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(54) **Title:** HIV NEUTRALIZING ANTIBODIES HAVING MUTATIONS IN CONSTANT DOMAIN (Fc)

(57) **Abstract:** IgG antibodies having one or more mutations in the Fc domain are disclosed. Monoclonal neutralizing antibodies having one or more mutations in the constant domain are disclosed that specifically bind to the CD4 binding site of HIV-1 gp120. In some examples, such antibodies have a longer half-life than the corresponding native antibodies. The use of these antibodies to diagnose HIV infection or treat an HIV infection are also disclosed.



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HIV NEUTRALIZING ANTIBODIES HAVING MUTATIONS IN CONSTANT DOMAIN (Fc)

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims priority to US Provisional Application No.
61/439,678 filed February 4, 2011, herein incorporated by reference.

FIELD OF THE DISCLOSURE

10 This relates to monoclonal neutralizing antibodies that bind to the CD4
binding site of HIV-1 gp120 having mutations in the constant domain (Fc), and
methods for their use.

BACKGROUND

15 An effective HIV-1 vaccine will likely need to induce neutralizing antibodies
(NAb)s that block HIV-1 entry into human cells. To be effective, vaccine induced
antibodies will have to be active against most circulating strains of HIV-1.
Unfortunately, current HIV-1 vaccines are unable to induce potent and broadly
reactive NAb)s. In addition, antibodies with increased half-life are desirable.

20 One previously characterized HIV-1 neutralizing mAb, called b12, can bind
to a site on gp120 that is required for viral attachment to its primary cellular
receptor, CD4. Another previously characterized HIV-1 neutralizing mAb, called
2F5, can bind to a site on gp41. mAb b12 was derived from a phage display library,
a process which makes it impossible to know if the antibody was naturally present in
an infected person, or was the result of a laboratory combination of antibody heavy
25 and light chains. b12 can neutralize about 75% of clade B strains of HIV-1 (those
most common in North America), but it neutralizes less than 50% of other strains of
HIV-1 found worldwide. Prior attempts to design a vaccine that induces NAb)s
similar to b12 have been unsuccessful. Therefore, there is a need to develop NAb)s
for HIV-1, including those that have an increased half-life.

30

SUMMARY OF THE DISCLOSURE

Isolated human monoclonal neutralizing antibodies that specifically bind HIV-1 gp120 and have one or more mutations in the constant region (Fc) are provided herein. In some examples such antibodies have an increased half-life
 5 relative to the same antibodies with native Fc sequences. Also disclosed herein are compositions including these antibodies, nucleic acids encoding these antibodies, expression vectors comprising the nucleic acids, and isolated host cells that express the nucleic acids.

One skilled in the art will appreciate based on this discovery, that any IgG
 10 antibody can be generated that has equivalent mutations in its Fc region. Thus, also provided are isolated IgG antibodies, wherein a heavy chain of the IgG includes one or more amino acid substitutions in the Fc region, wherein the one or more amino acid substitutions comprise: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E;
 15 S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A; S239D/A330L/I332E+ T307A/E380A/N434A; and S239D/A330L/I332E +
 20 M252Y/S254T/T256E. The numbering of these mutations is based on the VRC antibodies (see FIGS. 1 and 7), and one skilled in the art will appreciate how to make equivalent mutations in other IgG molecules, for example by aligning the Fc region of the IgG with the mutated Fc regions shown in FIG. 7. For example, an isolated IgG antibody can include a S239D/I332E or S239D/A330L/I332E
 25 substitution, and in some examples can further include one or more of T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; and M252Y/S254T/T256E substitutions. In some examples such variant IgG molecules have increased FcRn binding relative to the native IgG molecule, such as an increase of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%,
 30 at least 100%, at least 200%, or at least 500%. IgG molecules can include IgG1, IgG2, IgG3, or IgG4.

In some embodiments, the heavy chain of the isolated human monoclonal antibody (mAb) having one or more mutations in Fc has at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, and wherein the antibody comprises a light chain, and wherein the mAb specifically binds gp120 of HIV-1, and wherein the antibody is neutralizing. In some embodiments, the heavy chain of the isolated human mAb having one or more mutations in Fc has at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, wherein the antibody comprises a light chain, wherein the mAb specifically binds gp120 of HIV-1, wherein the antibody is neutralizing, and wherein the mAb includes the particular mutation provided by SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 (for example, a sequence having at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 2 includes the T250Q/M428L mutation and a sequence having at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 4 includes the M428L/N434S mutation, and so forth).

In some examples, the light chain of the mAb comprises amino acids 27-30 (CDR1), 48-50 (CDR2), and 87-91 (CDR3) of SEQ ID NO: 36. In some examples, the heavy chain of the mAb includes one or more of the following amino acid substitutions:

T250Q/M428L;
M428L/N434S;
N434A;
T307A/E380A/N434A;
M252Y/S254T/T256E;
S239D/I332E;
S239D/A330L/I332E;
S239D/I332E + T250Q/M428L;
S239D/I332E + M428L/N434S;
S239D/I332E + N434A;
S239D/I332E + T307A/E380A/N434A;

S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 S239D/A330L/I332E+ N434A;
 5 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E.

In some examples, the heavy chain of the mAb includes a sequence having at least 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to SEQ ID NO: 38 or 42, and wherein the antibody comprises a light chain (such as
 10 a sequence having at least 80%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to SEQ ID NO: 40 or 44), and wherein the antibody specifically binds gp120 of HIV-1, wherein the antibody is neutralizing, and wherein the heavy chain of the antibody has one or more of the following amino acid substitutions:

15 T250Q/M428L;
 M428L/N434S;
 N434A;
 T307A/E380A/N434A;
 M252Y/S254T/T256E;
 20 S239D/I332E;
 S239D/A330L/I332E;
 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 S239D/I332E + N434A;
 25 S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 S239D/A330L/I332E+ N434A;
 30 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E.

In some examples, the isolated mAb has a heavy chain as shown in SEQ ID NO: 38 comprising one or more of the amino acid substitutions shown above and a light chain as shown in SEQ ID NO: 40. In other examples, the isolated mAb has a heavy chain as shown in SEQ ID NO: 42 comprising one or more of the amino acid substitutions shown above and a light chain as shown in SEQ ID NO: 44.

In some examples, the disclosed neutralizing hmAbs having one or more Fc mutations and have the ability to bind gp120 have increased FcRn binding and/or increase antibody-dependent cellular cytotoxicity (ADCC) activity relative to the native IgG molecule (such as VRC01, VRC01, or VRC03), such as an increase of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 500%. In some examples, the disclosed neutralizing hmAbs having one or more Fc mutations and have the ability to bind gp120 have increased half-life relative to the native IgG molecule (such as VRC01, VRC01, or VRC03), such as an increase of at least 1.5-fold, 1.8-fold, 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 4.5 fold, or at least 5-fold.

Also provided are isolated functional fragments of the disclosed mAbs specific for gp120 that have one or more mutations in the Fc. Exemplary fragments include a Fab fragment, a Fab' fragment, a F(ab)₂ fragment, a single chain Fv protein (scFv), or a disulfide stabilized Fv protein (dsFv).

The disclosed antibodies and fragments thereof can be labeled, for example with a fluorescent, enzymatic, or radioactive label.

Compositions that include the disclosed antibodies are provided, for example those that include a pharmaceutically acceptable carrier, such as water or saline.

Also provided are isolated nucleic acid molecules that encode the human mAbs disclosed herein (or a functional fragment thereof). In some examples, such a nucleic acid molecule includes a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 98, or at least 99% sequence identity to any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37 or 41, as well as sequences that encode a sequence having at least 80%, at least 90%, at least 95%, or at least 98% sequence identity to SEQ ID NO: 38 or 42 which have one or more of the following amino acid substitutions:

T250Q/M428L;
 M428L/N434S;
 N434A;
 T307A/E380A/N434A;
 5 M252Y/S254T/T256E;
 S239D/I332E;
 S239D/A330L/I332E;
 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 10 S239D/I332E + N434A;
 S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 15 S239D/A330L/I332E+ N434A;
 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E.

The mAbs and compositions disclosed herein can be used for a variety of purposes, such as for detecting an HIV-1 infection or diagnosing AIDS in a subject.

20 These methods can include contacting a sample from the subject (such as one suspected of having or who has been diagnosed with HIV-1 or AIDS) with a human monoclonal antibody that specifically binds gp120 and has one or more mutations in the Fc region, and detecting binding of the antibody to the sample. An increase in binding of the antibody to the sample relative to binding of the antibody to a control

25 sample confirms that the subject has an HIV-1 infection and/or AIDS. In some embodiments, the methods further include contacting a second antibody that specifically binds gp120 or gp41 with the sample, and detecting binding of the second antibody. In some non-limiting examples an increase in binding of the antibody to the sample relative to a control sample detects HIV-1 in the subject. In

30 some non-limiting examples, the antibody specifically binds soluble gp120 in the sample. In some embodiments, the methods further include contacting a second

antibody that specifically recognizes the gp120- or gp41-specific antibody with the sample and detecting binding of the second antibody.

In additional embodiments, a method is disclosed for treating a subject with an HIV infection, such as, but not limited to, a subject with AIDS. The methods
 5 include administering a therapeutically effective amount of a human gp120 specific monoclonal antibody having one or more mutations in the Fc region disclosed herein to the subject.

Also provided is a method for testing a potential vaccine, wherein the method includes contacting the potential vaccine with a human mAb disclosed
 10 herein (or a functional fragment thereof) and detecting the binding of the antibody to an immunogen in the potential vaccine.

The foregoing and other features of this disclosure will become more apparent from the following detailed description of a several embodiments which proceeds with reference to the accompanying figures.

15

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a table showing mutations made to the Fc region of the heavy chain of the VRC01 antibody.

FIG. 2 is a schematic drawing showing the structures of the VRC01
 20 antibody and its Fc mutants. Blue (those at the top) and red spheres (those closer to the bottom) represent ADCC enhancing mutation and hFcRn-binding enhancing mutation, respectively.

FIG. 3 is a pair of graphs showing binding of the VRC01 antibody and its Fc mutants to hFcRn at pH 6 (left) and pH 7.4 (right).

FIG. 4 is a pair of graphs showing binding of the VRC01 antibody and its Fc
 25 mutants to FcγRIIIa (left) and RSC3 (right).

FIG. 5 shows a pair of graphs showing antibody-dependent cellular cytotoxicity (ADCC) of the VRC01 antibody and its Fc mutants.

FIG. 6 is a schematic drawing showing the binding of IgG to hFcRn (left)
 30 and FcγRIIIa (right).

FIG. 7 shows an alignment of the heavy chain amino acid sequences for mAbs VRC01 (SEQ ID NO: 46), VRC02 (SEQ ID NO: 38) and VRC03 (SEQ ID

NO: 42), and a consensus sequence (SEQ ID NO: 47). Specific Fc mutations are disclosed herein that were made to VRC01 (see FIG. 1). One skilled in the art will appreciate that equivalent mutations can be made to VRC02 and 03 at the equivalent positions (underlined).

5 **FIG. 8** is a bar graph showing that the LS mutant antibodies were transcytosed across MDCK cells expressing hFcRn more than other mutant groups.

FIG. 9 is a graph showing that the hFcRn-binding enhancing mutant antibody, LS, had a half-life about 2 times longer than native VRC01 in the hFcRn Tg/Tg mouse model.

10 **FIG. 10** is a graph showing that the hFcRn-binding enhancing mutant antibody, LS, had a half-life about 3 times longer than native VRC01 in the Rhesus macaques model.

SEQUENCE LISTING

15 The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence
20 listing:

SEQ ID NOS: 1 and 2 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a T250Q/M428L (QL) mutation in its Fc.

SEQ ID NOS: 3 and 4 are the nucleic acid and corresponding amino acid
25 sequence of the heavy chain of gp120-specific antibody VRC01 having a M428L/N434S (LS) mutation in its Fc.

SEQ ID NOS: 5 and 6 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a N434A (A) mutation in its Fc.

