, claims 1-11, 13-77, 79-90, 92-117, 173, 177-178, and 183-191, antibody (heterologous antigen binding domain), CD16A (naturally occurring receptor), SEQ ID NO: 3536 (peptide linker), CD19 (target antigen), SEQ ID NO: 2682 (heterologous antigen binding domain sequence), SEQ ID NO: 3743 (naturally occurring receptor sequence), SEQ ID NO: 9669 (hinge, transmembrane, and cytosolic domain sequence), SEQ ID NO: 3914 (membrane associated domain sequence), SEQ ID NO: 3944 (cytosolic domain sequence), SEQ ID NO: 9856 (activation domain sequence), Cytokine (accessory module), SEQ ID NO: 7833 (accessory module sequence).