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(54) Title: BROADLY NEUTRALIZING ANTIBODIES AGAINST HIV-1 AND USE THEREOF

(57) Abstract: Broadly neutralizing antibodies against HIV-1 which specifically bind to gp120 of HIV-1, and a method of preparing such antibodies and the use thereof are provided.



Broadly Neutralizing Antibodies against HIV-1 and Use thereof

Technical Field

The present invention relates to broadly neutralizing antibodies against HIV-1 which specifically bind to gp120 of HIV-1. The present invention also relates to the methods for preparing such antibodies and the use thereof.

Technical Background

HIV-1 neutralizing antibodies (NAbs) could effectively block HIV-1 entry into human CD4+T cells. As a therapeutic strategy, potent and broadly reactive NAbs have been developed in AIDS research.

Monoclonal antibody b12 was isolated from a phage display library, which neutralizes about 40% known HIV-1 isolates ^[1]. With the improvement of techniques including single B cell sorting and deep sequencing, a number of monoclonal broadly neutralizing antibodies have been isolated from HIV-1-infected donors which recognize a range of epitopes on HIV-1 viral spike since 2010. These sites include the CD4-binding site on gp120 (VRC01) ^[2], the glycan-containing regions of V1V2 on gp120 (PG9 and PG16) ^[3], the V3 region centered on the N332 glycan of gp120 (PGT121) ^[4] and the membrane-proximal external region (MPER) on gp41 (10E8) ^[5], and a conserved face on contiguous areas of gp41 and gp120 (35O22) ^[6].

Because HIV-1 has various clades and there are different major epidemic strains worldwide, combination of different broadly NAbs will be a more potential treatment strategy for AIDS. Therefore, it is still necessary to develop new potent NAbs for HIV-1.

Summary of Invention

The present invention provides human monoclonal antibodies and functional fragments thereof which are capable of specifically binding to gp120 of HIV-1 and blocking the entry of HIV-1 into target cells. The present invention also provides nucleic acid molecules encoding the above described antibodies or antibody fragments as well as an expression vector comprising at least one nucleic acid molecule as described above. The present invention also provides a host cell transformed with at least one nucleic acid molecule or expression vector as described above. The present invention also provides a method of producing an antibody by using at least one nucleic acid molecule or expression vector or host cell as described above. The present invention also provides a pharmaceutical composition comprising at least one antibody or antibody fragment as

described above. In some embodiments, the heavy chain variable domain (VH) of the antibody of the invention comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to amino acids 31-35, 50-66, and 99-109 of SEQ ID NO:2. In some embodiments, the light chain variable domain (VL) of the antibody of the invention comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:4. In some other embodiments, the VL of the antibody of the invention comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 22-32, 48-54, and 87-91 of SEQ ID NO:6. In some other embodiments, the VL of the antibody of the invention comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:8. The antibodies of the invention are broadly neutralizing antibodies which specifically bind to gp120 of HIV-1.

The present invention also provides method of detecting an HIV-1 infection in a human subject comprising contacting a biological sample from the subject with an antibody or antibody fragment of the present invention, and determining the presence of an immune complex formed by the antibody or the antibody fragment in said sample, wherein the presence of said immune complex indicates that the subject has an HIV-1 infection. In some embodiments, prior to the contacting, the sample is immobilized on a solid substrate. In some other embodiments, prior to the contacting, the antibody or antibody fragment is immobilized on a solid substrate. In some embodiments, the antibody or antibody fragment is labeled with a fluorescent, enzymatic, or radioactive label. In some embodiments, the immune complex is detected by using a second antibody capable of specifically binding to the antibody or antibody fragment. In some other embodiments, the immune complex is detected by using a second antibody capable of specifically binding to an antigen of HIV-1. The present invention also provides a kit for detecting an HIV-1 infection in a human subject, comprising an antibody or antibody fragment of the present invention.

The present invention further provides a method of preventing or treating an HIV-1 infection in a human subject, comprising administering to the subject an effective amount of at least one antibody or antibody fragment of the invention or pharmaceutical composition of the invention. In some embodiments, the subject has acquired immune deficiency syndrome (AIDS). In some embodiments, the method further comprises administering to the subject at least one anti-viral agent against HIV-1.

The present invention also relates to use of the antibody or antibody fragment of the invention in manufacture of a kit for detecting an HIV-1 infection in a human subject or in manufacture of a pharmaceutical composition for preventing or treating an HIV-1 infection

in a human subject.

Brief Description of the Drawings

Fig.1 shows the binding capacity of the monoclonal antibodies of the invention to HIV-1 antigens. (A) The binding capacity of DRVIA7 to gp140. (B) The binding capacity of antibody to gp120. (C) The binding capacity of antibody DRVIA7H+gDRVI01-L57 to gp140. (D) The binding capacity of antibody DRVIA7H+gDRVI01-L40 to gp140.

Fig.2 shows the ribbon diagrams of the crystal structure of DRVIA7 bound to gp120.

Fig.3 shows the residue-based energy evaluation of gp120 in complex with DRVIA7.

Fig.4 shows the crystal structure of N-terminus and CDRL1 of DRVIA7 bound to gp120.

Fig.5 shows the sequence analysis of antibody DRVIA7 and VRC01 heavy and light chains with alignment to respective germline genes.

Fig.6 shows the crystal structure of the DRVIA7 compared with VRC01-like antibodies.

Fig.7 shows the identity/divergence 2D analysis of the light chain repertoires from the HIV-1 infected donor.

Sequence Listing

SEQ ID NO:1 is the sequence encoding the DRVIA7 VH.

SEQ ID NO:2 is the amino acid sequence of the DRVIA7 VH.

SEQ ID NO:3 is the sequence encoding the DRVIA7 VL.

SEQ ID NO:4 is the amino acid sequence of the DRVIA7 VL.

SEQ ID NO:5 is the sequence encoding the VL gDRVI01-L57.

SEQ ID NO:6 is the amino acid sequence of the VL gDRVI01-L57.

SEQ ID NO:7 is the sequence encoding the VL gDRVI01-L40.

SEQ ID NO:8 is the amino acid sequence of the VL gDRVI01-L40.

Deposits of Biological Materials

Escherichia coli strain carrying the expression vector containing the heavy chain gene of DRVIA7 was deposited at CGMCC on December 14, 2015 under the Deposit Number CGMCC No.11879.

Escherichia coli strain carrying the expression vector containing the light chain gene of DRVIA7 was deposited at CGMCC on December 14, 2015 under the Deposit Number CGMCC No.11880.

Escherichia coli strain carrying the expression vector containing the light chain gene encoding the light chain variable domain gDRVI01-L57 was deposited at CGMCC on December 14, 2015 under the Deposit Number CGMCC No. 11881.

Escherichia coli strain carrying the expression vector containing the light chain gene encoding the light chain variable domain gDRVI01-L40 was deposited at CGMCC on December 14, 2015 under the Deposit Number CGMCC No. 11882.

Detailed Description

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the invention(s) belong. In addition, the terms and experimental procedures relating to protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology and immunology are those terms and common procedures widely used in the art. Meanwhile, for better understanding of the invention, definitions and explanations of relevant terms are provided below.

As used herein, “antibody” refers to immunoglobulins and immunoglobulin fragments, whether natural or partially or wholly synthetically, such as recombinantly, produced, including any fragment thereof containing at least a portion of the variable region of the immunoglobulin molecule that retains the binding specificity ability of the full-length immunoglobulin. Hence, an antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin antigen-binding domain (antibody combining site). Antibodies include antibody fragments, such as anti-HIV-1 antibody fragments. As used herein, the term antibody, thus, includes synthetic antibodies, recombinantly produced antibodies, multispecific antibodies (e.g., bispecific antibodies), human antibodies, non-human antibodies, humanized antibodies, chimeric antibodies, intrabodies, and antibody fragments, such as, but not limited to, Fab fragments, Fab' fragments, F(ab')₂ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments, single-chain Fvs (scFv), single-chain Fabs (scFab), diabodies, anti-idiotypic (anti-Id) antibodies, or antigen-binding fragments of any of the above. Antibodies provided herein include members of any immunoglobulin type (e.g., IgG, IgM, IgD, IgE, IgA and IgY), any class (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass (e.g., IgG2a and IgG2b).

As used herein, an “antibody fragment” or “antigen-binding fragment” of an antibody refers to any portion of a full-length antibody that is less than full length but contains at

least a portion of the variable region of the antibody that binds antigen (e.g. one or more CDRs and/or one or more antibody combining sites) and thus retains the binding specificity, and at least a portion of the specific binding ability of the full-length antibody. Hence, an antigen-binding fragment refers to an antibody fragment that contains an antigen-binding portion that binds to the same antigen as the antibody from which the antibody fragment is derived. Antibody fragments include antibody derivatives produced by enzymatic treatment of full-length antibodies, as well as synthetically, e.g. recombinantly produced derivatives. An antibody fragment is included among antibodies. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, single-chain Fv (scFv), Fv, dsFv, diabody, Fd and Fd' fragments and other fragments, including modified fragments (see, for example, *Methods in Molecular Biology*, Vol 207: *Recombinant Antibodies for Cancer Therapy Methods and Protocols* (2003); Chapter 1; p 3-25, Kipriyanov). The fragment can include multiple chains linked together, such as by disulfide bridges and/or by peptide linkers. An antibody fragment generally contains at least or about 50 amino acids and typically at least or about 200 amino acids. An antigen-binding fragment includes any antibody fragment that when inserted into an antibody framework (such as by replacing a corresponding region) results in an antibody that immunospecifically binds (i.e. exhibits K_a of at least or at least about 10^7 - 10^8 M⁻¹) to the antigen.

As used herein, a “neutralizing antibody” is any antibody or antigen-binding fragment thereof that binds to a pathogen and interferes with the ability of the pathogen to infect a cell and/or cause disease in a subject.

As used herein, “monoclonal antibody” refers to a population of identical antibodies, meaning that each individual antibody molecule in a population of monoclonal antibodies is identical to the others. This property is in contrast to that of a polyclonal population of antibodies, which contains antibodies having a plurality of different sequences. Monoclonal antibodies can be produced by a number of well-known methods (Smith et al. (2004) *J. Clin. Pathol.* 57, 912-917; and Nelson et al., *J Clin Pathol* (2000), 53, 111-117). For example, monoclonal antibodies can be produced by immortalization of a B cell, for example through fusion with a myeloma cell to generate a hybridoma cell line or by infection of B cells with virus such as EBV. Recombinant technology also can be used to produce antibodies *in vitro* from clonal populations of host cells by transforming the host cells with plasmids carrying artificial sequences of nucleotides encoding the antibodies.

