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(54) Title: UNIVERSAL CAR-NK CELL TARGETING VARIOUS EPITOPES OF HIV-1 GP160

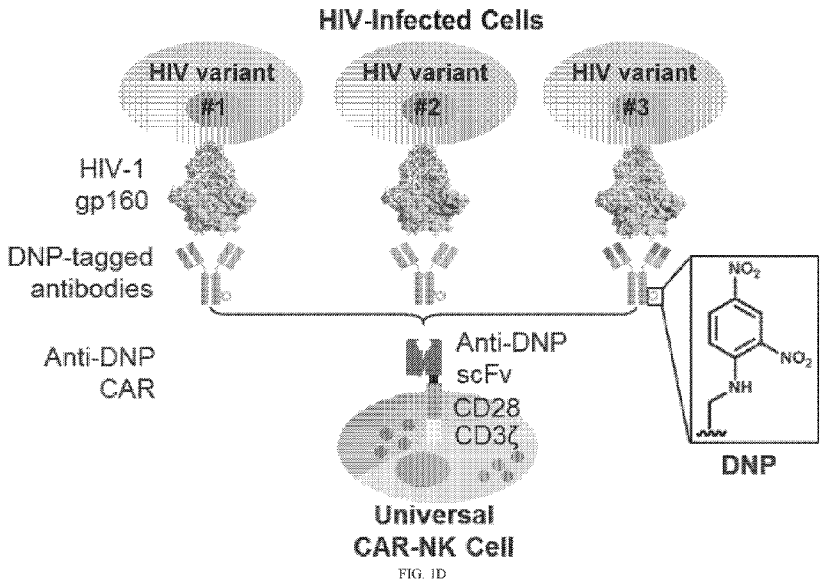

FIG. 1D

(57) Abstract: A universal chimeric antigen receptors (CAR)-modified immune cell is provided, including engineering natural killer (NK) cells and/or T cells which recognizes 2,4-dinitrophenyl (DNP) and can subsequently be redirected to target various epitopes of envelop protein gp160 using DNP-conjugated broad neutralizing antibodies as adaptor molecules. In preferred embodiments, the anti-gp160 antibodies target membrane-distal epitopes. This system can recognize and kill mimic HIV-infected cell lines expressing at least one of subtypes B and C gp160. Presently provided system containing universal CAR-NK cells and (bNAbs) overcome the limitations of conventional anti-HIV CARs, as the latter targets a single epitope of the HIV envelope glycoprotein gp160 and falls short of countering the enormous diversity and mutability of viruses.

[Continued on next page]

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/41469

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 47/55, A61K 35/17, A61K 47/48, A61P 31/16 (2021.01)

CPC - A61K 47/55, A61K 47/64, A61K 39/0013, A61K 36/17, A61K 47/545, A61K 2039/5156, A61K 2039/585

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	US 2015/0320799 A1 (PURDUE RESEARCH FOUNDATION) 12 November 2015 (12.11.2015) Claim 1, Claim 2, Claim 7, para [0012], [0102],	13-14
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Y		1-5
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A		15
Y	WO 2020/023323 A (PURDUE RESEARCH FOUNDATION) 13 February 2020 (13.02.2020) abstract, pg 6, para 7, pg 15, para 3, pg 17, para 4, pg 18, para 4,	1-5
A	US 2005/0152903 A1 (NEWMAN et al.) 14 July 2005 (14.07.2005) abstract, para [0035], SEQ ID NO:17	15
A	US 2019/0177415 A1 (PIERRE FABRE MEDICAMENT) 13 June 2019 (13.09.2019) Claim 183, para [0179], SEQ ID NO: 1289	15

☐ 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
30 November 2021	DEC 29 2021
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 21/41469

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 21/41469

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.: 6-12, 16-36
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 extra sheet for Box No. III Observations where unity of invention is lacking -

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.:
1-5, 13-15 limited to SEQ ID NOs: 11-12

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.

Groups I+: Claims 1-5, 13-15, drawn to a genetically engineered immune cell expressing a chimeric antigen receptor (CAR) specific for 2,4-dinitrophenyl (DNP), and a DNP-modified antibody having a binding affinity specific for a marker associated with human immunodeficiency virus (HIV) or malignant B cells and modified with a DNP moiety. The composition will be searched to the extent that the DNP specific targeting region encompasses VL of SEQ ID NO: 11 and VH of SEQ ID NO: 12. It is believed that claims 1-5, 13-15 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 11-12. Additional DNP specific targeting region(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected DNP specific targeting region(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 a DNP specific targeting region encompassing VL of SEQ ID NO: 15 and VH of SEQ ID NO: 16 (Claim 1-5, 13-15).

