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[Continued on next page]

(54) Title: ENGINEERED OUTER DOMAIN (EOD) OF HIV GP120, MUTANTS AND USE THEREOF

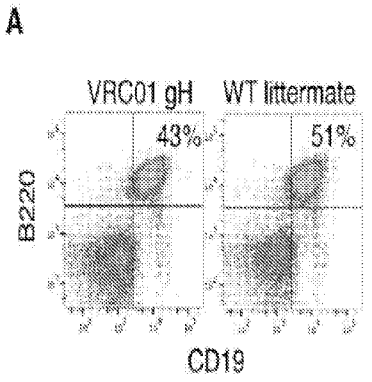

Fig. 1A

(57) Abstract: The present invention relates to engineered outer domain (eOD) immunogens of HIV gp120 and mutants thereof and methods of making and using the same. The present invention also includes fusions of eOD to various protein multimers to enhance immunogenicity. The mutant eODs bind to neutralizing antibody precursors. The mutant eODs can activate germline precursors on the pathway to eliciting a broadly neutralizing antibody (bnAb) response. The invention also relates to immunized knock-in mice expressing germline-reverted heavy chains. Induced antibodies showed characteristics of bnAbs and mutations that favored binding to near-native HIV-1 gp120 constructs. In contrast, native-like immunogens failed to activate precursors. The invention also relates to rational epitope design that can prime rare B cell precursors for affinity maturation to desired targets.

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Published:

— *with international search report (Art. 21(3))*

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

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16 March 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/038162

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/00; A61K 39/21; C07K 14/005 (2017.01)
CPC - A61K 38/00; A61K 39/12; G01N 33/564 (2017.01)
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)
IPC - A61K 38/00; A61K 39/21; C07K 14/005
CPC - A61K 38/00; A61K 39/12; G01N 33/564

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/208.1; 530/350; 536/23.72 (keyword delimited)

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

Search terms used: HIV, glycoprotein, antibody, envelope, engineering, outer domain, mutant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0322269 A1 (UNIVERSITY OF WASHINGTON et al) 30 October 2014 (30.10.2014) entire document	42
P, X	JARDINE et al. "HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen," Science, 25 March 2016 (25.03.2016), Vol. 351, Pgs. 1458-1463. entire document	1-11, 13, 14, 42
A	AZOITEI et al. "Computational design of protein antigens that interact with the CDR H3 loop of HIV broadly neutralizing antibody 2F5," Proteins, 31 July 2014 (31.07.2014), Vol. 82, No. 10, Pgs. 2770-2782. entire document	1-11, 13, 14, 42
A	US 2012/0282264 A1 (MASCOLA et al) 08 November 2012 (08.11.2012) entire document	1-11, 13, 14, 42
A	US 2011/0217338 A1 (PHOGAT et al) 08 September 2011 (08.09.2011) entire document	1-11, 13, 14, 42
A	US 2014/0302081 A1 (THE SCRIPPS RESEARCH INSTITUTE) 09 October 2014 (09.10.2014) entire document	1-11, 13, 14, 42

☐ 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
"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
18 January 2017	03 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/038162

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:

SEQ ID NO:10 was searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/038162

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.: 18-41
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(s).

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.:
1-11, 13, 14, and 42 to the extent that they read on an eOD of SEQ ID NO:10.

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/038162

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 need to be paid.

Group I+: claims 1-17, 42, and 43 are drawn to engineered outer domains (eODs), non-naturally occurring proteins and mouse comprising the same.

The first invention of Group I+ is restricted to an engineered outer domain (eOD), non-naturally occurring proteins and mouse comprising the same, wherein the eOD is selected to be SEQ ID NO:10 (eOD-GT8). It is believed that claims 1-11, 13, 14, and 42 read on this first named invention and thus these claims will be searched without fee to the extent that they read on an eOD of SEQ ID NO:10.

Applicant is invited to elect additional engineered outer domains (eODs) each with SEQ ID NO to be searched in a specific combination by paying an additional fee for each set of election. An exemplary election would be an engineered outer domain (eOD), non-naturally occurring proteins and mouse comprising the same, wherein the eOD is selected to be SEQ ID NO:11. Additional eODs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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.