As used herein, a “conventional antibody” refers to an antibody that contains two heavy chains (which can be denoted H and H') and two light chains (which can be denoted

L and L') and two antibody combining sites, where each heavy chain can be a full-length immunoglobulin heavy chain or any functional region thereof that retains antigen-binding capability (e.g. heavy chains include, but are not limited to, V_H , chains V_H - C_{H1} chains and V_H - C_{H1} - C_{H2} - C_{H3} chains), and each light chain can be a full-length light chain or any functional region of (e.g. light chains include, but are not limited to, V_L chains and V_L - C_L chains). Each heavy chain (H and H') pairs with one light chain (L and L', respectively)

As used herein, a full-length antibody is an antibody having two full-length heavy chains (e.g. V_H - C_{H1} - C_{H2} - C_{H3} or V_H - C_{H1} - C_{H2} - C_{H3} - C_{H4}) and two full-length light chains (V_L - C_L) and hinge regions, such as human antibodies produced naturally by antibody secreting B cells and antibodies with the same domains that are synthetically produced.

As used herein, a dsFv refers to an Fv with an engineered intermolecular disulfide bond, which stabilizes the V_H - V_L pair.

As used herein, a Fab fragment is an antibody fragment that results from digestion of a full-length immunoglobulin with papain, or a fragment having the same structure that is produced synthetically, *e.g.* by recombinant methods. A Fab fragment contains a light chain (containing a V_L and C_L) and another chain containing a variable domain of a heavy chain (V_H) and one constant region domain of the heavy chain (C_{H1}).

As used herein, a $F(ab')_2$ fragment is an antibody fragment that results from digestion of an immunoglobulin with pepsin at pH 4.0-4.5, or a fragment having the same structure that is produced synthetically, *e.g.* by recombinant methods. The $F(ab')_2$ fragment essentially contains two Fab fragments where each heavy chain portion contains an additional few amino acids, including cysteine residues that form disulfide linkages joining the two fragments.

As used herein, a Fab' fragment is a fragment containing one half (one heavy chain and one light chain) of the $F(ab')_2$ fragment.

As used herein, an scFv fragment refers to an antibody fragment that contains a variable light chain (V_L) and variable heavy chain (V_H), covalently connected by a polypeptide linker in any order. The linker is of a length such that the two variable domains are bridged without substantial interference. Exemplary linkers are $(Gly-Ser)_n$ residues with some Glu or Lys residues dispersed throughout to increase solubility.

As used herein, the term "epitope" refers to any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants typically contain chemically active surface groupings of molecules such as amino acids or sugar side chains and typically have specific three dimensional structural characteristics, as well as specific charge characteristics.

As used herein, a variable domain or variable region is a specific Ig domain of an antibody heavy or light chain that contains a sequence of amino acids that varies among different antibodies. Each light chain and each heavy chain has one variable region domain, V_L and V_H , respectively. The variable domains provide antigen specificity, and thus are responsible for antigen recognition. Each variable region contains CDRs that are part of the antigen-binding site domain and framework regions (FRs).

As used herein, “antigen-binding domain,” “antigen-binding site,” “antigen combining site” and “antibody combining site” are used synonymously to refer to a domain within an antibody that recognizes and physically interacts with cognate antigen. A native conventional full-length antibody molecule has two conventional antigen-binding sites, each containing portions of a heavy chain variable region and portions of a light chain variable region. A conventional antigen-binding site contains the loops that connect the anti-parallel beta strands within the variable region domains. The antigen combining sites can contain other portions of the variable region domains. Each conventional antigen-binding site contains three hypervariable regions from the heavy chain and three hypervariable regions from the light chain. The hypervariable regions also are called complementarity-determining regions (CDRs).

As used herein, “hypervariable region,” “HV,” “complementarity-determining region” and “CDR” and “antibody CDR” are used interchangeably to refer to one of a plurality of portions within each variable region that together form an antigen-binding site of an antibody. Each variable region domain contains three CDRs, named CDR1, CDR2 and CDR3. The three CDRs are non-contiguous along the linear amino acid sequence, but are proximate in the folded polypeptide. The CDRs are located within the loops that join the parallel strands of the beta sheets of the variable domain. As described herein, one of skill in the art knows and can identify the CDRs based on Kabat or Chothia numbering (see e.g., Kabat, E.A. *et al.* (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917).

As used herein, framework regions (FRs) are the domains within the antibody variable region domains that are located within the beta sheets; the FR regions are comparatively more conserved, in terms of their amino acid sequences, than the hypervariable regions.

As used herein, a “constant region” domain is a domain in an antibody heavy or light chain that contains a sequence of amino acids that is comparatively more conserved than that of the variable region domain. In conventional full-length antibody molecules, each

light chain has a single light chain constant region (C_L) domain and each heavy chain contains one or more heavy chain constant region (C_H) domains, which include, C_{H1} , C_{H2} , C_{H3} and C_{H4} . Full-length IgA, IgD and IgG isotypes contain C_{H1} , C_{H2} , C_{H3} and a hinge region, while IgE and IgM contain C_{H1} , C_{H2} , C_{H3} and C_{H4} . C_{H1} and C_L domains extend the Fab arm of the antibody molecule, thus contributing to the interaction with antigen and rotation of the antibody arms. Antibody constant regions can serve effector functions, such as, but not limited to, clearance of antigens, pathogens and toxins to which the antibody specifically binds, e.g., through interactions with various cells, biomolecules and tissues.

As used herein, a functional region of an antibody is a portion of the antibody that contains at least a V_H , V_L , C_H (e.g. C_{H1} , C_{H2} or C_{H3}), C_L or hinge region domain of the antibody, or at least a functional region thereof.

As used herein, a functional region of a V_H domain is at least a portion of the full V_H domain that retains at least a portion of the binding specificity of the full V_H domain (e.g. by retaining one or more CDR of the full V_H domain), such that the functional region of the V_H domain, either alone or in combination with another antibody domain (e.g. V_L domain) or region thereof, binds to antigen. Exemplary functional regions of V_H domains are regions containing the CDR1, CDR2 and/or CDR3 of the V_H domain.

As used herein, a functional region of a V_L domain is at least a portion of the full V_L domain that retains at least a portion of the binding specificity of the full V_L domain (e.g. by retaining one or more CDRs of the full V_L domain), such that the function region of the V_L domain, either alone or in combination with another antibody domain (e.g. V_H domain) or region thereof, binds to antigen. Exemplary functional regions of V_L domains are regions containing the CDR1, CDR2 and/or CDR3 of the V_L domain.

As used herein, “specifically bind” or “immunospecifically bind” with respect to an antibody or antigen-binding fragment thereof are used interchangeably herein and refer to the ability of the antibody or antigen-binding fragment to form one or more noncovalent bonds with a cognate antigen, by noncovalent interactions between the antibody combining site(s) of the antibody and the antigen. The antigen can be an isolated antigen or presented in a virus. Typically, an antibody that immunospecifically binds (or that specifically binds) to a virus antigen or virus is one that binds to the virus antigen (or to the antigen in the virus or to the virus) with an affinity constant K_a of about or $1 \times 10^7 \text{ M}^{-1}$ or $1 \times 10^8 \text{ M}^{-1}$ or greater (or a dissociation constant (K_d) of $1 \times 10^{-7} \text{ M}$ or $1 \times 10^{-8} \text{ M}$ or less). Affinity constants can be determined by standard kinetic methodology for antibody reactions, for example, immunoassays, surface plasmon resonance (SPR) (Rich and

Myszka (2000) *Curr. Opin. Biotechnol* 11:54; Englebienne (1998) *Analyst*. 123:1599), isothermal titration calorimetry (ITC) or other kinetic interaction assays known in the art (see, e.g., Paul, ed., *Fundamental Immunology*, 2nd ed., Raven Press, New York, pages 332-336 (1989); see also U.S. Pat. No. 7,229,619 for a description of exemplary SPR and ITC methods for calculating the binding affinity of antibodies). Instrumentation and methods for real time detection and monitoring of binding rates are known and are commercially available (e.g., BiaCore 2000, Biacore AB, Upsala, Sweden and GE Healthcare Life Sciences; Malmqvist (2000) *Biochem. Soc. Trans.* 27:335).

As used herein, "polypeptide" refers to two or more amino acids covalently joined. The terms "polypeptide" and "protein" are used interchangeably herein.

In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and generally can be made without altering a biological activity of a resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson et al., *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

As used herein, "nucleic acid" refers to at least two linked nucleotides or nucleotide derivatives, including a deoxyribonucleic acid (DNA) and a ribonucleic acid (RNA), joined together, typically by phosphodiester linkages.

Sequence "identity" has an art-recognized meaning and the percentage of sequence identity between two nucleic acid or polypeptide molecules or regions can be calculated using published techniques. Sequence identity can be measured along the full length of a polynucleotide or polypeptide or along a region of the molecule. (See, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptides, the term "identity" is well known to skilled artisans (Carrillo, H. & Lipman, D., *SIAM J Applied Math* 48:1073 (1988)).

As used herein, "operably linked" with reference to nucleic acid sequences, regions, elements or domains means that the nucleic acid regions are functionally related to each other. For example, a promoter can be operably linked to nucleic acid encoding a

polypeptide, whereby the promoter regulates or mediates the transcription of the nucleic acid.

As used herein, “expression” refers to the process by which polypeptides are produced by transcription and translation of polynucleotides. The level of expression of a polypeptide can be assessed using any method known in art, including, for example, methods of determining the amount of the polypeptide produced from the host cell. Such methods can include, but are not limited to, quantitation of the polypeptide in the cell lysate by ELISA, Coomassie blue staining following gel electrophoresis, Lowry protein assay and Bradford protein assay.

As used herein, a “host cell” is a cell that is used in to receive, maintain, reproduce and amplify a vector. A host cell also can be used to express the polypeptide encoded by the vector. The nucleic acid contained in the vector is replicated when the host cell divides, thereby amplifying the nucleic acids.