30 **SEQ ID NOS: 7 and 8** are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a T307A/E380A/N434A (AAA) mutation in its Fc.

SEQ ID NOS: 9 and 10 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a M252Y/S254T/T256E (YTE) mutation in its Fc.

SEQ ID NOS: 11 and 12 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
5 S239D/I332E (DE) mutation in its Fc.

SEQ ID NOS: 13 and 14 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/A330L/I332E (DLE) mutation in its Fc.

SEQ ID NOS: 15 and 16 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
10 S239D/I332E + T250Q/M428L (DE - QL) mutation in its Fc.

SEQ ID NOS: 17 and 18 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
15 S239D/I332E + M428L/N434S (DE - LS) mutation in its Fc.

SEQ ID NOS: 19 and 20 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/I332E + N434A (DE - A) mutation in its Fc.

SEQ ID NOS: 21 and 22 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
20 S239D/I332E + T307A/E380A/N434A (DE - AAA) mutation in its Fc.

SEQ ID NOS: 23 and 24 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/I332E + M252Y/S254T/T256E (DE - YTE) mutation in its Fc.

SEQ ID NOS: 25 and 26 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
25 S239D/A330L/I332E + T307A/E380A/N434A (DLE - AAA) mutation in its Fc.

SEQ ID NOS: 27 and 28 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a
30 S239D/A330L/I332E + M252Y/S254T/T256E (DLE - YTE) mutation in its Fc.

SEQ ID NOS: 29 and 30 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/A330L/I332E + T250Q/M428L (DLE - QL) mutation in its Fc.

SEQ ID NOS: 31 and 32 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/A330L/I332E + M428L/N434S (DLE - LS) mutation in its Fc.

SEQ ID NOS: 33 and 34 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC01 having a S239D/A330L/I332E + N434A (DLE - A) mutation in its Fc.

SEQ ID NOS: 35 and 36 are the nucleic acid and corresponding amino acid sequence of the light chain of gp120-specific antibody VRC01.

SEQ ID NOS: 37 and 38 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC02.

SEQ ID NOS: 39 and 40 are the nucleic acid and corresponding amino acid sequence of the light chain of gp120-specific antibody VRC02. CDRs are shown in amino acids 27-30 (CDR1), 48-50 (CDR2), and 87-91(CDR3) of SEQ ID NO: 40.

SEQ ID NOS: 41 and 42 are the nucleic acid and corresponding amino acid sequence of the heavy chain of gp120-specific antibody VRC03.

SEQ ID NOS: 43 and 44 are the nucleic acid and corresponding amino acid sequence of the light chain of gp120-specific antibody VRC03. CDRs are shown in amino acids 24-33 (CDR1), 49-55 (CDR2), and 88-92 (CDR3) of SEQ ID NO: 44.

SEQ ID NOS: 45 and 46 are the nucleic acid and corresponding amino acid sequence of the native sequence of the heavy chain of gp120-specific antibody VRC01.

SEQ ID NO: 47 is a VRC heavy chain consensus sequence.

DETAILED DESCRIPTION

I. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology can be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by

Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.),
Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published
by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8). Terms describing protein
structure and structural elements of proteins can be found in Creighton, Proteins,
5 Structures and Molecular Properties, W.H. Freeman & Co., New York, 1993 (ISBN
0-717-7030).

Unless otherwise explained, all technical and scientific terms used herein
have the same meaning as commonly understood by one of ordinary skill in the art
to which this disclosure belongs. The singular terms “a,” “an,” and “the” include
10 plural referents unless context clearly indicates otherwise. Similarly, the word “or”
is intended to include “and” unless the context clearly indicates otherwise.

It is further to be understood that all base sizes or amino acid sizes, and all
molecular weight or molecular mass values, given for nucleic acids or polypeptides
are approximate, and are provided for descriptive purposes, unless otherwise
15 indicated. Although many methods and materials similar or equivalent to those
described herein can be used, particular suitable methods and materials are described
below. In case of conflict, the present specification, including explanations of terms,
will control. In addition, the materials, methods, and examples are illustrative only
and not intended to be limiting. All references cited herein are incorporated by
20 reference.

To facilitate review of the various embodiments of this disclosure, the
following explanations of terms are provided:

Administration: The introduction of a composition, such as the antibodies
disclosed herein, into a subject by a chosen route, for example topically, orally,
25 intravascularly such as intravenously, intramuscularly, intraperitoneally,
intranasally, intradermally, transdermally, intrathecally, subcutaneously, via
inhalation or via suppository. Administration can be local or systemic, such as
intravenous or intramuscular. For example, if the chosen route is intravenous, the
composition is administered by introducing the composition into a vein of the
30 subject. In some examples a disclosed antibody specific for an HIV protein is
administered to a subject at an effective dose.

Amino acid substitution: The replacement of one amino acid in peptide with a different amino acid.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals, such as primates. Similarly, the term “subject” includes both human and veterinary subjects.

Antibody: A polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or antigen binding fragments thereof, which specifically binds and recognizes an analyte (antigen), such as gp120 or an antigenic fragment of gp120. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Exemplary neutralizing gp120 mAbs are provided herein that have mutations in the Fc region, which in some examples increases their half-life and/or antibody-dependent cellular cytotoxicity (ADCC) activity.

Antibodies exist, for example as intact immunoglobulins and as a number of well characterized fragments produced by digestion with various peptidases. For instance, Fabs, Fvs, and single-chain Fvs (scFvs) that specifically bind to gp120 or fragments of gp120 would be gp120-specific binding agents. A scFv protein is a fusion protein in which a light chain variable region of an immunoglobulin and a heavy chain variable region of an immunoglobulin are bound by a linker, while in dsFvs, the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains. The term also includes genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies), heteroconjugate antibodies such as bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

Antibody fragments include: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained

per antibody molecule; (3) (Fab')₂, the fragment of the antibody obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; (4) F(ab')₂, a dimer of two Fab' fragments held together by two disulfide bonds; (5) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (6) single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. The term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies.

Typically, a naturally occurring immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region and a variable region, (the regions are also known as "domains"). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs." The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991, hereby incorporated by reference in its entirety). Thus one of ordinary skill in the art will recognize the numbering of the residues in the disclosed antibodies is made with reference to the Kabat convention. The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3,

numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. Light chain CDRs are sometimes referred to as CDR L1, CDR L2, and CDR L3. Heavy chain CDRs are sometimes referred to as CDR H1, CDR H2, and CDR H3.

References to “V_H” or “VH” refer to the variable region of an immunoglobulin heavy chain, including that of an antibody fragment, such as Fv, scFv, dsFv or Fab. References to “V_L” or “VL” refer to the variable region of an immunoglobulin light chain, including that of an Fv, scFv, dsFv or Fab.

A “monoclonal antibody” (mAb) is an antibody produced by a single clone of B-lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. These fused cells and their progeny are termed “hybridomas.” Monoclonal antibodies include humanized monoclonal antibodies. In some examples monoclonal antibodies are isolated from a subject. The amino acid sequences of such isolated monoclonal antibodies can be determined.

A “humanized” immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a “donor,” and the human immunoglobulin providing the framework is termed an “acceptor.” In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they are substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A “humanized antibody” is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized

antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Humanized immunoglobulins can be constructed by means of genetic engineering (for example, see U.S. Patent No. 5,585,089).

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T cell response in an animal, including compositions that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous antigens, such as the disclosed antigens. "Epitope" or "antigenic determinant" refers to the region of an antigen to which B and/or T cells respond. In one embodiment, T cells respond to the epitope, when the epitope is presented in conjunction with an MHC molecule. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope can include at least 3, and more usually, at least 5, about 9, or about 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes are known in the art and include, for example, x-ray crystallography and nuclear magnetic resonance.

Examples of antigens include, but are not limited to, peptides, lipids, polysaccharides, and nucleic acids containing antigenic determinants, such as those recognized by an immune cell. In some examples, antigens include peptides derived from HIV, such as a gp120 polypeptide or antigenic fragment thereof, such as a gp120 outer domain or fragment thereof.

A "target epitope" is a specific epitope on an antigen that specifically binds an antibody of interest, such as a monoclonal antibody. In some examples, a target epitope includes the amino acid residues that contact the antibody of interest, such

that the target epitope can be selected by the amino acid residues determined to be in contact with the antibody of interest.

Antigenic surface: A surface of a molecule, for example a protein such as a gp120 protein or portion thereof, capable of eliciting an immune response. An antigenic surface includes the defining features of that surface, for example the three-dimensional shape and the surface charge. An antigenic surface includes both surfaces that occur on gp120 polypeptides as well as surfaces of compounds that mimic the surface of a gp120 polypeptide (mimetics). In some examples, an antigenic surface includes all or part of the surface of gp120 that binds to the CD4 receptor.

Binding affinity: Affinity of an antibody or antigen binding fragment thereof for an antigen. For example, under designated conditions, an antibody that binds preferentially to a particular target protein (such as gp120) and does not bind in a significant amount to other proteins or polysaccharides present in the sample or subject, is referred to an antibody that specifically binds to its target. In one embodiment, affinity is calculated by a modification of the Scatchard method described by Frankel et al., *Mol. Immunol.*, 16:101-106, 1979. In another embodiment, binding affinity is measured by an antigen/antibody dissociation rate. In yet another embodiment, a high binding affinity is measured by a competition radioimmunoassay. In several examples, a high binding affinity is at least about 1×10^{-8} M. In other embodiments, a high binding affinity is at least about 1.5×10^{-8} , at least about 2.0×10^{-8} , at least about 2.5×10^{-8} , at least about 3.0×10^{-8} , at least about 3.5×10^{-8} , at least about 4.0×10^{-8} , at least about 4.5×10^{-8} , or at least about 5.0×10^{-8} M.

CD4: Cluster of differentiation factor 4 polypeptide; a T-cell surface protein that mediates interaction with the MHC class II molecule. CD4 also serves as the primary receptor site for HIV on T-cells during HIV-I infection. CD4 binds to gp120 from HIV. The sequence of the CD4 precursor has a hydrophobic signal peptide, an extracellular region of approximately 370 amino acids, a highly hydrophobic stretch with significant identity to the membrane-spanning domain of the class II MHC beta chain, and a highly charged intracellular sequence of 40 residues (Maddon, *Cell* 42:93, 1985).

The term "CD4" includes polypeptide molecules that are derived from CD4 include fragments of CD4, generated either by chemical (for example enzymatic) digestion or genetic engineering means. Such a fragment may be one or more entire CD4 protein domains. The extracellular domain of CD4 consists of four contiguous immunoglobulin-like regions (D1, D2, D3, and D4, see Sakihama *et al.*, *Proc. Natl. Acad. Sci.* 92:6444, 1995; U.S. Patent No. 6,117,655), and amino acids 1 to 183 have been shown to be involved in gp120 binding. For instance, a binding molecule or binding domain derived from CD4 would comprise a sufficient portion of the CD4 protein to mediate specific and functional interaction between the binding fragment and a native or viral binding site of CD4. One such binding fragment includes both the D1 and D2 extracellular domains of CD4 (D1D2 is also a fragment of soluble CD4 or sCD4 which is comprised of D1 D2 D3 and D4), although smaller fragments may also provide specific and functional CD4-like binding. The gp120-binding site has been mapped to D1 of CD4.

Contacting: Placement in direct physical association; includes both in solid and liquid form, which can take place either *in vivo* or *in vitro*. Contacting includes contact between one molecule and another molecule, for example the amino acid on the surface of one polypeptide, such as an antigen, that contacts another polypeptide, such as an antibody. Contacting can also include contacting a cell, for example by placing an antibody in direct physical association with a cell.

Framework Region: Amino acid sequences interposed between CDRs. Includes variable light and variable heavy framework regions. The framework regions serve to hold the CDRs in an appropriate orientation for antigen binding.