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:

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

Additionally, even if the inventions of Groups I+ were considered to share the technical features of including: a chimeric antigen receptor specific for DNP, these shared technical features are previously disclosed by WO 2020/023323 A to Purdue Research Foundation (hereinafter "PRF '323") and US 2015/0320799 A1 to Purdue Research Foundation (hereinafter "PRF '799").

PRF '323 teaches (instant claim 1) a combination for use as a treatment (abstract, The disclosed drug conjugate may form an adaptor to recruit additional CAR T cells or other immune cells for precise elimination of influenza virus-infected cells in a subject.), comprising:
a genetically engineered immune cell expressing a chimeric antigen receptor (CAR) specific for fluorescein (pg 18, para 4, Fig. 13 depicts the CAR T cell strategy treating influenza virus infected cells. In Fig. 13, a zanamivir-FITC conjugate is produced and attached to an influenza virus-infected cell that expresses virus neuraminidase on the surface.....The presence of zanamivir-FITC conjugate at the infected cell surface may direct a T cell adapted with anti-FITC antibody to virus infected cell and form immunological synapse.....Activated CAR T cell therefore may secret cytokines and subsequently kill virus-infected cells, preventing virus replication.), and
wherein the genetically engineered immune cell expressing the CAR specific for the fluorescein recognizes the at least one fluorescein-modified conjugate, so as to target a cell expressing the marker to which the fluorescein-modified antibody has the binding affinity (pg 6, para 7, [a] method used a therapeutic drug comprising an adaptor molecule (i.e. fluorescein covalently bound to the TL), and an anti-fluorescein CAR T cell, wherein upon binding to the adaptor molecule, said CAR-T cell kills influenza virus infected cell that express neuraminidase that binds with TL, and thereby inhibits influenza virus replication in the subject.).

Although PRF '323 does not specifically teach treating HIV-infected cells, PRF '323 teaches that, similar to influenza neuraminidase and hemagglutinin, HIV gp120 is expressed on the exterior surface of cells (pg 15, para 3, For enveloped virus, the last step of its replication involves assembling of viral components on the infected cell membrane and budding from the infected cell surface. Meanwhile, some virus envelope glycoproteins, such as HIV gp120 and influenza neuraminidase/hemagglutinin, are expressed on the exterior surface of infected cells. In light of the fact that these exogenous viral proteins are exclusively expressed on the infected cells, they have the potential to be targeted by ligand targeted drug conjugates.). Thus, it would have been obvious to one of ordinary skill in the art to have developed a fluorescein-modified conjugate that specifically target HIV gp120.

PRF '323 does not specifically teach a DNP-modified antibody wherein the antibody having a binding affinity specific for a marker. PRF '323 teaches a DNP-modified conjugate having a binding affinity specific for a marker (pg 17, para 4, Because of the potential targeting ability of zanamivir to influenza virus or virus infected cells, a zanamivir-dinitrophcnyl (DNP) conjugate was also developed in our lab (Fig. 3). As shown in Figure 3b, zanamivir-DNP conjugate is believed to form a bispecific molecular "bridge" between influenza virus/virus infected cells and endogenous circulating anti-DNP antibodies. This "marking" step initiates the immune response leading to the clearance of the antibody-coated virus or virus infected cell via mechanisms such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).). Thus, it would have been obvious to one of ordinary skill in the art to have developed DNP modified antibody that specifically target HIV gp120.

PRF '323 does not specifically teach a CAR T cell specific for DNP. PRF '799 teaches a CAR T cell specific for DNP (Claim 1, A two component cancer therapeutic comprising: (a) a small conjugate molecule (SCM) comprising a targeted moiety conjugated to a tumor receptor ligand.....and (b) chimeric antigen receptor (CAR)-expressing cytotoxic lymphocytes.....wherein the CAR has binding specificity for the targeted moiety or can be bound by the targeted moiety.; Claim 2, wherein the targeted moiety is a molecule selected from the group consisting of 2,4-dinitrophenol (DNP).). Thus, it would have been obvious to one of ordinary skill in the art to have developed a CAR T cell specific for DNP that could work together with the DNP modified antibody that specifically target HIV gp120.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

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

*Item 4 (continued): Claims 6-12, 16-36 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).