As used herein, a “vector” is a replicable nucleic acid from which one or more heterologous proteins can be expressed when the vector is transformed into an appropriate host cell. Reference to a vector includes those vectors into which a nucleic acid encoding a polypeptide or fragment thereof can be introduced, typically by restriction digest and ligation. Reference to a vector also includes those vectors that contain nucleic acid encoding a polypeptide. The vector is used to introduce the nucleic acid encoding the polypeptide into the host cell for amplification of the nucleic acid or for expression/display of the polypeptide encoded by the nucleic acid. The vectors typically remain episomal, but can be designed to effect integration of a gene or portion thereof into a chromosome of the genome. Also contemplated are vectors that are artificial chromosomes, such as yeast artificial chromosomes and mammalian artificial chromosomes. Selection and use of such vehicles are well known to those of skill in the art.

As used herein, a vector also includes “virus vectors” or “viral vectors.” Viral vectors are engineered viruses that are operatively linked to exogenous genes to transfer (as vehicles or shuttles) the exogenous genes into cells.

As used herein, an “expression vector” includes vectors capable of expressing DNA that is operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Such additional segments can include promoter and terminator sequences, and optionally can include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or can contain elements of both. Thus, an expression vector refers to a recombinant DNA or

RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

As used herein, "treating" a subject with a disease or condition means that the subject's symptoms are partially or totally alleviated, or remain static following treatment. Hence treatment encompasses prophylaxis, therapy and/or cure. Prophylaxis refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease. Treatment also encompasses any pharmaceutical use of any antibody or antigen-binding fragment thereof provided or compositions provided herein.

As used herein, a "therapeutic effect" means an effect resulting from treatment of a subject that alters, typically improves or ameliorates the symptoms of a disease or condition or that cures a disease or condition.

As used herein, a "therapeutically effective amount" or a "therapeutically effective dose" refers to the quantity of an agent, compound, material, or composition containing a compound that is at least sufficient to produce a therapeutic effect following administration to a subject. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

As used herein, a "prophylactically effective amount" or a "prophylactically effective dose" refers to the quantity of an agent, compound, material, or composition containing a compound that when administered to a subject, will have the intended prophylactic effect, *e.g.*, preventing or delaying the onset, or reoccurrence, of disease or symptoms, reducing the likelihood of the onset, or reoccurrence, of disease or symptoms, or reducing the incidence of viral infection. The full prophylactic effect does not necessarily occur by administration of one dose, and can occur only after administration of a series of doses. Thus, a prophylactically effective amount can be administered in one or more administrations.

Anti-HIV-1 antibodies

The inventors isolated a monoclonal antibody from an HIV-1-infected Chinese donor sample and found that it has broadly neutralizing activities on a number of HIV-1 isolates. This monoclonal antibody is named DRVIA7. Based on the heavy chain of DRVIA7 and through light chain modification technique and light chain gene repertoire screening, additional antibodies with increased potency and broadly neutralizing activities were

obtained. The antibodies of the invention are capable of blocking the entry of HIV-1 into target cells.

In one aspect, the present invention provides an isolated human monoclonal antibody comprising a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to VHCDR1, VHCDR2, and VHCDR3 contained in the amino acid sequence of SEQ ID NO:2, wherein the antibody is a neutralizing antibody which specifically binds to gp120 of HIV-1.

In some embodiments, the heavy chain variable domain comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to amino acids 31-35, 50-66, and 99-109 of SEQ ID NO:2.

As used herein, when referring to the antibody of the invention, the expression “the heavy chain variable domain comprises VHCDR1 corresponding to VHCDR1 contained in the amino acid sequence of SEQ ID NO:2” means VHCDR1 in the heavy chain variable domain of said antibody has the same amino acid sequence with that of VHCDR1 contained in the amino acid sequence of SEQ ID NO:2. For example, as determined based on the Kabat numbering, VHCDR1 of antibody DRVIA7 consists of amino acids 31-35 (SSFIH) of the heavy chain variable domain as shown in SEQ ID NO:2, thus, the above expression means VHCDR1 of the heavy chain variable domain of the antibody of the invention also consists of SSFIH.

As used herein, VHCDR, HCDR, and CDRH have the same meaning and can be used interchangeably to refer to the complementary determining region of the antibody heavy chain variable domain.

In some embodiments, the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:2 or an amino acid sequence having at least 85%, at least 90%, at least 95% or even higher sequence identity with SEQ ID NO:2. Alternatively, the heavy chain variable domain consists of an amino acid sequence of SEQ ID NO:2 or an amino acid sequence having at least 85%, at least 90%, at least 95% or even higher sequence identity with SEQ ID NO:2. In some embodiments, the heavy chain variable domain comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with SEQ ID NO:2. In some preferred embodiments, the heavy chain variable domain comprises VHCDR1, VHCDR2, and VHCDR3 as set out in Table 1.

Table 1. VH/VL sequences and CDRs of exemplary antibodies of the invention (based on the Kabat

numbering)		
VH	QVQLVESGTQFRPGASVRLSCEASGYTFI <u>SSFIH</u> WIRQGPGQGLEWMG CDR1 <u>WMNPRHGAVNYPRRFQG</u> KVTMTRDTSIDTAYMELRDLRSDDTAMYFCVT CDR2 <u>SRTKDYDWDFV</u> WGQGTLLVVSS CDR3	SEQ ID NO:2
VL	DIQMTQSPVTLASISIGDRVITITC <u>RASQRIDNWVA</u> WYQQKPGRAPKLLIY CDR1 <u>KASILET</u> GVPSRFSGSGSGTEFTLSINSLQPDDVATYYC <u>QOFEE</u> FGRGT CDR2 CDR3 KIDIK	SEQ ID NO:4
	QMTQSPVTLASISIGDRVITITC <u>RASQRIDNWVA</u> WYQQKPGRAPKLLIY <u>KA</u> CDR1 <u>SILET</u> GVPSRFSGSGSGTEFTLSINSLQPDDVATYYC <u>QOFEE</u> FGRGTKI CDR2 CDR3 DIK	SEQ ID NO:6
	SIWLTQSPSTLASVGDVRTITTC <u>RASESIYPWLA</u> WYQQKPGMAPKLLIY CDR1 <u>KVSTLES</u> GVPSRFSGSGSGTEFTLTISSLQPDDLASYIC <u>QIYES</u> FGQGT CDR2 CDR3 KVEIK	SEQ ID NO:8

In some embodiments, the light chain variable domain comprises:

(i) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:4,

(ii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:6, or

(iii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:8.

In some embodiments, the light chain variable domain comprises:

(i) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:4,

(ii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 22-32, 48-54, and 87-91 of SEQ ID NO:6, or

(iii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:8.

As used herein, when referring to the antibody of the invention, the expression “the light chain variable domain comprises VLCDR1 corresponding to VLCDR1 contained in the amino acid sequence of SEQ ID NO:4” means VLCDR1 in the light chain variable

domain of said antibody has the same amino acid sequence with that of VLCDR1 contained in the amino acid sequence of SEQ ID NO:4. For example, as determined based on the Kabat numbering, VLCDR1 of antibody DRVIA7 consists of amino acids 22-32 (RASQRIDNWVA) of the light chain variable domain as shown in SEQ ID NO:4, thus, the above expression means VLCDR1 of the light chain variable domain of the antibody of the invention also consists of RASQRIDNWVA.

As used herein, VLCDR, LCDR, and CDRL have the same meaning and can be used interchangeably to refer to a complementary determining region of the antibody light chain variable domain.

In some other embodiments, the light chain variable domain comprises or consists of an amino acid sequence selected from a group consisting of (i) an amino acid sequence of SEQ ID NO:4 or an amino acid sequence having at least 85%, at least 90%, at least 95% or even higher sequence identity with SEQ ID NO:4, (ii) an amino acid sequence of SEQ ID NO:6 or an amino acid sequence having at least 85%, at least 90%, at least 95% or even higher sequence identity with SEQ ID NO:6, and (iii) an amino acid sequence of SEQ ID NO:8 or an amino acid sequence having at least 85%, at least 90%, at least 95% or even higher sequence identity with SEQ ID NO:8. In some embodiments, the light chain variable domain comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with SEQ ID NO:4, 6, or 8. In some preferred embodiments, the light chain variable domain comprises VLCDR1, VLCDR2, and VLCDR3 as set out in Table 1.

The present invention also provides an isolated antibody capable of specifically binding to an epitope on HIV-1 gp120 which is recognized by an antibody of the invention as described above.

The present invention also provides an isolated antibody capable of competing with an antibody of the invention as described above to bind to an epitope on HIV-1 gp120.

The ability of two antibodies to bind to the same epitope can be determined by known assays in the art such as, for example, surface plasmon resonance assays and antibody competition assays. Typically, antibodies that immunospecifically bind to the same epitope can compete for binding to the epitope, which can be measured, for example, by an in vitro binding competition assay (e.g. competition ELISA), using techniques known the art.

In some embodiments, the antibody of the invention is IgG. In some other embodiments, the antibody of the invention is IgM. In some other embodiments, the antibody of the invention is IgA.

The present invention further provides an isolated antibody fragment, which is a

functional fragment of the above described antibody of the invention and capable of specifically binding to gp120 of HIV-1. In some embodiments, the antibody fragment of the invention is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab)'₂ fragment, a single chain Fv protein (scFv), and a disulfide stabilized Fv protein (dsFv). Preferably, the antibody fragment of the invention has broadly neutralizing activities against a number of HIV-1 isolates.

Polypeptides

The present invention also provides an isolated polypeptide which is the heavy or light chain variable domain of immunoglobulin. The polypeptide of the invention can be used to construct an antibody capable of specifically binding to gp120 of HIV-1 and having broadly neutralizing activities on HIV-1. In some embodiments, the polypeptide is a heavy chain variable domain of immunoglobulin and comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to amino acids 31-35, 50-66, and 99-109 of SEQ ID NO:2. In some other embodiments, the polypeptide is a heavy chain variable domain of immunoglobulin and comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with SEQ ID NO:2. In some embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:4. In some other embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with SEQ ID NO:4. In some embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 22-32, 48-54, and 87-91 of SEQ ID NO:6. In some other embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with SEQ ID NO:6. In some embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:8. In some other embodiments, the polypeptide is a light chain variable domain of immunoglobulin and comprises an amino acid sequence having about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or

about 99% sequence identity with SEQ ID NO:8.