Fc region: The protein sequence that includes the constant region of an antibody excluding the first constant region immunoglobulin domain. Fc region generally refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM. An Fc region may also include part or all of the flexible hinge N-terminal to these domains. For IgA and IgM, an Fc region may or may not include the tailpiece, and may or may not be bound by the J chain. For IgG, the Fc region comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the

Fc region may vary, the human IgG heavy chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. For IgA, the Fc region comprises immunoglobulin domains Calpha2 and Calpha3 (Ca2 and Ca3) and the lower part of the hinge between Calpha1 (Ca1) and Ca2.

gp120: An envelope protein from Human Immunodeficiency Virus (HIV). This envelope protein is initially synthesized as a longer precursor protein of 845-870 amino acids in size, designated gp160. gp160 is cleaved by a cellular protease into gp120 and gp41. gp120 contains most of the external, surface-exposed, domains of the HIV envelope glycoprotein complex, and it is gp120 which binds both to cellular CD4 receptors and to cellular chemokine receptors (such as CCR5).

The mature gp120 wild-type proteins have about 500 amino acids in the primary sequence. gp120 is heavily N-glycosylated giving rise to an apparent molecular weight of 120 kD. The protein is comprised of five conserved regions (C1-C5) and five regions of high variability (V1-V5). Exemplary sequence of wt gp120 proteins are shown on GENBANK®, for example accession numbers AAB05604 and AAD12142 (as available on February 4, 2011), incorporated by reference herein. It is understood that there are numerous variation in the sequence of gp120 from what is given in GENBANK®, for example accession numbers AAB05604 and AAD12142, and that these variants are skill recognized in the art as gp120.

The numbering used in gp120 polypeptides disclosed herein is relative to the HXB2 numbering scheme as set forth in *Numbering Positions in HIV Relative to HXB2CG* Bette Korber *et al.*, Human Retroviruses and AIDS 1998: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences. Korber B, Kuiken CL, Foley B, Hahn B, McCutchan F, Mellors JW, and Sodroski J, Eds. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM which is incorporated by reference herein in its entirety.

Host cells: Cells in which a vector can be propagated and its DNA expressed, for example a disclosed antibody can be expressed in a host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell

since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Immunoaderhesin: A molecular fusion of a protein with the Fc region of an immunoglobulin, wherein the immunoglobulin retains specific properties, such as Fc receptor binding and increased half-life. An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general can be any protein, polypeptide, peptide, or small molecule. In one example, and immunoaderhesin includes the hinge, CH₂, and CH₃ domains of the immunoglobulin gamma 1 heavy chain constant region. In another example, the immunoaderhesin includes the CH₂, and CH₃ domains of an IgG.

Immunologically reactive conditions: Includes reference to conditions which allow an antibody raised against a particular epitope (such as an HIV gp120 epitope) to bind to that epitope to a detectably greater degree than, and/or to the substantial exclusion of, binding to substantially all other epitopes.

Immunologically reactive conditions are dependent upon the format of the antibody binding reaction and typically are those utilized in immunoassay protocols or those conditions encountered *in vivo*. See Harlow & Lane, *supra*, for a description of immunoassay formats and conditions. The immunologically reactive conditions employed in the methods are "physiological conditions" which include reference to conditions (*e.g.*, temperature, osmolarity, pH) that are typical inside a living mammal or a mammalian cell. While it is recognized that some organs are subject to extreme conditions, the intra-organismal and intracellular environment normally lies around pH 7 (*e.g.*, from pH 6.0 to pH 8.0, more typically pH 6.5 to 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. Osmolarity is within the range that is supportive of cell viability and proliferation.

IgA: A polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin alpha gene. In humans, this class or isotype includes IgA₁ and IgA₂. IgA antibodies can exist as monomers, polymers (referred to as pIgA) of predominantly dimeric form, and secretory IgA. The constant chain of wild-type IgA contains an 18-amino-acid extension at its C-terminus called the tail piece (tp). Polymeric IgA is secreted by plasma cells with a

15-kDa peptide called the J chain linking two monomers of IgA through the conserved cysteine residue in the tail piece.

IgG: A polypeptide belonging to the class or isotype of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans,
5 this class includes IgG₁, IgG₂, IgG₃, and IgG₄, in mice IgG₁, IgG_{2a}, IgG_{2b}, IgG₃.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease such as acquired immunodeficiency syndrome (AIDS). "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological
10 condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease,
15 a reduction in the viral load, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

Isolated: An "isolated" biological component (such as a cell, for example a
20 B cell, a nucleic acid, protein or antibody) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, such as, other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids
25 and proteins which have been "isolated" thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids. In some examples an antibody, such as an antibody specific for gp120 can be isolated, for example isolated from a subject infected with
30 HIV or from a cell that expresses the antibody.

K_d: The dissociation constant for a given interaction, such as a polypeptide ligand interaction or an antibody antigen interaction. For example, for the

bimolecular interaction of an antibody (such as VRC01, VRC02, or VRC03 or a mutant thereof) and an antigen (such as gp120) it is the concentration of the individual components of the bimolecular interaction divided by the concentration of the complex.

5 **Label:** A detectable compound or composition that is conjugated directly or indirectly to another molecule, such as an antibody or a protein, to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, quantum dots, and radioactive isotopes. In some examples, a disclosed antibody is labeled.

10 **Neutralizing antibody:** An antibody which reduces the infectious titer of an infectious agent by binding to a specific antigen on the infectious agent. In some examples the infectious agent is a virus. In some examples, an antibody that is specific for gp120 neutralizes the infectious titer of HIV. In specific examples, HIV-1 resists neutralization by most antibodies. However, the disclosed hmAbs
15 having one or more Fc mutations successfully neutralize at least 50% of current circulating HIV-1 isolates, such as at least 75%, at least 80%, or at least 90% of current circulating HIV-1 isolates.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W.
20 Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the antibodies herein disclosed.

 In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually
25 comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (*e.g.*, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or
30 magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary

substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide preparation is one in which the protein (such as an antibody) is more enriched than the protein is in its natural environment within a cell. In one embodiment, a preparation is purified such that the protein represents at least 50% of the total protein content of the preparation.

Recombinant: A recombinant nucleic acid molecule or protein is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by methods known in the art, such as chemical synthesis or by the artificial manipulation of isolated segments of nucleic acids, *e.g.*, by genetic engineering techniques. Cells that express such molecules are referred to as recombinant or transgenic cells.

Sequence identity: The similarity between amino acid or nucleic acid sequences are expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet et al., *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul et al., *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx,

tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Variants of the disclosed mAbs that specifically bind gp120 and have one or more mutations in the Fc region are encompassed by this disclosure typically characterized by possession of at least about 75%, for example at least about 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity counted over the full length alignment with the amino acid or nucleic acid sequence of interest, such as any of SEQ ID NOS: 1-47. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Therapeutically effective amount: A quantity of a specific substance, such as a disclosed mAb (or combinations of mAbs), sufficient to achieve a desired effect in a subject administered the mAb. For instance, this can be the amount necessary to inhibit HIV replication or treat AIDS. In several embodiments, a therapeutically effective amount is the amount necessary to reduce a sign or symptom of AIDS, and/or to decrease viral titer in a subject (such as a decrease of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, or at least 95% as compared to an absence of the mAb). When administered to a subject, a dosage will generally be used that will achieve target tissue concentrations that has been shown to achieve a desired *in vitro* effect.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, CD4⁺ T cells and CD8⁺ T cells. A CD4⁺ T lymphocyte is an

immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. CD8⁺ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a CD8 T cells is a cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Virus: A virus consists essentially of a core of a single nucleic acid surrounded by a protein coat, and has the ability to replicate only inside a living cell. "Viral replication" is the production of additional virus by the occurrence of at least one viral life cycle. A virus may subvert the host cells' normal functions, causing the cell to behave in a manner determined by the virus. For example, a viral infection may result in a cell producing a cytokine, or responding to a cytokine, when the uninfected cell does not normally do so.

"Retroviruses" are RNA viruses wherein the viral genome is RNA. When a host cell is infected with a retrovirus, the genomic RNA is reverse transcribed into a DNA intermediate which is integrated very efficiently into the chromosomal DNA of infected cells. The integrated DNA intermediate is referred to as a provirus. The term "lentivirus" is used in its conventional sense to describe a genus of viruses containing reverse transcriptase. The lentiviruses include the "immunodeficiency viruses" which include human immunodeficiency virus (HIV) type 1 and type 2 (HIV-I and HIV-II), simian immunodeficiency virus (SIV), and feline immunodeficiency virus (FIV).

HIV-I is a retrovirus that causes immunosuppression in humans (HIV disease), and leads to a disease complex known as the acquired immunodeficiency syndrome (AIDS). "HIV disease" refers to a well-recognized constellation of signs and symptoms (including the development of opportunistic infections) in persons who are infected by an HIV virus, for example, as determined by antibody or

western blot studies. Laboratory findings associated with this disease are a progressive decline in T cells.

II. Description of Several Embodiments

5 A. Neutralizing Monoclonal Antibodies

Isolated human monoclonal antibodies (hmAbs) that specifically bind gp120 are disclosed herein, which include one or more mutations in their Fc region. In one example, such mutations include one or more of the following: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E;
 10 S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A; S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E +
 15 M252Y/S254T/T256E. The numbering of amino acid residue in IgG1 follows the Eu system (Edelman *et al.*, *PNAS* 63:78-85, 1969).

It has been unexpectedly found that hmAbs that specifically bind gp120 and have a S239D/I332E or S239D/A330L/I332E substitution, in combination with one or more of T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; or
 20 M252Y/S254T/T256E, have greater FcRn binding, and/or half-life, than any of these mutations alone. It has also been unexpectedly found that mAbs that specifically bind gp120 and have a M428L/N434S substitution, have a greater half-life and increased transcytosis activity, as compared to the native VRC01 antibody, and that the combination M428L/N434S and S239D/I332E substitutions provide a mutant
 25 antibody with better transcytosis and a similar half-life relative to the native VRC01 antibody. In some examples, hFcRn-binding enhancing mutants (hFcRn mutant) have lower FcγRIIIa-binding affinity and ADCC activity, and FcγRIIIa-binding mutants (FcγR mutants) have lower hFcRn-binding affinity than wild type. When an FcγR mutation is introduced into an hFcRn mutant, the binding affinity of the
 30 combined mutant to FcγRIIIa and ADCC activity becomes similar to wild type and vice versa. In some examples, combining an hFcRn mutant with an FcγR-binding

enhancing mutation increases ADCC activity relative to a wildtype antibody (*e.g.*, VRC01).

Methods of introducing such mutations into the Fc region are routine. In some examples such hmAbs that specifically bind gp120 that include one or more mutations in their Fc region have increased FcRn binding relative to the native hmAb (such as VRC01), such as an increase of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 500%. In some examples such hmAbs that specifically bind gp120 that include one or more mutations in their Fc region have increased FcγRIIIa binding relative to the native hmAb (such as VRC01), such as an increase of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 500%. In some examples such hmAbs that specifically bind gp120 that include one or more mutations in their Fc region have increased antibody-dependent cellular cytotoxicity (ADCC) relative to the native hmAb (such as VRC01), such as an increase of at least 20%, at least 50%, at least 60%, at least 75%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 500%. In some examples such hmAbs that specifically bind gp120 that include one or more mutations in their Fc region have increased half-life relative to the native hmAb (such as VRC01), such as an increase of at least 1.5-fold, at least 1.8 fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, or at least 10-fold.

One skilled in the art will appreciate based on this discovery, that any IgG antibody can be generated that has equivalent mutations in its Fc region. Thus, also provided are isolated IgG antibodies, wherein a heavy chain of the IgG includes one or more amino acid substitutions in the Fc region, wherein the one or more amino acid substitutions comprise: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A; S239D/A330L/I332E+ T307A/E380A/N434A; S239D/A330L/I332E + M252Y/S254T/T256E, or combinations thereof. The numbering of these mutations

is based on the VRC antibodies (see FIGS. 1 and 7), and one skilled in the art will appreciate how to make equivalent mutations in other IgG molecules, for example by aligning the Fc region of the IgG with the mutated Fc regions shown in FIG. 7. For example, an isolated IgG antibody can include a S239D/I332E or

5 S239D/A330L/I332E substitution, and in some examples can further include one or more of T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; and M252Y/S254T/T256E substitutions. In some examples such variant IgG molecules have increased FcRn binding relative to the native IgG molecule (such as IgG1, IgG2, IgG3, or IgG4), such as an increase of at least 20%, at least 50%, at least 60%,
10 at least 75%, at least 80%, at least 90%, at least 100%, at least 200%, or at least 500%.