The inventions listed in 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 Groups I+ formulas do not share a significant structural element responsible for eliciting an immune response and/or binding to VRC01-class antibodies, requiring a selection of alternatives for the engineered outer domain, where the "engineered outer domain (eOD) with at least 90% homology or identity with SEQ ID NO: 10 (eOD-GT8) or 11 (eOD-GT10)" or "the protein comprises any one of SEQ ID NO: 10 to 40 or any combination thereof".

The Groups I+ share the technical features of a non-naturally occurring protein comprising an engineered outer domain (eOD); a non-naturally occurring protein comprising an eOD variant comprising at least one mutation; a non-naturally occurring protein capable of binding to VRC01-class antibodies comprising a HIV gp120-derived variant; a non-naturally occurring protein comprising a monomeric engineered outer domain (eOD); a non-naturally occurring fused to a multimerization motif; a non-naturally occurring protein comprising an eOD 60mer fused to a lumazine synthase protein; a knock-in mouse for testing immunogens capable of activating germline precursors of VRCO1 class antibodies, said mouse comprising a substitution of the VRCO1 gH VDJ exon, wherein the CDRH3 comprises a mutation to remove an unpaired cysteine; a non-naturally occurring protein comprising an HIV boosting molecule. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2014/0322269 A1 to University of Washington et al. discloses a non-naturally occurring protein (The invention relates to an engineered outer domain (eOD) of HIV gp 120 and mutants thereof, Abstract) comprising an engineered outer domain (eOD) (naturally occurring protein which may comprise an engineered outer domain (eOD) mutation, Para. [0015]); a non-naturally occurring protein comprising an eOD variant comprising at least one mutation (naturally occurring protein which may comprise an engineered outer domain (eOD) mutation, Para. [0015]); a non-naturally occurring protein capable of binding to VRC01-class antibodies comprising a HIV gp120-derived variant (The present invention relates to engineering of the eOD to: (a) improve binding to mature VRC01, in which Applicants use mature VRC01 binding affinity as a readout for structural mimicry of HIV, Para. [0014]); a non-naturally occurring protein comprising a monomeric engineered outer domain (eOD) (naturally occurring protein which may comprise an engineered outer domain (eOD) mutation, Para. [0015]); a non-naturally occurring fused to a multimerization motif (designed fusions of eOD to various protein multimers to enhance innnunogenicity, including 4 mers, 8 mers, 24 mers, 60 mers, and 180 mers, most important of which are the 60 mers that present eOD on a virus-like particle, Para. [0014]); a non-naturally occurring protein comprising an eOD 60mer fused to a lumazine synthase protein (designed fusions of eOD to various protein multimers to enhance immunogenicity, including 4 mers, 8 mers, 24 mers, 60 mers, and 180 mers, most important of which are the 60 mers that present eOD on a virus-like particle, Para. [0014]; Applicants formed larger virus-like particles of eOD by engineering fusions to proteins that assemble into 24mers...lumazine, Para. [0267]); a knock-in mouse for testing immunogens capable of activating germline precursors of VRC01 class antibodies (determine therefor: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse, Para. [0312]; The fact that removal of the glycan at 276 allows measureable binding by germline VRC01 indicates that this mutation may be essential for germline-targeting immunogens. It also indicates that the HIV strains that infected the Patients, Para. [0225]), said mouse comprising a substitution of the VRC01 gH VDJ exon (In the development of germline-targeting eODs described below, two additional modifications were identified for improving the binding of mature VRC01. One is the mutation L260F, Para. [0228]), wherein the CDRH3 comprises a mutation to remove an unpaired cysteine (human germline VRC01 antibody uses the mature VRC01 HCDR3, Para. [0256]; Furthermore, the V4 is bounded in sequence by two cysteines that are paired in disulfide bonds., Para. [0179]); a non-naturally occurring protein comprising an HIV boosting molecule (naturally occurring protein which may comprise an engineered outer domain (eOD) mutation, Para. [0015]; the vaccine may incorporate set booster doses. For example, booster doses may comprise variants in order to provide protection against multiple clades of HIV, Para. [0310]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.