Nucleic acids, vectors and method for producing antibodies

In another aspect, the present invention provides an isolated nucleic acid molecule encoding an antibody or antibody fragment or polypeptide of the invention. In some specific embodiments, the nucleic acid molecule of the invention comprises the nucleotide sequence of SEQ ID NO:1 encoding a heavy chain variable domain. In some other specific embodiments, the nucleic acid molecule of the invention comprises the nucleotide sequence of SEQ ID NO:3, 5, or 7 encoding a light chain variable domain. In some embodiments, the nucleic acid molecule of the invention is operatively linked to a promoter.

The present invention also provides an expression vector comprising at least one nucleic acid molecule of the invention as described above.

The present invention also provides an isolated host cell which has been transformed with at least one nucleic acid molecule or expression vector of the invention as described above.

In another aspect, the present invention provides a method of producing an antibody, comprising:

- (i) transforming a host cell with at least one nucleic acid molecule or expression vector of the invention as described above,
- (ii) culturing the transformed host cell under conditions suitable for the expression of the nucleic acid molecule or the expression vector, and
- (iii) isolating and purifying the antibody or antibody fragment expressed from the nucleic acid molecule or the expression vector.

The present invention also relates to an isolated antibody or antibody fragment obtained by performing the method of the invention as described above, which is capable of specifically binding to HIV-1 gp120. Preferably, the isolated antibody or antibody fragment obtained by the method of the invention has broadly neutralizing activities on various HIV-1 isolates.

Detection and diagnosis

In another aspect, the present invention provides a method of detecting an HIV-1 infection in a human subject, comprising:

- (i) contacting a biological sample from the subject with an antibody or antibody fragment of the invention, and

(ii) determining the presence of an immune complex formed by the antibody or the antibody fragment in said sample,

wherein the presence of said immune complex indicates that the subject has an HIV-1 infection.

In some embodiments of the method of the invention for detecting an HIV-1 infection in a human subject, in step (i), the sample is immobilized on a solid substrate prior to the contacting, and the contacting comprises applying the antibody or antibody fragment to the solid substrate on which the sample is immobilized. In some embodiments, the antibody or antibody fragment is labeled with a fluorescent, enzymatic, or radioactive label. In some other embodiments, in step (ii), the solid substrate is brought into contact with a first binding partner capable of specifically binding to the antibody or antibody fragment. In some embodiments, the first binding partner is a second antibody capable of specifically binding to the antibody or antibody fragment.

In some other embodiments of the method of the invention for detecting an HIV-1 infection in a human subject, in step (i), the antibody or antibody fragment is immobilized on a solid substrate prior to the contacting, and the contacting comprises applying the sample to the solid substrate on which the antibody or antibody fragment is immobilized. In some embodiments, in step (ii), the solid substrate is brought into contact with a second binding partner capable of specifically binding to an antigen of HIV-1. In some embodiments, the second binding partner is a second antibody capable of specifically binding to an antigen of HIV-1. In some embodiments, the second antibody is capable of specifically binding to gp120 of HIV-1. In some embodiments, the antibody or antibody fragment and the second antibody capable of specifically binding to an antigen of HIV-1 bind to different epitopes on HIV-1.

In some embodiments of the method of the invention for detecting an HIV-1 infection in a human subject, the biological sample from the subject is a blood, plasma, serum, blood cell, or blood cell lysate sample.

In some embodiments of the method of the invention for detecting an HIV-1 infection in a human subject, the biological sample from the subject contains blood cells, and the method further comprises, prior to, during, or after step (i), contacting the biological sample with a third binding partner capable of specifically binding to the blood cells. In some embodiments, the third binding partner is an antibody capable of specifically binding to the blood cells. In some specific embodiments, the blood cells are lymphocytes, for example T cells, such as CD4⁺ T cells. In some other specific embodiments, the blood cells are monocytes. In some specific embodiments, the third binding partner is an

antibody capable of specifically binding to a marker on the blood cells which is characteristic for the blood cells.

In another aspect, the present invention relates to use of the antibody or antibody fragment of the invention in manufacture of a kit for detecting an HIV-1 infection in a human subject.

In another aspect, the present invention also provides a kit for detecting an HIV-1 infection in a human subject, comprising an antibody or antibody fragment of the invention.

Treatment and pharmaceutical composition

In another aspect, the present invention also provides a method of preventing or treating an HIV-1 infection in a human subject, comprising administering to the subject an effective amount of an antibody or antibody fragment or a pharmaceutical composition of the invention. In some embodiments, the subject has acquired immune deficiency syndrome (AIDS). In some embodiments, the method further comprises administering to the subject at least one anti-viral agent against HIV-1.

A subject or candidate for treatment with an antibody or antigen-binding fragment thereof provided herein includes, but is not limited to, a human subject that has been exposed to a HIV-1 virus, a human subject who exhibits one or more symptoms of a HIV-1 infection and a human subject who is at risk of a HIV-1 infection.

In another aspect, the present invention also relates to use of the antibody or antibody fragment of the invention in manufacture of a pharmaceutical composition for preventing or treating an HIV-1 infection in a human subject. In some embodiments, the subject has acquired immune deficiency syndrome (AIDS).

In another aspect, the present invention provides a pharmaceutical composition, which comprises at least one antibody or antibody fragment of the invention as described above, and a pharmaceutically acceptable carrier.

An effective amount of antibody or antigen-binding fragment thereof to be administered therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. In addition, the attending physician takes into consideration various factors known to modify the action of drugs, including severity and type of disease, patient's health, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Accordingly, it will be necessary for the therapist to titer the dosage of the antibody or antigen-binding fragment thereof and modify the route of administration as required to obtain the optimal

therapeutic effect. Typically, the clinician will administer the antibody or antigen-binding fragment thereof until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays. Exemplary assays for monitoring treatment of a viral infection are known in the art and include for example, viral titer assays.

Generally, the dosage ranges for the administration of the antibodies or antigen-binding fragments thereof provided herein are those large enough to produce the desired effect in which the symptom(s) of the pathogen-mediated disease (e.g. viral disease) are ameliorated or the likelihood of virus infection is decreased. In some examples, the antibodies or antigen-binding fragments thereof provided herein are administered in an amount effective for inducing an immune response in the subject. The dosage is not so large as to cause adverse side effects, such as hyperviscosity syndromes, pulmonary edema or congestive heart failure. Generally, the dosage will vary with the age, condition, sex and the extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of the appearance of any adverse side effect. Exemplary dosages for the prevention or treatment of a HIV-1 infection include, but are not limited to, about or 0.01 mg/kg to about or 300 mg/kg, such as for example, about or 0.01 mg/kg, about or 0.1 mg/kg, about or 0.5 mg/kg, about or 1 mg/kg, about or 5 mg/kg, about or 10 mg/kg, about or 15 mg/kg, about or 20 mg/kg, about or 25 mg/kg, about or 30 mg/kg, about or 35 mg/kg, about or 40 mg/kg, about or 45 mg/kg, about or 50 mg/kg, about or 100 mg/kg, about or 150 mg/kg, about or 200 mg/kg, about or 250 mg/kg, or about or 300 mg/kg.

For treatment of a viral infection, the dosage of the antibodies or antigen-binding fragments thereof of the invention can vary depending on the type and severity of the disease. The antibodies or antigen-binding fragments thereof can be administered single dose, in multiple separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment can be repeated until a desired suppression of disease symptoms occurs or the desired improvement in the patient's condition is achieved. Repeated administrations can include increased or decreased amounts of the antibody or antigen-binding fragment thereof depending on the progress of the treatment. Other dosage regimens also are contemplated.

The antibodies or antigen-binding fragments thereof provided herein can be administered to a subject by any method known in the art for the administration of polypeptides, including for example systemic or local administration. The antibodies or antigen-binding fragments thereof can be administered by routes, such as parenteral (e.g.,

intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, or intracavity), topical, epidural, or mucosal (e.g. intranasal or oral). The antibodies or antigen-binding fragments thereof can be administered externally to a subject, at the site of the disease for exertion of local or transdermal action. Compositions containing the antibodies or antigen-binding fragments thereof can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa).

Pharmaceutical compositions provided herein can be in various forms, e.g., in solid, semi-solid, liquid, powder, aqueous, or lyophilized form. Examples of suitable pharmaceutically acceptable carriers are known in the art and include but are not limited to water, buffering agents, saline solutions, phosphate buffered saline solutions, various types of wetting agents, sterile solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, gelatin, glycerin, carbohydrates such as lactose, sucrose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, powders, among others. Pharmaceutical compositions provided herein can contain other additives including, for example, antioxidants, preservatives, antimicrobial agents, analgesic agents, binders, disintegrants, coloring, diluents, excipients, extenders, glidants, solubilizers, stabilizers, tonicity agents, vehicles, viscosity agents, flavoring agents, emulsions, such as oil/water emulsions, emulsifying and suspending agents, such as acacia, agar, alginic acid, sodium alginate, bentonite, carbomer, carrageenan, carboxymethylcellulose, cellulose, cholesterol, gelatin, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, octoxynol 9, oleyl alcohol, povidone, propylene glycol monostearate, sodium lauryl sulfate, sorbitan esters, stearyl alcohol, tragacanth, xanthan gum, and derivatives thereof, solvents, and miscellaneous ingredients such as crystalline cellulose, microcrystalline cellulose, citric acid, dextrin, dextrose, liquid glucose, lactic acid, lactose, magnesium chloride, potassium metaphosphate, starch, among others (see, generally, Alfonso R. Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins). Such carriers and/or additives can be formulated by conventional methods and can be administered to the subject at a suitable dose. Stabilizing agents such as lipids, nuclease inhibitors, polymers, and chelating agents can preserve the compositions from degradation within the body.

The following examples are illustrative and should not be construed to limit the scope of the invention in any way.

Examples

Example 1: Identification of broadly neutralizing antibody DRVIA7

The isolation of antibody DRVIA7 from an HIV-1-infected Chinese donor

A monoclonal antibody DRVIA7 was isolated from a sample from an HIV-1-infected Chinese donor and has been identified as having broadly neutralizing activities against HIV-1 isolates. Two *Escherichia coli* strains carrying the heavy chain gene expression vector (DRVIA7H) or light chain gene expression vector (DRVIA7L) were deposited at China General Microbiological Culture Collection Center (CGMCC) (NO.1 West Beichen Road, Chaoyang District, Beijing, China) under the accession number of CGMCC No. 11879 and CGMCC No. 11880, respectively. The sequence encoding the heavy chain variable region contained in DRVIA7H is SEQ ID NO:1, and the encoded amino acid sequence of the heavy chain variable region is SEQ ID NO:2. The sequence encoding the light chain variable region contained in DRVIA7L is SEQ ID NO:3, and the encoded amino acid sequence of the light chain variable region is SEQ ID NO:4.