Also disclosed herein are compositions including these variant IgGs or hmAbs and a pharmaceutically acceptable carrier. Nucleic acids encoding these antibodies, expression vectors comprising these nucleic acids, and isolated host cells
15 that express the nucleic acids are also provided.

Compositions comprising the disclosed hmAbs specific for gp120 and having one or more Fc mutations can be used for research, diagnostic and therapeutic purposes. For example, the hmAbs disclosed herein can be used to diagnose or treat a subject having an HIV-1 infection and/or AIDS. For example,
20 the hmAbs can be used to determine HIV-1 titer in a subject. The hmAbs disclosed herein also can be used to study the biology of HIV.

In some embodiments, an isolated hmAb specifically binds gp120, and includes one or more (such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) of the following mutations (relative to the native Ab Fc sequence): T250Q/M428L; M428L/N434S; N434A;
25 T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A;
30 S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E + M252Y/S254T/T256E. In some embodiments, the isolated hmAbs specifically bind gp120 (and may neutralize HIV), and include a heavy chain having one or more of

the mutations listed above (relative to the native Ab Fc sequence) and has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

5 In specific examples, the heavy chain of the hmAb has at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 and specifically binds gp120 of HIV-1 and is neutralizing. For example, the antibody can include a heavy chain that comprises or consists of any of SEQ ID
10 NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34.

In some examples, the light chain of the hmAb includes amino acids 27-30 (CDR1), 48-50 (CDR2), and 87-91 (CDR3) of SEQ ID NO: 36, for example, a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 36, or a sequence comprising or consisting of SEQ ID NO: 36. In some
15 examples, the light chain of the hmAb includes amino acids 27-30 (CDR1), 48-50 (CDR2), and 87-91 (CDR3) of SEQ ID NO: 40, for example, a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 40, or a sequence comprising or consisting of SEQ ID NO: 40. In some examples, the light chain of the hmAb includes amino acids 24-33 (CDR1), 49-55 (CDR2), and 88-92
20 (CDR3) of SEQ ID NO: 44, for example, a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 44, or a sequence comprising or consisting of SEQ ID NO: 44.

In one example, the isolated hmAb has a heavy chain with a sequence that includes or consists of the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8, 10,
25 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 and the light chain of the antibody includes or consists of or consists of SEQ ID NO: 36. In a specific example, the isolated human monoclonal antibody of has a heavy chain comprising SEQ ID NO: 4 or 18.

In some examples the disclosed antibodies having Fc amino acid
30 substitutions (mutations) include a variant of a native heavy chain of the VRC01 hmAb (SEQ ID NO: 46) having one or more mutations in the Fc disclosed herein. For example, FIG. 1 shows several amino acid substitutions made to a native

VRC01 sequence. One skilled in the art will appreciate that the equivalent mutation can be made to other antibodies, such as VRC02 and VRC03. As shown in FIG. 7, an amino acid alignment of the heavy chain is provided for VRC01, VRC02 and VRC03. The amino acid in the Fc region mutated in VRC01 is underlined, as is the equivalent amino acid in VRC02 and VRC03. Based on this alignment and the mutations provided herein, equivalent mutations are provided for VRC02 and VRC03. For example, provided herein are hmAbs, wherein a heavy chain of the antibody includes a sequence having at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, or at least 99%, sequence identity to SEQ ID NO: 38 or 42, wherein the heavy chain of the antibody has one or more of the following amino acid substitutions: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A; S239D/A330L/I332E+ T307A/E380A/N434A; S239D/A330L/I332E + M252Y/S254T/T256E, or combinations thereof, wherein the antibody comprises a light chain, and wherein the antibody specifically binds gp120 of HIV-1 and is neutralizing. In some examples, the light chain of such hmAbs include a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 40 or 44. For example, the disclosed hmAbs can include a heavy chain that includes SEQ ID NO: 38 with one or more of the amino acid substitutions listed above and a light chain that includes SEQ ID NO: 40. In another example, a disclosed hmAb has a heavy chain comprising SEQ ID NO: 42 with one or more of the amino acid substitutions listed above and a light chain that includes SEQ ID NO: 44.

In further embodiments, the isolated human monoclonal antibody specifically binds gp120, and includes a heavy chain with at most one, at most two, at most three or at most four amino acid substitutions in the CDR1, CDR2, or CDR3 of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 (or SEQ ID NO: 38 or 42 with one or more of the mutations provided herein) and a light chain. In some examples, these antibodies retain the binding affinity of the parental

antibody (VRC01 or VRC02 or VRC03) for the antigenic epitope. Thus, in some examples, these antibodies have a KD of < 3 nM for the antigenic epitope of gp120.

Fully human monoclonal antibodies include human framework regions. Thus, any of the antibodies that specifically bind gp120 and have a mutated Fc region provided herein can include the human framework region and can include the framework regions of the amino acid sequence set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 (or SEQ ID NO: 38 or 42 with one or more of the mutations provided herein). However, the framework regions can be from another source. Additional examples of framework sequences that can be used include the amino acid framework sequences of the heavy and light chains disclosed in PCT Publication No. WO 2006/074071 (see, for example, SEQ ID NOs: 1-16), herein incorporated by reference.

The monoclonal antibody can be of any isotype. The monoclonal antibody can be, for example, an IgM or an IgG antibody, such as IgG₁ or an IgG₂. The class of an antibody that specifically binds gp120 can be switched with another. In one aspect, a nucleic acid molecule encoding V_L or V_H is isolated using methods well-known in the art, such that it does not include any nucleic acid sequences encoding the constant region of the light or heavy chain, respectively. The nucleic acid molecule encoding V_L or V_H can be operatively linked to a nucleic acid sequence encoding a C_L or C_H from a different class of immunoglobulin molecule. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as known in the art. For example, a hmAbs disclosed herein that was originally IgM may be class switched to an IgG. Class switching can be used to convert one IgG subclass to another, such as from IgG₁ to IgG₂.

In some examples, the disclosed hmAbs are oligomers of antibodies, such as dimers trimers, tetramers, pentamers, hexamers, septamers, octomers and so on. In some examples, the hmAbs are pentamers.

By definition, the CDRs of the light chain are bounded by the residues at positions 24 and 34 (L-CDR1), 50 and 56 (L-CDR2), 89 and 97 (L-CDR3); the CDRs of the heavy chain are bounded by the residues at positions 31 and 35b (H-CDR1), 50 and 65 (H-CDR2), 95 and 102 (H-CDR3), using the numbering convention delineated by Kabat *et al.*, (1991) *Sequences of Proteins of*

Immunological Interest, 5th Edition, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD (NIH Publication No. 91-3242, incorporated herein by reference in its entirety).

Antibody fragments of the disclosed neutralizing hmAbs that specifically
5 bind gp120 and have one or more mutations in the Fc region are encompassed by the present disclosure, such as Fab, F(ab')₂, and Fv which include a heavy chain and light chain variable region. These antibody fragments retain the ability to selectively bind with the antigen. These fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding
10 fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by
treating whole antibody with pepsin, followed by reduction, to yield an intact light
15 chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) (Fab')₂, the fragment of the antibody that can be obtained by treating
whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a
dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region
20 of the light chain and the variable region of the heavy chain expressed as two chains;
and

(5) Single chain antibody (such as scFv), defined as a genetically
engineered molecule containing the variable region of the light chain, the variable
25 region of the heavy chain, linked by a suitable polypeptide linker as a genetically
fused single chain molecule.

(6) A dimer of a single chain antibody (scFV₂), defined as a dimer of a
scFV. This has also been termed a "miniantibody."

Methods of making these fragments are known in the art (see for example,
30 Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor
Laboratory, New York, 1988).

In further embodiments, the antibodies are Fv antibodies, which are typically about 25 kDa and contain a complete antigen-binding site with three CDRs per each heavy chain and each light chain. To produce these antibodies, the V_H and the V_L can be expressed from two individual nucleic acid constructs in a host cell. In particular examples, the V_H amino acid sequence includes the CDRs from one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 (or SEQ ID NO: 38 or 42 with one or more of the mutations provided herein). In other examples, the V_L amino acid sequence includes the CDRs from SEQ ID NO: 36, 40 or 44.

If the V_H and the V_L are expressed non-contiguously, the chains of the Fv antibody are typically held together by noncovalent interactions. However, these chains tend to dissociate upon dilution, so methods have been developed to crosslink the chains through glutaraldehyde, intermolecular disulfides, or a peptide linker. Thus, in one example, the Fv can be a disulfide stabilized Fv (dsFv), wherein the heavy chain variable region and the light chain variable region are chemically linked by disulfide bonds.

In an additional example, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271, 1993; and Sandhu, *supra*). Dimers of a single chain antibody (scFV₂), are also contemplated.

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted

F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

One of skill will realize that conservative variants of the disclosed antibodies can be produced. Such conservative variants employed in antibody fragments, such as dsFv fragments or in scFv fragments, will retain critical amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions, and will retain the charge characteristics of the residues in order to preserve the low pI and low toxicity of the molecules. Amino acid substitutions (such as at most one, at most two, at most three, at most four, at most five, or at most 10 amino acid substitutions, such as 1 to 10 or 1 to 5 conservative substitutions) can be made in the V_H and the V_L regions to increase yield. In particular examples, the V_H sequence is from one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 (or SEQ ID NO: 38 or 42 with one or more of the mutations provided herein). In other examples, the V_L sequence is from one of SEQ ID NOs: 36, 40 or 44. Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);

- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The antibodies disclosed herein can be isolated using cloaked antigens, as described in PCT Publication No. WO 2009/100376. Briefly, antigens are cloaked to target antigenicity of the antigen to a specific epitope that specifically bound by the antibody of interest, such as a neutralizing antibody.

In some examples, the amino acid substitutions result in the antigen not being bound by antibodies in a polyclonal serum that specifically bind surface exposed amino acid residues of the wild-type antigen located exterior of the target epitope. In some embodiments, the amino acid substitutions alter antigenicity of the antigen *in vivo* as compared to the wild-type antigen (unsubstituted antigen) but do not introduce additional glycosylation sites as compared to the wild-type antigen. In some embodiments, that antigen is glycosylated. In some embodiments the cloaked antigen is modified to substitute one or more residues recognized by the antibody of interest to abolish antigen recognition. In some examples, a biotinylation peptide can be fused to the cloaked antigen. Biotinylated cloaked antigen can then be used to stain and thus identify cells, such as PBMC, expressing an antibody of interest.

The hmAbs or antibody fragments disclosed herein can be derivatized or linked to another molecule (such as another peptide or protein). In general, the antibody or portion thereof is derivatized such that the binding to gp120 is not affected adversely by the derivatization or labeling. For example, the antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bispecific antibody or a diabody), a detection agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

One type of derivatized antibody is produced by cross-linking two or more antibodies (of the same type or of different types, such as to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-

maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company (Rockford, IL).

5 An antibody that specifically binds gp120 and has an Fc mutation (or a functional fragment thereof) can be labeled with a detectable moiety. Useful detection agents include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein, Yellow fluorescent protein. An antibody can also be labeled with enzymes that are useful for detection, such as
10 horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase
15 is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. An antibody may also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be labeled with an enzyme or a fluorescent label. In one example, an antibody or fragment thereof
20 disclosed herein can be labeled with a quantum dot.