The expression and purification of antibody DRVIA7

Escherichia coli strains carrying the heavy chain gene expression vector DRVIA7H or the light chain gene expression vector DRVIA7L were separately inoculated in 100ml LB culture medium (Amersham) containing kanamycin at a final concentration of 50µg/ml, and then cultured at 37°C for 16 hours with shaking at 200rpm. The expression vector plasmids were extracted from the cultures using Plasmid Midi Kit (Omega) following the manufacturer's instructions. 293F cells were co-transfected with equal amount of heavy and light chain plasmids using PEI transfection reagent (Polysciences), and then cultured at 8% CO₂ and 37°C for 6 days. Monoclonal antibody DRVIA7 was purified from the culture supernatant using Protein-A columns (GE health), and the concentration of antibody was determined by NanoDrop2000 (Thermo). The purified antibody samples were stored at 4°C for further detection.

Binding capacity of antibody DRVIA7

The binding capacity of antibody DRVIA7 was detected by Enzyme Linked Immunosorbent Assay (ELISA). Briefly, 96-well flat-bottom plates were coated with either purified CN54 GP140, wild-type (WT) YU2 gp120 or T278G YU2 gp120 (YU2 gp120 with T278G mutation) protein at 2µg/ml in PBS (100µl/well) at 4°C overnight. The plates were washed five times with 0.05% Tween 20 in PBS (PBS-T), and then blocked with 250µl/well of blocking buffer (5% skim milk and 2% bovine albumin in PBS) for 1h at RT.

After three times of washing with PBS-T, 100µl of antibody DRVIA7 serially diluted (5-time dilution, starting from 10µg/ml) in blocking buffer was added to the antigen-coated plates, and incubated at 37°C for 1 hour. Plates were then washed five times with PBS-T, 100µl of HRP conjugated goat anti-human IgG (H+L) (ZSGB-BIO) diluted at 1:5000 in blocking buffer was added and incubated at 37°C for 1h. The plates were washed five times with PBS-T and 100µl TMB (Kinghawk) substrate was added and incubated for 20mins at RT and the reaction was stopped by the addition of 50µl 1N H₂SO₄ (Kinghawk) to each well and the readout (OD) was measured at a wave length of 450nm and 630nm. As shown in Fig.1, monoclonal antibody DRVIA7 can specifically bind with CN54 GP140 protein (Fig.1A) as well as wild-type and mutant gp120 proteins (Fig. 1B).

Neutralizing activity of antibody DRVIA7

The neutralizing activity of antibody DRVIA7 was measured using TZM-bl/pseudovirus neutralizing assay ^[7]. Briefly, antibody DRVIA7 was serially diluted in DMEM growth medium (Hyclone). The diluted antibody (100µl/well) and the pseudovirus (50µl/well, containing pseudovirus of 200 TCID₅₀) were added to each well of the 96-well flat bottom plates. The plates were then incubated at 37°C, 5% CO₂ for 1 hour. TZM-bl cells were added at 1×10^4 /well to the plates in DMEM growth medium containing 11µg/ml DEAE-dextran (Sigma). Cell controls (TZM-bl cell only) and virus controls (TZM-bl and pseudovirus) were set. The plates were then incubated at 37°C, 5% CO₂ for 48 hours. The luciferase reaction was detected using Bright-Glo luciferase reagent kit (Promega) and the 50% inhibitory dose (ID₅₀) was calculated. As shown in Tables 2A and 2B, DRVIA7 can neutralize different subtypes of HIV-1 viruses, and thus is a broadly neutralizing antibody.

Table 2A. Neutralizing breadth and potency of three monoclonal antibodies on the global panel of HIV-1 Env strains.					
Global Panel ^a			monoclonal antibodies		
Clade	Tier	Pseudovirus	DRVIA7	DRVIA7H+ gDRV101-L57	DRVIA7H+ gDRV101-L40
A	2	398-F1	>20 ^{AA}	7.69	8.93
B	2	TRO.11	15.81	14.81	2.92
	2	X2278	0.57	5.97	1.56
C	2	25710	>20	14.81	9.52
	2	Ce0217	3.29	9.30	2.22
	2	Ce1176	19.78	>20	13.51
G	2	X1632	>20	12.90	>20

CRF01_AE	2	CNE55	>20	13.79	19.23
	2	CNE8	>20	14.29	10.20
CRF07_BC	2	BJOX2000	>20	>20	>20
	2	CH119.10	>20	>20	10.75
AC	2	246	>20	11.76	>20
Potency(GMTs)			4.92	11.21	6.62
Breadth%(<20µg/ml)			33.3 ^{☆☆}	75	75
^a The global panel was developed by the Montefiori group at Duke University Medical Center. ^{AA} The neutralizing potency is measured as IC ₅₀ in µg/ml of the monoclonal antibodies. ^{☆☆} The neutralizing breadth is calculated as the percentage of viruses neutralized with IC ₅₀ <20µg/ml.					

Table 2B. Neutralizing breadth and potency of three monoclonal antibodies on the DRV1 panel of HIV-1 Env strains.					
DRV1 Panel ^a			monoclonal antibodies		
Clade	Tier	Pseudovirus	DRV1A7	DRV1A7H+ gDRV101-L57	DRV1A7H+ gDRV101-L40
B	1A	SF162	>20 ^{AA}	7.55	>20
	2	QH0692	12.64	>20	14.24
	2	SC422661	2.25	4.71	3.79
	3	PVO.4	1.57	11.76	4.17
	2	AC10	>20	18.18	>20
	2	RHPA4259	>20	1.85	1
	2	REJO4541	>20	2.46	6.1
	3	TRJO4551	>20	3.36	>20
	2	CAAN5342	14.22	17.39	9.82
CRF07_BC	2	CH110	0.12	8.51	0.24
	2	CH117	>20	4.04	>20
	2	CH181	>20	9.75	2.14
	3	CH120	>20	>20	>20
C	1A	MW965	>20	2.67	0.54
	1B	ZM109F	>20	5.48	>20
	2	Du422	>20	>20	6.17
	2	ZM249M	>20	5.8	>20
	2	CAP45	>20	>20	>20
A	2	Q461	>20	8.7	7.97
	2	Q769	7.35	1.19	0.87
	2	Q259	11.36	3.5	1.67
	2	Q842	2.6	1.29	0.68
CRF01_AE	NA	BJMSM2249	>20	>20	>20
	NA	BJMSM2316	8.7	2.86	1.18
	NA	BJMSM2498	>20	16.67	>20

Potency(GMTs)	3.74	5.07	2.51
Breadth%(<20μg/ml)	36 ^{☆☆}	80	60
^a The DRVI panel was compiled by the Shao group in the Department of Virology and Immunology at China CDC. ^{ΔΔ} The neutralizing potency is measured as IC ₅₀ in μg/ml of the monoclonal antibodies. ^{☆☆} The neutralizing breadth is calculated as the percentage of viruses neutralized with IC ₅₀ <20μg/ml.			

The crystal structure of antibody DRVIA7

Crystallographic characterization of unliganded DRVIA7 and DRVIA7+gp120 complex were determined using X-ray crystallography (Table 3). Ribbon diagrams of the crystal structure of DRVIA7 bound to gp120 was displayed in Fig.2.

Table 3. X-ray crystallographic data collection and refinement statistics.

Data collection	UnligandedDRVIA7	DRVIA7+gp120 complex
X-ray Source	APS 23ID-B	APS 23ID-B
Wavelength (Å)	1.033	1.033
Space group	P1	P4 ₁ 2 ₁ 2
Unit cell parameters	a = 66.8, b = 73.8, c = 101.2 Å α = 89.8°, β = 89.4°, γ = 90.0°	a = b = 72.3, c = 338.8 Å α = β = γ = 90.0°
Resolution (Å)	50.0-2.90 (2.95-2.90) ^a	29.0-3.39(3.67-3.39) ^a
Observations	74,827	205,736
Unique reflections	41,586 (2,062) ^a	13,362 (2,662) ^a
Redundancy	1.8 (1.8) ^a	15.4 (15.7) ^a
Completeness (%)	97.4 (98.4) ^a	99.7 (99.3) ^a
<I/σ _I > ^b	3.8(1.1) ^a	10.0 (1.2) ^a
R _{sym} ^c	0.24 (0.89) ^a	0.31 (2.92) ^a
R _{pim} ^c	0.17 (0.63) ^a	0.08 (0.76) ^a
CC _{1/2}	0.55 ^a	0.62 ^a
Refinement statistics		
Resolution (Å)	49.54-2.90(2.97-2.90) ^a	28.97-3.40(3.66-3.40) ^a
Reflections (work)	39,396 (2,443) ^a	12,585 (2,394) ^a
Reflections (test)	2,109 (151) ^a	653 (141) ^a
R _{cryst} (%) ^c	23.4(28.4) ^a	25.3(37.6) ^a
R _{free} (%) ^d	28.4(36.0) ^a	30.6(39.8) ^a
Average B-value (Å ²)	34	148
DRVIA7 Fab	34	126
gp120 core	N/A	174
Wilson B-value (Å ²)	36	121
RMSD from ideal geometry		
Bond length (Å)	0.005	0.006
Bond angles (°)	1.23	1.33
Ramachandran statistics (%) ^f		
Favored	97.9	97.9
Outliers	0.0	0.0
PDB ID	5CD3	5CD5

^a Numbers in parentheses refer to the highest resolution shell.

^b Calculated as average(I)/average(σI)

^c $R_{sym} = \sum_{hkl} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection h, k, l, $\langle I_{hkl} \rangle$ is the average intensity for that reflection, and n is the redundancy. R_{pim} is a redundancy-independent measure of the quality of intensity measurements. $R_{pim} = \sum_{hkl} (1/(n-1))^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$, where $I_{hkl,i}$ is the scaled intensity of the i^{th} measurement of reflection h, k, l, $\langle I_{hkl} \rangle$ is the

average intensity for that reflection, and n is the redundancy.