An antibody or fragment thereof disclosed herein can be labeled with a magnetic agent, such as gadolinium. Antibodies can also be labeled with lanthanides (such as europium and dysprosium), and manganese. Paramagnetic particles such as superparamagnetic iron oxide are also of use as labels. An antibody
25 may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

30 An antibody or fragment thereof disclosed herein can also be labeled with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. Examples of labels for polypeptides include, but are not

limited to, the following radioisotopes or radionucleotides: ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I .

An antibody or fragment thereof disclosed herein can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody, such as to increase serum half-life or to increase tissue binding.

Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

B. Polynucleotides and Expression

Nucleic acid molecules encoding the peptides provided herein (including, but not limited to antibodies) can readily be produced by one of skill in the art. For example, these nucleic acids can be produced using the amino acid sequences provided herein (such as the CDR sequences, heavy chain and light chain sequences), sequences available in the art (such as framework sequences), and the genetic code.

Thus, the disclosure provides isolated nucleic acid molecules that encoding any of the neutralizing hmAbs disclosed herein (or a functional fragment thereof). In one example, such a nucleic acid molecule comprises a nucleic acid sequence having at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37 or 41. In some examples the nucleic acid molecule comprises or consists of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37 or 41.

In one example, the isolated nucleic acid molecule encodes a heavy chain and a light chain of an antibody, wherein (a) the heavy chain of the antibody

- comprises a sequence having at least 80%, at least 90%, at least 95% or at least 99% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, and having one or more of the following substitutions/mutations: T250Q/M428L; M428L/N434S; N434A;
- 5 T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+
- 10 N434A; S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E + M252Y/S254T/T256E, and wherein the light chain of the antibody comprises a sequence having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 36; (b) the heavy chain of the antibody comprises the heavy chain of the antibody comprises any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18,
- 15 20, 22, 24, 26, 28, 30, 32, or 34, and wherein the light chain of the antibody comprises SEQ ID NO: 36; (c) the heavy chain of the antibody comprises SEQ ID NO: 36 or 42 having one or more of the following substitutions/mutations: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E; S239D/A330L/I332E; S239D/I332E +
- 20 T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L; S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+ N434A; S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E + M252Y/S254T/T256E, and wherein the light chain of the
- 25 antibody comprises a sequence having at least 80%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 40 or 44; (d) the heavy chain of the antibody comprises SEQ ID NO: 36 having one or more of the following substitutions/mutations: T250Q/M428L; M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E; S239D/I332E;
- 30 S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A; S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L;

S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+
 N434A; S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E +
 M252Y/S254T/T256E, and wherein the light chain of the antibody comprises SEQ
 ID NO: 40; (e) the heavy chain of the antibody comprises SEQ ID NO: 42 having
 5 one or more of the following substitutions/mutations: T250Q/M428L;
 M428L/N434S; N434A; T307A/E380A/N434A; M252Y/S254T/T256E;
 S239D/I332E; S239D/A330L/I332E; S239D/I332E + T250Q/M428L; S239D/I332E
 + M428L/N434S; S239D/I332E + N434A; S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E; S239D/A330L/I332E+ T250Q/M428L;
 10 S239D/A330L/I332E+ M428L/N434S; S239D/A330L/I332E+
 N434A; S239D/A330L/I332E+ T307A/E380A/N434A; or S239D/A330L/I332E +
 M252Y/S254T/T256E, and wherein the light chain of the antibody comprises SEQ
 ID NO: 44, wherein the encoded antibody specifically binds gp120 of HIV-1, and
 wherein the encoded antibody is neutralizing.

15 Such isolated nucleic acid molecules can be operably linked to a promoter.
 The disclosed isolated nucleic acid molecules can be part of an expression vector,
 such as a plasmid. In one example, the expression vector includes a promoter and
 an enhancer, such as a cytomegalovirus promoter or cytomegalovirus enhancer.
 The expression vector can include RNA splicing donor sites (such as HTLV-1 or
 20 CMV RNA splicing donor sites), RNA splicing acceptor sites (such as HTLV-1 or
 CMV RNA splicing acceptor sites) and/or internal ribosomal binding sequences.
 Such expression vectors can be used to express the heavy chain of the antibody and
 the light chain of the antibody as a fusion polypeptide following the introduction of
 the expression vector in a host cell. For example, the expression vector can include
 25 a nucleic acid sequence encoding a furin cleavage site between the heavy chain and
 the light chain of the antibody. In some examples, the expression vector also
 encodes a selectable marker. Isolated host cells transformed with the disclosed
 nucleic acid molecules or vectors are also provided.

30 One of skill in the art can readily use the genetic code to construct a variety
 of functionally equivalent nucleic acids, such as nucleic acids which differ in
 sequence but which encode the same antibody sequence, or encode a conjugate or
 fusion protein including the heavy or light chain nucleic acid sequence.

Nucleic acid sequences encoding the neutralizing hmAbs that specifically bind gp120 and have one or more Fc mutations can be prepared by any suitable method including, for example, cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al.*,
5 *Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for
10 example, using an automated synthesizer as described in, for example, Needham-VanDevanter *et al.*, *Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that
15 longer sequences may be obtained by the ligation of shorter sequences.

Exemplary nucleic acids can be prepared by routine cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Sambrook *et al.*, *supra*, Berger and Kimmel (eds.), *supra*, and Ausubel, *supra*.
20 Product information from manufacturers of biological reagents and experimental equipment also provide useful information. Such manufacturers include the SIGMA Chemical Company (Saint Louis, MO), R&D Systems (Minneapolis, MN), Pharmacia Amersham (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen
25 Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen (Carlsbad, CA), and Applied Biosystems (Foster City, CA), as well as many other commercial sources.

Nucleic acids can also be prepared by amplification methods. Amplification
30 methods include but are not limited to polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription-based amplification system (TAS), and the self-

sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known.

Any of the nucleic acids encoding any of the antibodies, V_H and/or V_L, disclosed herein (or fragment thereof) can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and mammalian cells. These antibodies can be expressed as individual V_H and/or V_L chain, or can be expressed as a fusion protein. An immunoadhesin can also be expressed. Thus, nucleic acids encoding a V_H and V_L, and immunoadhesin are provided. The nucleic acid sequences can optionally encode a leader sequence.

To create a single chain antibody, (scFv) the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly₄-Ser)₃, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker (see, *e.g.*, Bird *et al.*, *Science* 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; McCafferty *et al.*, *Nature* 348:552-554, 1990). Optionally, a cleavage site can be included in a linker, such as a furin cleavage site.

The nucleic acid encoding the V_H and/or the V_L optionally can encode an Fc domain (immunoadhesin). The Fc domain can be an IgA, IgM or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100/093979. In one example, the immunoadhesin is an IgG₁ Fc.

The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to gp120 and to another molecule, such as gp41. The encoded V_H and V_L optionally can include a furin cleavage site between the V_H and V_L domains.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of proteins including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

The host cell can be a gram positive bacteria including, but are not limited to, *Bacillus*, *Streptococcus*, *Streptomyces*, *Staphylococcus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Clostridium*, *Geobacillus*, and *Oceanobacillus*.

Methods for expressing protein in gram positive bacteria, such as *Lactobacillus* are well known in the art, see for example, U.S. Published Patent Application No. 20100/080774. Expression vectors for lactobacillus are described, for example in U.S. Pat. Nos. 6,100,388 and 5,728,571. Leader sequences can be included for expression in *Lactobacillus*. Gram negative bacteria include, but not limited to, *E. coli*, *Pseudomonas*, *Salmonella*, *Campylobacter*, *Helicobacter*, *Flavobacterium*, *Fusobacterium*, *Ilyobacter*, *Neisseria*, and *Ureaplasma*.

One or more DNA sequences encoding the antibody or fragment thereof can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art. Hybridomas expressing the mutant mAbs provided herein are also encompassed by this disclosure.

The expression of nucleic acids encoding the isolated proteins described herein can be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression cassette. The promoter can be any promoter, including a cytomegalovirus promoter and a human T cell lymphotropic virus promoter (HTLV)-1. Optionally, an enhancer, such as a cytomegalovirus enhancer, is included in the construct. The cassettes can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression cassettes contain specific sequences useful for regulation of the expression of the DNA encoding the protein. For example, the expression cassettes can include appropriate promoters, enhancers, transcription and translation terminators, initiation sequences, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, sequences for the maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and

stop codons. The vector can encode a selectable marker, such as a marker encoding drug resistance (for example, ampicillin or tetracycline resistance).

To obtain high level expression of a cloned gene, expression cassettes can include a strong promoter to direct transcription, a ribosome binding site for
5 translational initiation (internal ribosomal binding sequences), and a transcription/translation terminator. For *E. coli*, this includes a promoter such as the T7, trp, lac, or lambda promoters, a ribosome binding site, and preferably a transcription termination signal. For eukaryotic cells, the control sequences can include a promoter and/or an enhancer derived from, for example, an
10 immunoglobulin gene, HTLV, SV40 or cytomegalovirus, and a polyadenylation sequence, and can further include splice donor and/or acceptor sequences (for example, CMV and/or HTLV splice acceptor and donor sequences). The cassettes can be transferred into the chosen host cell by well-known methods such as transformation or electroporation for *E. coli* and calcium phosphate treatment,
15 electroporation or lipofection for mammalian cells. Cells transformed by the cassettes can be selected by resistance to antibiotics conferred by genes contained in the cassettes, such as the amp, gpt, neo and hyg genes.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as
20 microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with polynucleotide sequences encoding the antibody, labeled antibody, or functional fragment thereof, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to
25 use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). One can readily use an expression system, such as plasmids and vectors, to produce proteins in cells including higher eukaryotic cells such as the COS, CHO,
30 HeLa, and myeloma cell lines.

Modifications can be made to a nucleic acid encoding a polypeptide described herein without diminishing its biological activity. Some modifications

can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation, site, additional amino acids placed on either terminus to create
5 conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps. In addition to recombinant methods, the immunoconjugates, effector moieties, and antibodies of the present disclosure can also be constructed in whole or in part using standard peptide synthesis.

Once expressed, the recombinant immunoconjugates, antibodies, and/or
10 effector molecules can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, R. Scopes, PROTEIN PURIFICATION, Springer-Verlag, N.Y., 1982). The antibodies, immunoconjugates and effector molecules need not be 100% pure. Once purified, partially or to
15 homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of antibodies and/or refolding to an appropriate active form, including single chain antibodies, from bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed
20 herein. See, Buchner *et al.*, *Anal. Biochem.* 205:263-270, 1992; Pluckthun, *Biotechnology* 9:545, 1991; Huse *et al.*, *Science* 246:1275, 1989 and Ward *et al.*, *Nature* 341:544, 1989.

Often, functional heterologous proteins from *E. coli* or other bacteria are isolated from inclusion bodies and require solubilization using strong denaturants,
25 and subsequent refolding. During the solubilization step, a reducing agent is present to separate disulfide bonds. An exemplary buffer with a reducing agent is: 0.1 M Tris pH 8, 6 M guanidine, 2 mM EDTA, 0.3 M DTE (dithioerythritol). Reoxidation of the disulfide bonds can occur in the presence of low molecular weight thiol reagents in reduced and oxidized form, as described in Saxena *et al.*, *Biochemistry*
30 9:5015-21, 1970, and by Buchner *et al.*, *supra*.

Renaturation is typically accomplished by dilution (for example, 100-fold) of the denatured and reduced protein into refolding buffer. An exemplary buffer is 0.1

M Tris, pH 8.0, 0.5 M L-arginine, 8 mM oxidized glutathione (GSSG), and 2 mM EDTA.

As a modification to the two chain antibody purification protocol, the heavy and light chain regions are separately solubilized and reduced and then combined in the refolding solution. An exemplary yield is obtained when these two proteins are mixed in a molar ratio such that a 5-fold molar excess of one protein over the other is not exceeded. Excess oxidized glutathione or other oxidizing low molecular weight compounds can be added to the refolding solution after the redox-shuffling is completed.

In addition to recombinant methods, the antibodies, labeled antibodies and functional fragments thereof that are disclosed herein can also be constructed in whole or in part using standard peptide synthesis. Solid phase synthesis of the polypeptides of less than about 50 amino acids in length can be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany & Merrifield, *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.* pp. 3-284; Merrifield *et al.*, *J. Am. Chem. Soc.* 85:2149-2156, 1963, and Stewart *et al.*, *Solid Phase Peptide Synthesis, 2nd ed.*, Pierce Chem. Co., Rockford, Ill., 1984. Proteins of greater length may be synthesized by condensation of the amino and carboxyl termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxyl terminal end (such as by the use of the coupling reagent N, N'-dicyclohexylcarbodiimide) are well known.

C. Compositions and Therapeutic Methods

Methods are disclosed herein for the treatment of an HIV infection, such as an HIV-1 infection. In one example, the methods include prevention or inhibition of infection with HIV-1. The methods include contacting a cell with an effective amount of one or more of the hmAbs disclosed herein that specifically bind gp120 and have one or more Fc mutations, or a functional fragment thereof. The method can also include administering to a subject a therapeutically effective amount of the hmAbs to a subject. In some examples, the method includes selecting a subject

infected with HIV, such as HIV-1, or who is at risk for such infection (such as medical personnel).

In one example the subject is an HIV-positive pregnant woman, or child recently born to such a woman. Studies have shown that the rate of HIV transmission from mother to infant is reduced significantly when zidovudine is administered to HIV-infected women during pregnancy and delivery and to the offspring after birth (Connor *et al.*, 1994 *Pediatr Infect Dis J* 14: 536-541). Several studies of mother-to-infant transmission of HIV have demonstrated a correlation between the maternal virus load at delivery and risk of HIV transmission to the child. The present disclosure provides isolated hmAbs that can decrease HIV-transmission from mother to infant. Thus, in some examples a therapeutically effective amount one or more of the disclosed hmAbs specific for gp120- is administered in order to prevent transmission of HIV, or decrease the risk of transmission of HIV, from a mother to an infant. In some examples, a therapeutically effective amount of the antibody is administered to mother and/or to the child at childbirth. In other examples, a therapeutically effective amount of the antibody is administered to the mother and/or infant prior to breast feeding in order to prevent viral transmission to the infant or decrease the risk of viral transmission to the infant. In some embodiments, both a therapeutically effective amount of the antibody and a therapeutically effective amount of another agent, such as zidovudine, is administered to the mother and/or infant.

Methods to assay for neutralization activity, such as HIV neutralization, include, but are not limited to, a single-cycle infection assay as described in Martin *et al.* (2003) *Nature Biotechnology* 21:71-76. In this assay, the level of viral activity is measured via a selectable marker whose activity is reflective of the amount of viable virus in the sample, and the IC₅₀ is determined. In other assays, acute infection can be monitored in the PM1 cell line or in primary cells (normal PBMC). In this assay, the level of viral activity can be monitored by determining the p24 concentrations using ELISA. See, for example, Martin *et al.* (2003) *Nature Biotechnology* 21:71-76.

In one example, the cell is also contacted with an effective amount of an additional agent, such as anti-viral agent. The cell can be *in vivo* or *in vitro*. For

example, the disclosed mAbs can be combined with anti-retroviral therapy.

Antiretroviral drugs are broadly classified by the phase of the retrovirus life-cycle that the drug inhibits. The disclosed antibodies can be administered in conjunction with nucleoside and nucleotide reverse transcriptase inhibitors (nRTI), non-

5 nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, entry inhibitors (or fusion inhibitors), maturation inhibitors, or a broad spectrum inhibitors, such as natural antivirals. Exemplary agents include lopinavir, ritonavir, zidovudine, lamivudine, tenofovir, emtricitabine and efavirenz. In some examples the mAbs and the anti-retroviral therapy is administered simultaneously or
10 contemporaneously. In other examples the therapies are administered separately.

Compositions are provided that include one or more of the hmAbs or functional fragments thereof disclosed herein in a carrier. The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating physician to achieve the
15 desired purposes. The antibody can be formulated for systemic or local administration. In one example, the hmAbs or functional fragments thereof disclosed herein is formulated for parenteral administration, such as intravenous administration.

The compositions for administration can include a solution of the hmAbs or
20 functional fragments thereof in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable
25 auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body
30 weight and the like in accordance with the particular mode of administration selected and the subject's needs.

Controlled-release parenteral formulations containing the therapeutic mAb can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic mAb as a central core. In microspheres the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the mAb compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al.*, *Pharm. Res.* 9:425-434, 1992; and Pec *et al.*, *J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al.*, *Int. J. Pharm.* 112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al.*, *Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins, such as mAbs, are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S.

Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

5 A typical pharmaceutical composition for intravenous administration includes at least 1 mg/kg (such as at least 5 mg/kg, at least 10 mg/kg, at least 20 mg/kg or at least 40 mg/kg, such as 1 to 100 mg/kg, 1 to 50 mg/kg, 5 to 50 mg/kg, or 10-40 mg/kg) administered every two to four weeks. In one example, the dosage for a small rodent is about 50 to 500 µg mAb, such as 200 µg mAb (*e.g.*, 10 mg/kg). In
10 another example, the dosage for a large mammal, such as a human or non-human primate is about 1 to 100 mg mAb /kg subject, such as 1 to 50, 10 to 24 or 20 mg/kg. Dosages from 0.1 up to about 100 mg per subject per day may be used, particularly if the agent is administered to a secluded site and not into the circulatory or lymph system, such as into a body cavity or into a lumen of an organ. Actual methods for
15 preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995).

Antibodies may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of
20 known concentration. The antibody solution is then added to an infusion bag containing 0.9% sodium chloride, USP, and typically administered at a dosage of from 0.5 to 75 mg/kg of body weight (such as 5 to 50 or 10 to 40 mg/kg). Considerable experience is available in the art in the administration of antibody drugs, which have been marketed in the U.S. since the approval of RITUXAN® in
25 1997. Antibodies can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused
30 over a 30 minute period if the previous dose was well tolerated. In some examples the Abs are administered at least once a week, at least once a month, at least twice a month, or at least once every two months. or In one example, at least 1 mg mAb/kg

(such as at least 5 mg/kg, at least 10 mg/kg, at least 20 mg/kg or at least 40 mg/kg, such as 1 to 100 mg/kg, 1 to 50 mg/kg, 5 to 50 mg/kg, or 10-40 mg/kg) is administered to the patient every two to four weeks. In some examples, the mAbs are administered iv, subcutaneously or im.

5 A therapeutically effective amount of a mAb disclosed herein will depend upon the severity of the disease and/or infection and the general state of the patient's health. A therapeutically effective amount of the antibody is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. These compositions can be
10 administered in conjunction with another therapeutic agent, either simultaneously or sequentially.

 In one embodiment, administration of the antibody results in a reduction in the establishment of HIV infection and/or reducing subsequent HIV disease progression in a subject. A reduction in the establishment of HIV infection and/or a
15 reduction in subsequent HIV disease progression encompass any statistically significant reduction in HIV activity. In some embodiments, methods are disclosed for treating a subject with an HIV-1 infection. These methods include administering to the subject a therapeutically effective amount of an antibody, or a nucleic acid encoding the antibody, thereby preventing or treating the HIV-1 infection.

20 HIV infection does not need to be completely eliminated for the composition to be effective. For example, a composition can decrease HIV infection by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination of detectable HIV infected cells), as compared to HIV infection
25 in the absence of the composition.

 In additional examples, HIV replication can be reduced or inhibited by use of the disclosed mAbs. HIV replication does not need to be completely eliminated for the composition to be effective. For example, a composition can decrease HIV replication by a desired amount, for example by at least 10%, at least 20%, at least
30 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination of detectable HIV), as compared to HIV replication in the absence of the composition.

Single or multiple administrations of the compositions including the mAbs disclosed herein are administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of at least one of the antibodies disclosed herein to effectively
5 treat the patient. The dosage can be administered once but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. In one example, a dose of the antibody is infused for thirty minutes every other day. In this example, about one to about ten doses can be administered, such as three or six doses can be administered every other day. In a
10 further example, a continuous infusion is administered for about five to about ten days. The subject can be treated at regular intervals, such as monthly, until a desired therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

15 ***D. Diagnostic Methods and Kits***

Methods are provided herein for the detection of gp120 or *in vitro* or *in vivo*. In one example, gp120 is detected in a biological sample, and can be used to detect HIV-1 infection. The sample can be any sample, including, but not limited to, tissue from biopsies or check swabs, autopsies and pathology specimens. Biological
20 samples also include sections of tissues, for example, frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, plasma, semen, sputum, breast milk, spinal fluid or urine.

In one embodiment, a method is provided for detecting AIDS and/or an HIV-1 infection in a subject. The disclosure provides a method for detecting HIV-1 in a
25 biological sample, wherein the method includes contacting a biological sample with a hmAb or functional fragment thereof disclosed herein under conditions conducive to the formation of an immune complex, and detecting the immune complex to detect the gp120 in the biological sample. In one example, the detection of gp120 in the sample (for example above a background level) indicates that the subject has an
30 HIV infection. In another example, the detection of gp120 in the sample indicates that the subject has AIDS. In another example, detection of gp120 in the sample confirms a diagnosis of AIDS and/or an HIV-1 infection in a subject. In some

examples the method includes comparing the gp120-hmAb complex detected to an amount of such complex detected in a control, such as a sample known to not contain HIV or gp120, or known to be infected with HIV or contain gp120. In some examples the method includes comparing an absolute or relative amount of the
5 gp120-hmAb complex detected to a reference value or range of values expected of such complex expected if the sample does not contain gp120, or expected if the sample does contain gp120.

In some embodiments, the disclosed antibodies are used to test vaccines, for example to determine if a vaccine composition assumes the same conformation as a
10 native gp120 peptide. Thus provided herein is a method for detecting testing a vaccine, wherein the method includes contacting a sample containing the test vaccine, such as a gp120 immunogen, with an antibody disclosed herein under conditions conducive to the formation of an immune complex, and detecting the immune complex, to detect the vaccine in the sample. In one example, the detection
15 of the immune complex in the sample indicates that vaccine component, such as such as a gp120 immunogen assumes a conformation capable of binding the antibody.

In one embodiment, the anti-gp120 antibody is directly labeled with a detectable label. In another embodiment, the anti-gp120 antibody (the first
20 antibody) is unlabeled and a second antibody or other molecule that can bind the anti-gp120 antibody is utilized. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the specific species and class of the first antibody. For example, if the first antibody is a human IgG, then the secondary antibody may be an anti-human-IgG. Other molecules that can bind to
25 antibodies include, without limitation, Protein A and Protein G, both of which are available commercially.

Suitable labels for the antibody or secondary antibody are described above, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, quantum dots, magnetic agents and radioactive materials. Non-limiting
30 examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Non-

limiting examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. A non-limiting exemplary luminescent material is luminol; a non-limiting exemplary a magnetic agent is gadolinium, and non-limiting exemplary radioactive labels include ^{125}I , ^{131}I , ^{35}S or ^3H .

The immunoassays and method disclosed herein can be used for a number of purposes. Kits for detecting a polypeptide will typically include one or more hmAbs or functional fragments thereof disclosed herein. In some embodiments, an antibody fragment, such as an Fv fragment or a Fab is included in the kit. In a further embodiment, the antibody is labeled (for example, with a fluorescent, radioactive, or an enzymatic label).

Thus, the disclosure provides a kit that includes one more of the mAbs provided herein, for example in separate containers. In one example the kit includes an antibody having a heavy chain comprising the sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, and can further include a light chain comprising the sequence of SEQ ID NO: 36. Such antibodies may include a label. In some examples such a kit further includes a labeled secondary antibody in a separate container, which permits detection of the primary antibody. In some examples such a kit further includes additional therapeutic agents, such as one or more antiretroviral compounds, in separate containers.

In one embodiment, a kit includes instructional materials disclosing means of use. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

In one embodiment, the diagnostic kit comprises an immunoassay. Although the details of the immunoassays may vary with the particular format employed, the method of detecting gp120 in a biological sample generally includes the steps of contacting the biological sample with an antibody which specifically reacts, under
5 immunologically reactive conditions, to gp120. The antibody is allowed to specifically bind under immunologically reactive conditions to form an immune complex, and the presence of the immune complex (bound antibody) is detected directly or indirectly.