^d $R_{\text{cryst}} = \sum_{hkl} |F_o - F_c| / \sum_{hkl} |F_o| \times 100$

^e R_{free} was calculated as for R_{cryst} , but on a test set comprising 5% of the data excluded from refinement.

^fThese values were calculated using MolProbity(<http://molprobity.biochem.duke.edu/>).

Example 2: Redesigned antibody DRVIA7H+gDRVI01-L57

Residue-based energy evaluation

Residue-based energy evaluation ^[8] of the complex formed between DRVIA7 and gp120 revealed that there might be a likelihood of structural interference between the N-terminus, CDRL1 and CDRL3 of DRVIA7 light chain and gp120 which may affect the binding thereof (Fig.3).

Modified variable region of light chain gDRVI01-L57

Based on the above discovery, a series of modified DRVIA7 antibodies with mutations on the light chain were generated. Antibodies with modifications on CDRL1 failed to show an improved neutralizing activity. However, a modified DRVIA7 antibody obtained by deleting two residues from the N-terminus of DRVIA7 light chain variable region significantly increased the binding capacity and neutralizing breadth. The modified light chain variable region is named as gDRVI01-L57. *Escherichia coli* strain carrying the expression vector (gDRVI01-L57) containing the light chain gene encoding light chain variable region gDRVI01-L57 was deposited at CGMCC under the accession number of CGMCC No. 11881. The sequence encoding the light chain variable region contained in the expression vector gDRVI01-L57 is SEQ ID NO:5, and the encoded amino acid sequence of the light chain variable region is SEQ ID NO:6.

Binding capacity of antibody DRVIA7H+gDRVI01-L57

Antibody DRVIA7H+gDRVI01-L57 was obtained by an antibody expression and purification process similar to that described in Example 1 by using *Escherichia coli* strains carrying the heavy chain expression vector DRVIA7H and the light chain expression vector gDRVI01-L57. The binding capacity of DRVIA7H+gDRVI01-L57 was measured by ELISA as described in Example 1. As shown in Fig. 1C, the binding capacity of DRVIA7H+gDRVI01-L57 to CN54 GP140 antigen was markedly improved as compared with DRVIA7.

Neutralizing activity of antibody DRVIA7H+gDRVI01-L57

The neutralizing activity of DRVIA7H+gDRVI01-L57 was measured using TZM-bl/pseudovirus neutralizing assay as described in Example 1. As shown in Tables 2A

and 2B, the neutralizing breadth of DRVIA7H+gDRVI01-L57 was increased significantly in both the tested Global Panel and DRVI Panel as compared with that of DRVIA7.

The mechanism of improved neutralizing activity of DRVIA7H+gDRVI01-L57 was preliminarily discussed by analyzing the crystal structure of N-terminus (LC-Nt) and CDRL1 of DRVIA7 light chain bound to gp120. As shown in Fig. 4, the N-terminus of wild-type DRVIA7 light chain was very adjacent to the N461 glycosylation site on gp120. As it is believed that N-linked glycosylation sites may protect the virus against neutralizing activity of antibody, it is possible that the deletion of two N-terminal residues from the light chain variable region may help to avoid the interference of the N461 glycosylation site on the binding of the antibody with gp120 and thereby facilitate the neutralizing of virus by the antibody.

Example 3: Antibody DRVIA7H+gDRVI01-L40 obtained by antibody light chain gene repertoire screening

Sequence analysis of antibody DRVIA7

The sequence analysis of antibody DRVIA7 was performed using IMGT V-QUEST server (http://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg) assisted with manual inspection. The CDR3 length was determined based on the Kabat numbering. As shown in Fig. 5, DRVIA7 heavy chain is derived from IgHV1-02*02 allele, and CDRH3 contains 11 amino acids. DRVIA7 light chain is derived from IgKV1-5*03 allele, and CDRL3 contains 5 amino acids. DRVIA7 heavy chain is derived from the same allele as VRC01 and CDRL3 of light chain has the same length as VRC01 ^[1], suggesting that DRVIA7 may be a VRC01-like antibody.

Structural comparison analysis between DRVIA7 and VRC01-like antibodies

The crystal structure of DRVIA7 was determined in example 1, which strongly resembles some published VRC01-like antibodies such as VRC01 (PDBID: 3NGB), VRC03 (PDBID: 3SE8), PG04 (PDBID: 3SE9) and 12A21 (PDBID: 4JPW) (Fig. 6). The results also suggest that DRVIA7 may be a VRC01-like antibody.

The establishment of antibody light chain repertoire

The antibody light chain gene repertoire was established using deep sequencing technology from the blood sample of the HIV-1 infected donor from whom DRVIA7 was isolated, and a large number of DRVIA7-like light chain genes were found in this

repertoire. Based on the sequence analysis of published VRC01-like antibodies, some common characteristics were identified. The CDRL3 signatures (5 amino acids in length, hydrophobic residue at position 3 and Q/E at position 4) among VRC01-like antibody light chains were summarized and used to select candidate light chain genes from the antibody light chain repertoire. As shown in Fig.7, 703 VRC01-like light chains (blue dots) were identified and 22 candidate light chains (red dots) were synthesized and the binding capacity and neutralizing activity were tested.

Antibody DRVIA7H+gDRVI01-L40

By pairing the above twenty-two candidate light chains with the heavy chain of DRVIA7, a series of antibodies were then prepared according to the expression and purification process as described Example 1, and binding and neutralizing assays were also performed. A light chain variable region gDRVI01-L40 was finally identified. Antibody DRVIA7H+gDRVI01-L40 generated by pairing antibody light chain comprising gDRVI01-L40 with the heavy chain of DRVIA7 showed an increased CN54 GP140 binding capacity (Fig. 1D) and an improved neutralizing breadth (Table 2A and 2B) as compared with DRVIA7. *Escherichia coli* strain carrying the expression vector (gDRVI01-L40) containing the light chain gene encoding light chain variable region gDRVI01-L40 was deposited at CGMCC under the accession number of CGMCC No. 11882. The sequence encoding the light chain variable region contained in the expression vector gDRVI01-L40 is SEQ ID NO:7, and the encoded amino acid sequence of the light chain variable region is SEQ ID NO:8.

Although the invention has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the application of the principles of the invention. Numerous modifications made be made therein without departing from the spirit and scope of the invention.

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PCT

Print Out (Original in Electronic Form)

0-1	Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT-SAFE Version 3.51.061.237 MT/FOP 20140101/0.20.5.21
0-2	International Application No.	PCT/CN2017/072749
0-3	Applicant's or agent's file reference	P2016TC319
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	3
1-2	line	28
1-3	Identification of deposit	
1-3-1	Name of depositary institution	CGMCC China General Microbiological Culture Collection Center
1-3-2	Address of depositary institution	Institute of Microbiology, Chinese Academy of Sciences, No. 1, West Beichen Road, Chaoyang District, Beijing 100101, China
1-3-3	Date of deposit	14 December 2015 (14.12.2015)
1-3-4	Accession Number	CGMCC 11879
1-5	Designated States for Which Indications are Made	All designations
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	3
2-2	line	31
2-3	Identification of deposit	
2-3-1	Name of depositary institution	CGMCC China General Microbiological Culture Collection Center
2-3-2	Address of depositary institution	Institute of Microbiology, Chinese Academy of Sciences, No. 1, West Beichen Road, Chaoyang District, Beijing 100101, China
2-3-3	Date of deposit	14 December 2015 (14.12.2015)
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3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
3-1	page	4
3-2	line	3
3-3	Identification of deposit	
3-3-1	Name of depositary institution	CGMCC China General Microbiological Culture Collection Center
3-3-2	Address of depositary institution	Institute of Microbiology, Chinese Academy of Sciences, No. 1, West Beichen Road, Chaoyang District, Beijing 100101, China
3-3-3	Date of deposit	14 December 2015 (14.12.2015)
3-3-4	Accession Number	CGMCC 11881
3-5	Designated States for Which Indications are Made	All designations
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
4-1	page	4
4-2	line	6
4-3	Identification of deposit	
4-3-1	Name of depositary institution	CGMCC China General Microbiological Culture Collection Center
4-3-2	Address of depositary institution	Institute of Microbiology, Chinese Academy of Sciences, No. 1, West Beichen Road, Chaoyang District, Beijing 100101, China
4-3-3	Date of deposit	14 December 2015 (14.12.2015)
4-3-4	Accession Number	CGMCC 11882
4-5	Designated States for Which Indications are Made	All designations

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0-4	This form was received with the international application: (yes or no)	
0-4-1	Authorized officer	

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Claims

1. An isolated human monoclonal antibody comprising a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to VHCDR1, VHCDR2, and VHCDR3 contained in the amino acid sequence of SEQ ID NO:2, and wherein the antibody is a neutralizing antibody which specifically binds to gp120 of HIV-1.

2. The antibody of claim 1, wherein the heavy chain variable domain comprises VHCDR1, VHCDR2, and VHCDR3 respectively corresponding to amino acids 31-35, 50-66, and 99-109 of SEQ ID NO:2.

3. The antibody of claim 1 or 2, wherein the heavy chain variable domain comprises an amino acid sequence of SEQ ID NO:2 or an amino acid sequence at least 85% identical to SEQ ID NO:2.

4. The antibody of any one of claims 1-3, wherein the light chain variable domain comprises:

(i) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:4,

(ii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:6, or

(iii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to VLCDR1, VLCDR2, and VLCDR3 contained in the amino acid sequence of SEQ ID NO:8.

5. The antibody of claim 4, wherein the light chain variable domain comprises:

(i) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:4,

(ii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 22-32, 48-54, and 87-91 of SEQ ID NO:6, or

(iii) VLCDR1, VLCDR2, and VLCDR3 respectively corresponding to amino acids 24-34, 50-56, and 89-93 of SEQ ID NO:8.

6. The antibody of claim 4 or 5, wherein the light chain variable domain comprises:

(i) an amino acid sequence of SEQ ID NO:4 or an amino acid sequence at least 85% identical to SEQ ID NO:4,

(ii) an amino acid sequence of SEQ ID NO:6 or an amino acid sequence at least 85% identical to SEQ ID NO:6, and

(iii) an amino acid sequence of SEQ ID NO:8 or an amino acid sequence at least 85%

identical to SEQ ID NO:8.

7. An isolated antibody which specifically binds to an epitope on gp120 of HIV-1 recognized by the antibody of any one of claims 1-6.

8. An isolated antibody which competes with the antibody of any one of claims 1-6 to bind to an epitope on gp120 of HIV-1.

9. The antibody of any one of claims 1-8, wherein the antibody is an IgG, IgM, or IgA.

10. An isolated antibody fragment, which is a functional fragment of the antibody of any one of claims 1-9.

11. The antibody fragment of claim 10, selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab)'₂ fragment, a single chain Fv protein (scFv), and a disulfide stabilized Fv protein (dsFv).

12. An isolated nucleic acid molecule encoding the antibody or antibody fragment of any one of claims 1-11.

13. The nucleic acid molecule of claim 12 comprising the nucleotide sequence of SEQ ID NO:1 encoding a heavy chain variable domain.

14. The nucleic acid molecule of claim 12 or 13 comprising the nucleotide sequence of SEQ ID NO:3, 5, or 7 encoding a light chain variable domain.

15. The nucleic acid molecule of any one of claims 12-14, which is operatively linked to a promoter.

16. An expression vector comprising at least one nucleic acid molecule of any one of claims 12-15.

17. An isolated host cell transformed with the nucleic acid molecule of any one of claims 12-15 or the expression vector of claim 16.

18. A method of producing an antibody, comprising:

(i) transforming a host cell with the nucleic acid molecule of any one of claims 12-15 or the expression vector of claim 16,

(ii) culturing the transformed host cell under conditions suitable for the expression of the nucleic acid molecule or the expression vector, and

(iii) isolating and purifying the antibody or antibody fragment expressed from the nucleic acid molecule or the expression vector.

19. An isolated antibody or antibody fragment obtained by the method of claim 18.

20. A pharmaceutical composition comprising the antibody or antibody fragment of any one of claims 1-11 and 19, and a pharmaceutically acceptable carrier.

21. A method of detecting an HIV-1 infection in a human subject comprising:

(i) contacting a biological sample from the subject with the antibody or antibody fragment of any one of claims 1-11 and 19, and

(ii) determining the presence of an immune complex formed by the antibody or the antibody fragment in said sample,

wherein the presence of said immune complex indicates that the subject has an HIV-1 infection.

22. The method of claim 21, wherein in step (i), the sample is immobilized on a solid substrate, and the contacting comprises applying the antibody or antibody fragment to the solid substrate on which the sample is immobilized.

23. The method of claim 21 or 22, wherein the antibody or antibody fragment is labeled with a fluorescent, enzymatic, or radioactive label.

24. The method of claim 22, wherein in step (ii), the solid substrate is brought into contact with a first binding partner capable of specifically binding to the antibody or antibody fragment.

25. The method of claim 24, wherein the first binding partner is a second antibody capable of specifically binding to the antibody or antibody fragment.

26. The method of claim 21, wherein in step (i), the antibody or antibody fragment is immobilized on a solid substrate, and the contacting comprises applying the sample to the solid substrate on which the antibody or antibody fragment is immobilized.

27. The method of claim 26, wherein in step (ii), the solid substrate is brought into contact with a second binding partner capable of specifically binding to an antigen of HIV-1.

28. The method of claim 27, wherein the second binding partner is a second antibody capable of specifically binding to an antigen of HIV-1.

29. The method of claim 28, wherein the antibody or antibody fragment and the second antibody capable of specifically binding to an antigen of HIV-1 bind to different epitopes on HIV-1.

30. The method of any one of claims 21-29, wherein the biological sample from the subject is a blood, plasma, serum, blood cell, or blood cell lysate sample.

31. The method of claim 21, wherein the biological sample from the subject contains blood cells, and wherein the method further comprises, prior to, during, or after step (i), contacting the biological sample with a third binding partner capable of specifically binding to the blood cells.

32. The method of claim 31, wherein the third binding partner is an antibody capable of specifically binding to the blood cells.

33. Use of the antibody or antibody fragment of any one of claims 1-11 and 19 in manufacture of a kit for detecting an HIV-1 infection in a human subject.

34. A method of preventing or treating an HIV-1 infection in a human subject, comprising administering to the subject an effective amount of at least one antibody or antibody fragment of any one of claims 1-11 and 19 or the pharmaceutical composition of claim 20.

35. The method of claim 34, wherein the subject has acquired immune deficiency syndrome (AIDS).

36. The method of claim 34 or 35, further comprising administering to the subject at least one anti-viral agent against HIV-1.

37. Use of the antibody or antibody fragment of any one of claims 1-11 and 19 in manufacture of a pharmaceutical composition for preventing or treating an HIV-1 infection in a human subject.

38. The use of claim 37, wherein the subject has acquired immune deficiency syndrome (AIDS).

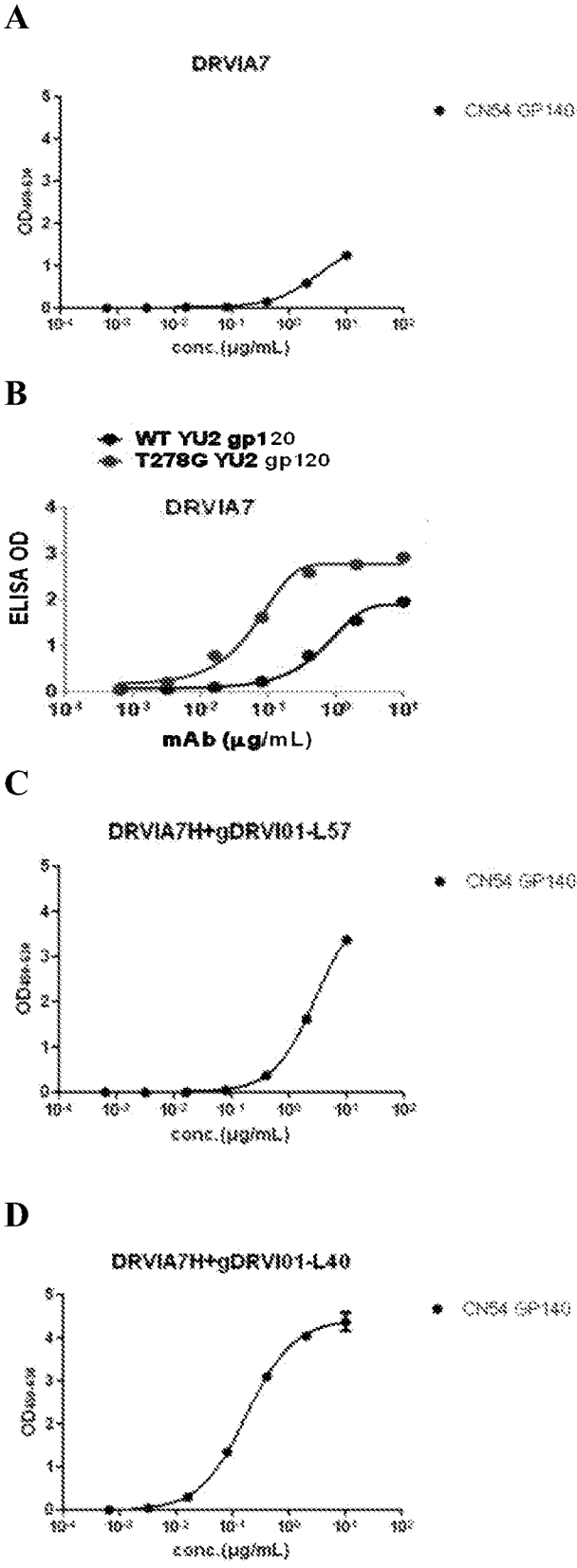


Figure 1

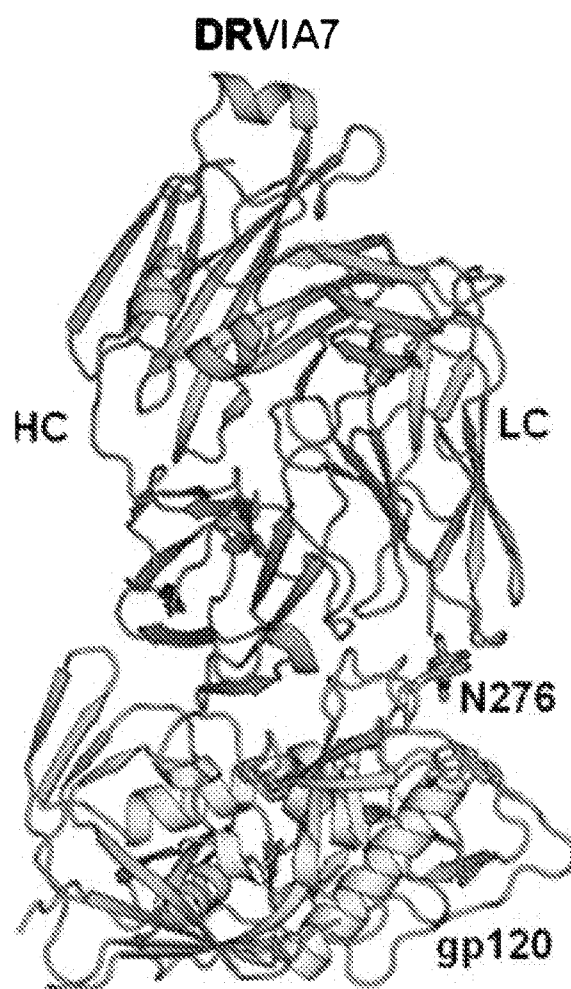
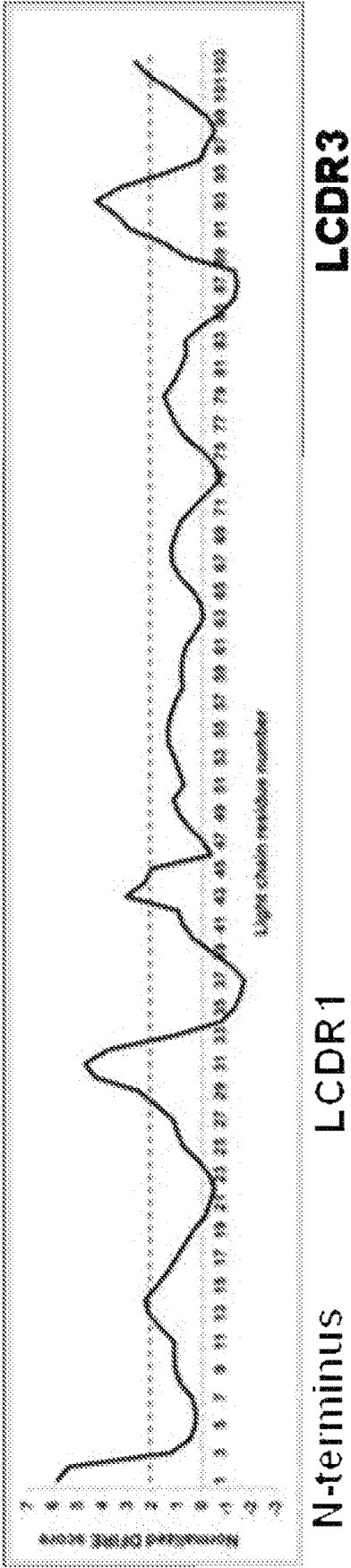