The following examples are provided to illustrate certain particular features
10 and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

EXAMPLES

Example 1

15 **Mutation of the Fc Region of VRC01**

This example describes the mutation of the Fc region of VRC01, a neutralizing human monoclonal antibody that specifically binds to gp120. One skilled in the art will appreciate that similar mutations can be made to the heavy chains of VRC02 (SEQ ID NOS: 37-38) or VRC03 (SEQ ID NOS: 41-42), for
20 example based on the alignment shown in FIG. 7.

Mutations were made in the Fc region of VRC01 using site-directed mutagenesis PCR in the heavy chain of VRC01. The resulting VRC01 mutant heavy chain and native light chain (SEQ ID NOS: 35 and 36) were transiently transfected to 293F cells and the supernatant collected six days later. The resulting
25 VRC01 antibodies having one or more Fc mutations were purified by Protein A from the supernatant. The mutations made are shown in SEQ ID NOS: 1-34 and in the Table below and FIGS. 1 and 2.

Table 1. VRC01 Fc mutations

Abbreviation	Mutation Sites	SEQ ID NOS:
QL	T250Q/M428L	1 and 2 (Q at position 273 and L at position 451 of SEQ ID NO: 2)
LS	M428L / N434S	3 and 4 (L at position 451 and S at position 457 of SEQ ID NO: 4)
A	N434A	5 and 6 (A at position 457 of SEQ ID NO: 6)
AAA	T307A / E380A / N434A	7 and 8 (As at positions 330, 403 and 457 of SEQ ID NO: 8)
YTE	M252Y / S254T / T256E	9 and 10 (Y at position 275, T at position 277, E at position 279 of SEQ ID NO: 10)
DE	S239D / I332E	11 and 12 (D at position 262 and E at position 355 of SEQ ID NO: 12)
DLE	S239D / A330L / I332E	13 and 14 (D at position 262, L at position 353, E at position 355 of SEQ ID NO: 14)
DE - QL	S239D / I332E + T250Q / M428L	15 and 16 (D at position 262, E at position 355, Q at position 273, L at position 451, of SEQ ID NO: 16)
DE - LS	S239D / I332E + M428L / N434S	17 and 18 (D at position 262, E at position 355, L at position 451, S at position 457, of SEQ ID NO: 18)
DE - A	S239D / I332E + N434A	19 and 20 (D at position 262, E at position 355, A at position 457, of SEQ ID NO: 20)
DE - AAA	S239D / I332E + T307A / E380A / N434A	21 and 22 (D at position 262, E at position 355, As at positions 330, 403 and 457, of SEQ ID NO: 22)
DE - YTE	S239D / I332E + M252Y / S254T / T256E	23 and 24 (D at position 262, E at position 355, Y at position 275, T at position 277, E at position 279, of SEQ ID NO: 24)
DLE - AAA	S239D / A330L / I332E + T307A / E380A / N434A	25 and 26 (D at position 262, L at position 353, E at position 355, As at positions 330, 403 and 457, of SEQ ID NO: 26)
DLE - YTE	S239D / A330L / I332E + M252Y /	27 and 28 (D at position 262, L

	S254T / T256E	at position 353, E at position 355, Y at position 275, T at position 277, E at position 279, of SEQ ID NO: 28)
DLE-QL	S239D / A330L / I332E + T250Q / M428L	29 and 30 (D at position 262, L at position 353, E at position 355, Q at position 273, L at position 451 of SEQ ID NO: 30)
DLE-LS	S239D / A330L / I332E + M428L / N434S	31 and 32 (D at position 262, L at position 353, E at position 355, L at position 451, S at position 457, of SEQ ID NO: 32)
DLE-A	S239D / A330L / I332E + N434A	33 and 34 (D at position 262, L at position 353, E at position 355, A at position 457, of SEQ ID NO: 34)

Viral entry, neutralization and protein competition assays. Neutralization was measured using single round infection by HIV-1 Env-pseudoviruses and TZM-bl target cells. Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation. The 50% and 80% inhibitory concentrations (IC₅₀ and IC₈₀) were reported as the antibody concentrations required to inhibit infection by 50% and 80% respectively. Competition of serum or mAb neutralization was assessed by adding a fixed concentration (25 µg/ml) of the RSC3 or ΔRSC3 glycoprotein to serial dilutions of antibody for 15 min prior to the addition of virus. The resulting IC₅₀ values were compared to the control with mock protein added. The neutralization blocking effect of the proteins was calculated as the percent reduction in the IC₅₀ value of the antibody in the presence of protein compared to PBS. Synergistic or additive neutralization was assessed by mixing a fixed concentration (10 µg/ml) of the test antibody with serial dilutions of sCD4, CD4-Ig or VRC01 for 15 min prior to the addition of virus. The baseline of viral entry at each concentration of sCD4, CD4-Ig or VRC01 was used to calculate the adjusted percent neutralization. Neutralization was also assessed using Env-pseudoviruses generated by 293T transfection using the pNL4-3 ΔEnv HIV-1 backbone containing a luciferase reporter gene to infect activated PBMC. Neutralizations using uncloned

PBMC-derived HIV-1 primary isolates were performed by single-round infection of either TZM-bl cells using luciferase as readout, or activated PBMC using flow cytometry staining for HIV-1 p24 antigen. CD4-facilitated virus entry was performed in the CCR5+/CD4- cell line Cf2Th/syn CCR5 with Env-pseudoviruses containing the luciferase pNL4-3 Δ Env HIV-1 backbone. A mixture of 40 μ l of viral stock and 10 μ l of serial dilutions of sCD4, CD4-Ig or VRC01 was incubated at 37°C for 30 min before adding 1×10^4 Cf2Th/syn CCR5 cells. Virus entry was measured 2 days later by luciferase activity in cell lysates.

As shown in Tables 2-5, the VRC01 Fc mutants have similar neutralization activity as the wild-type VRC01 antibody. Table 2 provides a comparison of the IC_{50} between wild type VRC01 and the mutant VRC01 antibodies. Table 3 provides a comparison of IC_{80} between wild type and the mutants. Tables 4 and 5 show the fold increase/decrease of IC_{50} and IC_{80} of the Fc mutants comparing with wild type.

Table 2: IC_{50} values (mg/mL)

	Source of data	Q23.17	Q842.d12	YU2	JR-FL	7165.18	Du156.12	ZM109.4	SVA.MLV	SIVmac 251.30.SIV
VRC01	CVL1062.001	0.063	0.020	0.139	0.026	>50	0.090	0.094	>50	>50
QL	CVL1062.001	0.067	0.020	0.144	0.027	>50	0.085	0.109	>50	>50
LS	CVL1062.001	0.082	0.028	0.166	0.031	>50	0.097	0.154	>50	>50
A	CVL1062.001	0.074	0.019	0.168	0.027	>50	0.098	0.136	>50	>50
AAA	CVL1062.001	0.080	0.019	0.154	0.027	>50	0.110	0.084	>50	>50
YTE	CVL1062.001	0.079	0.021	0.176	0.032	>50	0.093	0.188	>50	>50
DE	CVL1062.001	0.093	0.025	0.197	0.037	>50	0.103	0.156	>50	>50
DE-QL	CVL1062.001	0.078	0.021	0.172	0.026	>50	0.119	0.123	>50	>50
DE-LS	CVL1062.001	0.087	0.023	0.176	0.024	>50	0.098	0.112	>50	>50
DE-A	CVL1062.001	0.080	0.019	0.173	0.025	>50	0.090	0.115	>50	>50
DE-AAA	CVL1062.001	0.098	0.023	0.163	0.026	>50	0.099	0.134	>50	>50
VRC01	CVL1062.002	0.048	0.018	0.118	0.025	>50	0.067	0.096	>50	>50
DE-YTE	CVL1062.002	0.058	0.025	0.132	0.032	>50	0.097	0.107	>50	>50
DLE	CVL1062.002	0.066	0.021	0.122	0.030	>50	0.073	0.121	>50	>50
DLE-QL	CVL1062.002	0.079	0.031	0.142	0.033	>50	0.085	0.144	>50	>50
DLE-LS	CVL1062.002	0.052	0.022	0.118	0.024	>50	0.077	0.104	>50	>50

DLE-A	CVL1062.002	0.061	0.025	0.141	0.029	>50	0.094	0.132	>50	>50
DLE-AAA	CVL1062.002	0.079	0.026	0.138	0.031	>50	0.094	0.132	>50	>50
DLE-YTE	CVL1062.002	0.063	0.030	0.139	0.030	>50	0.085	0.121	>50	>50

Table 3: IC₈₀ values (mg/mL)

	Source of data	Q23.17	Q842.d12	YU2	JR-FL	7165.18	Du156.12	ZM109.4	SVA.MLV	SIV ^{mac} 251.30.SIV
VRC01	CVL1062.001	0.182	0.078	0.341	0.081	>50	0.255	0.414	>50	>50
QL	CVL1062.001	0.195	0.082	0.387	0.092	>50	0.263	0.466	>50	>50
LS	CVL1062.001	0.228	0.099	0.439	0.099	>50	0.279	0.548	>50	>50
A	CVL1062.001	0.204	0.079	0.482	0.093	>50	0.290	0.538	>50	>50
AAA	CVL1062.001	0.227	0.076	0.409	0.093	>50	0.295	0.429	>50	>50
YTE	CVL1062.001	0.239	0.089	0.478	0.105	>50	0.284	0.612	>50	>50
DE	CVL1062.001	0.267	0.095	0.470	0.105	>50	0.301	0.597	>50	>50
DE-QL	CVL1062.001	0.251	0.082	0.462	0.096	>50	0.323	0.516	>50	>50
DE-LS	CVL1062.001	0.274	0.082	0.475	0.098	>50	0.298	0.493	>50	>50
DE-A	CVL1062.001	0.244	0.077	0.477	0.093	>50	0.275	0.514	>50	>50
DE-AAA	CVL1062.001	0.273	0.088	0.454	0.092	>50	0.263	0.525	>50	>50
VRC01	CVL1062.002	0.160	0.073	0.307	0.079	>50	0.218	0.400	>50	>50
DE-YTE	CVL1062.002	0.199	0.093	0.359	0.100	>50	0.294	0.509	>50	>50
DLE	CVL1062.002	0.195	0.081	0.324	0.096	>50	0.232	0.444	>50	>50
DLE-QL	CVL1062.002	0.236	0.102	0.399	0.104	>50	0.263	0.550	>50	>50
DLE-LS	CVL1062.002	0.179	0.082	0.353	0.089	>50	0.238	0.442	>50	>50
DLE-A	CVL1062.002	0.216	0.090	0.435	0.101	>50	0.283	0.563	>50	>50
DLE-AAA	CVL1062.002	0.251	0.101	0.361	0.094	>50	0.291	0.526	>50	>50
DLE-YTE	CVL1062.002	0.207	0.093	0.420	0.092	>50	0.262	0.565	>50	>50

Table 4: IC₅₀ values (fold-difference)