Figure 2

Figure 3



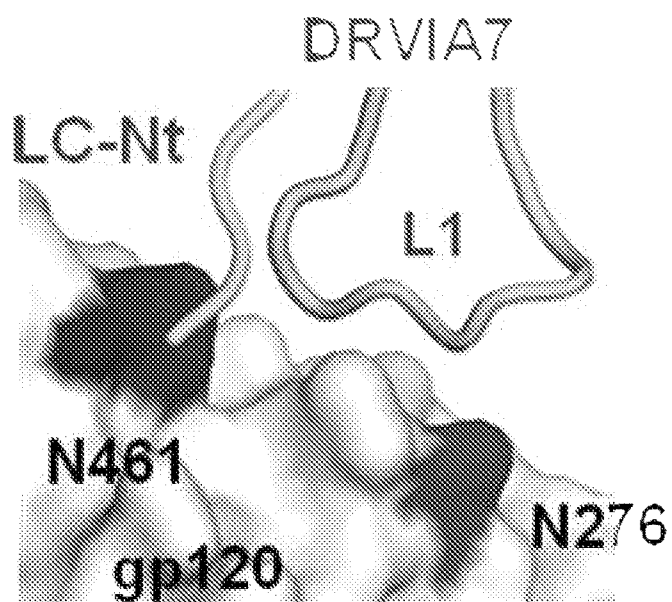


Figure 4

Figure 5

Heavy chain

-----FR1-----CDR1-----FR2-----CDR2-----FR3-----FR4-----
IgH1-7*01 IgHJ5*02
IgKV1-02*02 QVQLVQSGAEVKKPQASVKSCXKSGYTFGYTHGVVQAPQCGLEWVGWINDPNSCGTNFAQKFGQGVMTTRDT SI STAYMEL SRLSDDTA VYYCAR CDR3
DRVIA? QVQLVESGTFQRFEPGASVRLSCRASGYTFISSEFLNVIHQGPGCGGLEWVGWANDPEMAVNPFRPFGQGVMTTRDT SI DTAYMELRLDLRSDDTA NYTCVT SRTADYDYDF VWCQGT LVPVSS
IgH3-16*02 IgHJ1*01
-----FR3-----FR4-----
CDR3
IgKV1-02*02 QVQLVQSGAEVKKPQASVKSCXKSGYTFGYTHGVVQAPQCGLEWVGWINDPNSCGTNFAQKFGQGVMTTRDT SI STAYMEL SRLSDDTA VYYCAR
VRC01 QVQLVQSGGQMKTPGESMRISCRASGYTFIDCTLNWIELAPCKPPEWVGWIKTPGCAVNYAPPLQGVMTTRDYV SDTAFLELRSLTYDTAVYECTRGKNC DYNWDFEHWKRGTPVIVSS

Light chain

-----FR1-----CDR1-----FR2-----FR3-----FR4-----
IgKJ1*01
IgKV1-5*03 DIQHTQSPSTLSASGGRVTITCRASQSTLSMLAYQOKPGKAPKILLYKASSIESGVPSRFSSCSGSEFTLTISLSQPHDPFVYTCQ CDR3
DRVIA? DIQHTQSPVTLASISGDRVTITCRASQRIDNVWVAYQOKPGRAPKILLYKASSILETGVPSPRFSSCSGSEFTLTISLSQPHDPFVYTCQVKK FCKGTFKIDIK
IgKJ2*01
-----FR3-----FR4-----
CDR3
IgKV3-11*01 EIVLTQSPATLSLSPGERATLSCRASQSVSYLWYQOKPGQAPPELLTYDASNEKTCIPARFSSCSGSEFTLTISLKPEDFVYTCQ
VRC01 EIVLTQSPCTLSLSPGETATLSCTISQYGS LMYQQRPGQAPPELLTYSGSTEAGCIPDRFSSKMGCPDYNLTISNLSKDFGYYTCQVKK FCGCTKVQVNIK

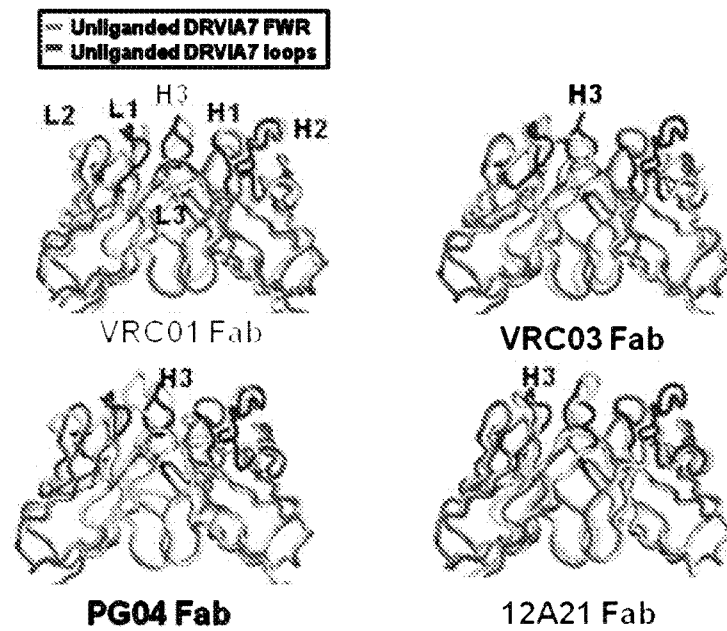


Figure 6

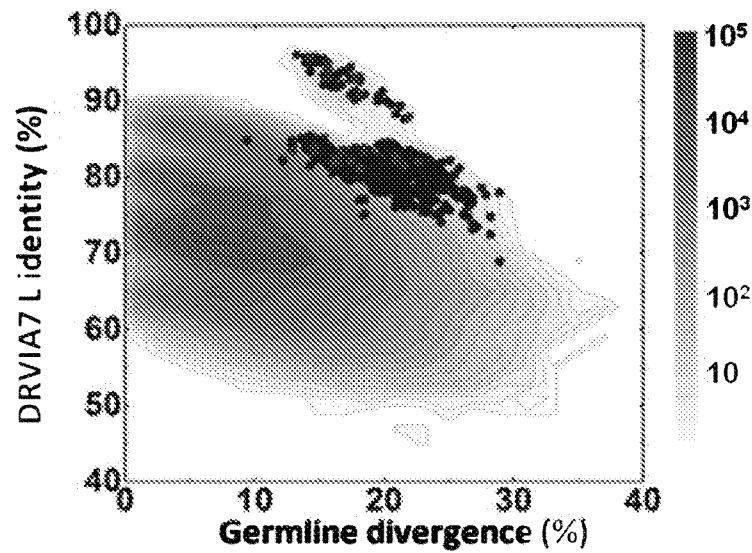


Figure 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/072749

A. CLASSIFICATION OF SUBJECT MATTER		
C07K 16/10(2006.01)i; C12N 15/13(2006.01)i; A61K 39/42(2006.01)i; A61P 31/18(2006.01)i; G01N 33/577(2006.01)i; G01N 33/569(2006.01)i		
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,C12N,A61K,A61P,G01N		
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) CPEA, DWPI, JPABS, SIPOABS, VEN, CPRSABS, MOABS, CNABS, TWABS, CJFD, CSCD, SIPONPL USTXT, JPTXT, EPTXT, WOTXT, CNTXT, cnki, isi web of knowledge:hiv-1,gp120,isolated antibody; GenBank+EMBL+DDBJ:sequence search on SEQ ID NOs:1-8		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 104271597 A (US DEPT HEALTH & HUMAN SERVICES) 07 January 2015 (2015-01-07) see the whole document	1-33,37,38
A	CN 103403026 A (US DEPT HEALTH & HUMAN SERVICES ET AL.) 20 November 2013 (2013-11-20) see the whole document	1-33,37,38
A	Jean-Philippe Julien et al. "Broadly Neutralizing Antibody PGT121 Allosterically Modulates CD4 Binding via Recognition of the HIV-1 gp120 V3 Base and Multiple Surrounding Glycans" <i>PLOS Pathogens</i> , Vol. 9, No. 5, 31 May 2013 (2013-05-31), ISSN: 1553-7374, e1003342, see the whole document	1-33,37,38
PX	Leopold Kong et al. "Key gp120 Glycans Pose Roadblocks to the Rapid Development of VRC01-Class Antibodies in an HIV-1- Infected Chinese Donor" <i>Immunity</i> , Vol. 44, No. 4, 19 April 2016 (2016-04-19), ISSN: 1074-7613, pages 930-950 see the whole document	1-33,37,38
☐ 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 29 March 2017		Date of mailing of the international search report 09 May 2017
Name and mailing address of the ISA/CN STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA 6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088 China		Authorized officer WANG,Qiyang
Facsimile No. (86-10)62019451		Telephone No. (86-10)62088409

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/072749

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 filed or furnished:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☒ in the international application as filed
☐ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
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 in 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/CN2017/072749

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.: **34-36**
because they relate to subject matter not required to be searched by this Authority, namely:
[1] Methods for Treatment of the Human or Animal Body by Surgery or Therapy.
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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2017/072749

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	104271597	A	07 January 2015	EP	2788376	A4	09 September 2015
				EP	2788376	A1	15 October 2014
				WO	2013086533	A1	13 June 2013
				AU	2012347453	A1	19 June 2014
				CA	2858716	A1	13 June 2013
				US	2014322163	A1	30 October 2014
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				AU	2010298025	A1	12 April 2012
				US	2012237523	A1	20 September 2012
				US	2016009789	A1	14 January 2016
				AU	2010298025	B2	21 April 2016
				WO	2011038290	A8	28 April 2011
				US	8637036	B2	28 January 2014
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