	Source of data	Q23.17	Q842.d12	YU2	JR-FL	7165.18	Du156.12	ZM109.4	SVA.MLV	SIVmac 251.30.SIV
VRC01	CVL1062.001	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
QL	CVL1062.001	1.06	1.00	1.04	1.04	1.00	0.94	1.16	1.00	1.00
LS	CVL1062.001	1.30	1.40	1.19	1.19	1.00	1.08	1.64	1.00	1.00
A	CVL1062.001	1.17	0.95	1.21	1.04	1.00	1.09	1.45	1.00	1.00
AAA	CVL1062.001	1.27	0.95	1.11	1.04	1.00	1.22	0.89	1.00	1.00
YTE	CVL1062.001	1.25	1.05	1.27	1.23	1.00	1.03	2.00	1.00	1.00
DE	CVL1062.001	1.48	1.25	1.42	1.42	1.00	1.14	1.66	1.00	1.00
DE-QL	CVL1062.001	1.24	1.05	1.24	1.00	1.00	1.32	1.31	1.00	1.00
DE-LS	CVL1062.001	1.38	1.15	1.27	0.92	1.00	1.09	1.19	1.00	1.00
DE-A	CVL1062.001	1.27	0.95	1.24	0.96	1.00	1.00	1.22	1.00	1.00
DE-AAA	CVL1062.001	1.56	1.15	1.17	1.00	1.00	1.10	1.43	1.00	1.00
VRC01	CVL1062.002	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DE-YTE	CVL1062.002	1.21	1.39	1.12	1.28	1.00	1.45	1.11	1.00	1.00
DLE	CVL1062.002	1.38	1.17	1.03	1.20	1.00	1.09	1.26	1.00	1.00
DLE-QL	CVL1062.002	1.65	1.72	1.20	1.32	1.00	1.27	1.50	1.00	1.00
DLE-LS	CVL1062.002	1.08	1.22	1.00	0.96	1.00	1.15	1.08	1.00	1.00
DLE-A	CVL1062.002	1.27	1.39	1.19	1.16	1.00	1.40	1.38	1.00	1.00
DLE-AAA	CVL1062.002	1.65	1.44	1.17	1.24	1.00	1.40	1.38	1.00	1.00
DLE-YTE	CVL1062.002	1.31	1.67	1.18	1.20	1.00	1.27	1.26	1.00	1.00

Table 5: IC₈₀ values (fold-difference)

	Source of data	Q23.17	Q842.d12	YU2	JR-FL	7165.18	Du156.12	ZM109.4	SVA.MLV	SIVmac 251.30.SIV
VRC01	CVL1062.001	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
QL	CVL1062.001	1.07	1.05	1.13	1.14	1.00	1.03	1.13	1.00	1.00
LS	CVL1062.001	1.25	1.27	1.29	1.22	1.00	1.09	1.32	1.00	1.00
A	CVL1062.001	1.12	1.01	1.41	1.15	1.00	1.14	1.30	1.00	1.00
AAA	CVL1062.001	1.25	0.97	1.20	1.15	1.00	1.16	1.04	1.00	1.00
YTE	CVL1062.001	1.31	1.14	1.40	1.30	1.00	1.11	1.48	1.00	1.00
DE	CVL1062.001	1.47	1.22	1.38	1.30	1.00	1.18	1.44	1.00	1.00
DE-QL	CVL1062.001	1.38	1.05	1.35	1.19	1.00	1.27	1.25	1.00	1.00
DE-LS	CVL1062.001	1.51	1.05	1.39	1.21	1.00	1.17	1.19	1.00	1.00
DE-A	CVL1062.001	1.34	0.99	1.40	1.15	1.00	1.08	1.24	1.00	1.00
DE-AAA	CVL1062.001	1.50	1.13	1.33	1.14	1.00	1.03	1.27	1.00	1.00
VRC01	CVL1062.002	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DE-YTE	CVL1062.002	1.24	1.27	1.17	1.27	1.00	1.35	1.27	1.00	1.00
DLE	CVL1062.002	1.22	1.11	1.06	1.22	1.00	1.06	1.11	1.00	1.00
DLE-QL	CVL1062.002	1.48	1.40	1.30	1.32	1.00	1.21	1.38	1.00	1.00
DLE-LS	CVL1062.002	1.12	1.12	1.15	1.13	1.00	1.09	1.11	1.00	1.00
DLE-A	CVL1062.002	1.35	1.23	1.42	1.28	1.00	1.30	1.41	1.00	1.00
DLE-AAA	CVL1062.002	1.57	1.38	1.18	1.19	1.00	1.33	1.32	1.00	1.00
DLE-YTE	CVL1062.002	1.29	1.27	1.37	1.16	1.00	1.20	1.41	1.00	1.00

Example 2**Binding of the VRC01 Mutants to hFcRn**

5 This example describes the methods used to measure the binding of the VRC01 antibody mutants to the human MHC class I-related Fc γ -receptor (hFcRn) as compared to the native VRC01 antibody. Binding to hFcRn provides an indication as to the likely degradation of the antibody; antibodies while bound to hFcRn avoid degradation.

10 Nickel-coated plates were incubated with His tagged recombinant hFcRn for one hour, and washed with PBS, pH7.4 + 0.05 % Tween20. VRC01 and its Fc

mutants were diluted with 2.5%-BSA in PBS, pH 6.0 or PBS, pH 7.4 were incubated for two hours and washed with PBS, pH6.0 + 0.05 % Tween20 or PBS, pH7.4 + 0.05 % Tween20. Anti-human IgG-HRP diluted with 2.5%-BSA in PBS, pH6.0 or PBS, pH7.4 were incubated for one hour and washed with PBS, pH6.0 + 0.05 %
5 Tween20 or PBS, pH7.4 + 0.05 % Tween20. HRP substrate TMB was added and the color development was stopped by 0.1N H₂SO₄. Optical density at 450 nm was measured by ELISA reader.

As shown in FIG. 3, all of the VRC01 Fc mutants have a higher binding to hFcRN at pH 6 and 7.4 relative to VRC01, except for the combined A mutants.
10 Thus, these mutants are expected to have an increase half-life relative to the native VRC01 antibody (FIG. 1).

Example 3

Binding of the VRC01 Mutants to FcγRIIIa and RSC3

15 This example describes the methods used to measure the binding of the VRC01 antibody mutants to the Fc fragment of IgG low affinity IIIa receptor (FcγRIIIa) and resurfaced stabilized core 3 (RSC3) as compared to the native VRC01 antibody. Antibody binding to FcγRIIIa provides an indication of ADCC activity. RSC3 is the resurfaced stabilized core from gp120, and is the preserved
20 antigenic structure of the neutralizing surface of the CD4 binding site but does not include other antigenic regions of HIV-1. Antibody binding to RSC3 provides an indication of the ability of the antibody to specifically bind gp120.

Nickel-coated plates were incubated with His tagged recombinant FcγRIIIa for one hour, and washed with PBS, pH7.4 + 0.05 % Tween20 (washing buffer)
25 (left). ELISA plates were coated with RSC3 for overnight, blocked with 5 % BSA in PBS, pH7.4, and washed with washing buffer (right). VRC01 and its Fc mutants diluted with 2.5%-BSA in PBS, pH7.4 were incubated for two hours and washed. Anti-human IgG-HRP in PBS, pH7.4 were incubated for one hour and washed. HRP substrate TMB was added and the color development was stopped by 0.1N H₂SO₄.
30 Optical density at 450 nm was measured by ELISA reader.

As shown in FIG. 4, all of the combined VRC01 antibody Fc mutants had higher binding to FcγRIIIa than the native VRC01 antibody, while all VRC01

antibody Fc mutants had higher binding to RSC3 than the native VRC01. Thus, the combined mutants are indicated to have higher ADCC activity than the native VRC01 antibody, and all mutants bind to the gp120 target the same as the native VRC01 antibody.

5

Example 4

ADCC Activity of VRC01 Mutants

This example describes the methods used to measure the antibody-dependent cellular cytotoxicity (ADCC) of the VRC01 antibody mutants as compared to the native VRC01 antibody.

HIV-infected CD4+CEM-NK_r cells were double-stained with PKH26 and CFSE, and suspended in RPMI with 10%-FBS (Target cells). Human PBMC was prepared from human buffy coat by Ficoll gradient centrifugation, and suspended in RPMI with 10%-FBS (Effector cells). Target cells (10,000 cells/well) and serial dilutions of VRC01 Fc mutants were incubated together at R.T. for 15 min. Effector cells (500,000 cells/well) were added and incubated at 37 °C, CO₂ incubator for four hours. Percent killing was calculated from % CFSE negative within PKH26hi population.

As shown in FIG. 5, ADCC was lower for the hFcRn-binding enhancing mutations, while the combined LS, A and AAA mutants had stronger ADCC activities.

In summary, in the ADCC assay and ELISA binding assay, the combined LS and A mutants showed higher ADCC activities and higher binding to FcγRIIIa. That is, DE or DLE mutants combined with LS or A mutations have higher affinity to FcγRIIIa and functional ADCC activities. As shown in FIG. 2, LS and A mutants have mutations only at CH3, whereas DE and DLE mutations are made in CH2 region close to hinge. FIG. 6 shows the binding of IgG to hFcRn and FcγRIIIa. hFcRn binds to the CH2 and CH3 region of IgG, while FcγRIIIa bind to the hinge and CH2 regions of IgG. Therefore, hFcRn-binding enhancing mutations did not affect (or affected less) the enhanced binding to FcγRIIIa by LS and A mutants.

This may be the reason why combined LS and A mutants showed higher ADCC activities than other mutants.

Example 5

5

Transcytosis of the Mutant mAbs

This example describes methods used to measure transcytosis of the antibody mutants as compared to the native antibody.

hFcRn is expressed in the epithelial cells in mucosa and mediates bidirectional transcytosis of human IgG across cellular epithelium. To demonstrate that hFcRn-binding enhancing mutants of VRC01 can increase transcytosis of the HIV neutralizing antibody, VRC01, a monolayer of Madin Darby canine kidney (MDCK) cells expressing hFcRn was grown in transwell plates. The native VRC01 antibody or the Fc mutants were added to the basolateral side of the cells at 1.0 mg/mL and incubated for 2 hours. The concentrations of the antibodies were then measured on the apical side by ELISA coated with RSC3 to compare the levels of transcytosis.

As shown in FIG. 8, the LS mutant groups (LS, DE-LS, and DLE-LS) showed a higher level of transcytosis than VRC01 or other mutant antibodies.

20

Example 6

Half-Life of the Mutant mAbs

This example describes methods used to measure the half-life of the VRC01 antibody mutants as compared to the native VRC01 antibody.

2 mg/Kg of each mAb (native VRC01, DE, LS, or DE-LS) was injected into a hFcRn Tg/Tg mouse by i.v. The concentrations of the mAbs in the sera were examined by indirect ELISA with RSC3-coated plates over time, and their half-life was calculated.

As shown in FIG. 9 and Table 6, the hFcRn-binding enhancing mutant antibody, LS, had a half-life about 2 times longer than VRC01.

30

Table 6. Half-life of antibodies *in vivo*

		Half-life (days)
	WT	2.65 ± 0.28
	DE	2.20 ± 0.08
5	LS	4.47 ± 0.52
	DE-LS	2.15 ± 0.11

Thus, in some examples the disclosed mutant antibodies, such as the LS antibodies, have a half-life that is greater than the native antibody (such as native VRC01), such as an at least 1.5 fold, at least 1.8 fold, at least 2-fold, at least 2.5-
 10 fold, or at least 3-fold.

Example 7**Half-Life of the Mutant mAbs in Non-Human Primates**

This example describes methods used to measure the half-life and the
 15 clearance of the VRC01 antibody mutants as compared to the native VRC01 antibody.

10 mg/Kg of each mAb (native VRC01, DE, LS, or DE-LS) was injected into rhesus macaques by i.v. The concentrations of the mAbs in the sera were examined by indirect ELISA with RSC3-coated plates over time, and their half-life
 20 was calculated by WinNonlin software.

As shown in FIG. 10 and Table 7, the hFcRn-binding enhancing mutant antibody, LS had a half-life about 3 times longer than VRC01, and the clearance rate of LS is about 2 times slower than VRC01.

Table 7. Half-life of antibodies *in vivo*

		Half-life (days)	Clearance (mL/day/Kg)
	WT	4.88 ± 0.71	13.34 ± 1.03
	LS	14.32 ± 6.15	5.79 ± 1.61
	DE	3.40 ± 0.41	24.27 ± 4.35
30	DE-LS	5.17 ± 1.08	12.51 ± 2.75

Thus, in some examples the disclosed mutant antibodies, such as the LS antibodies, have a half-life that is greater than the native antibody (such as native

VRC01), such as an at least 1.5 fold, at least 1.8 fold, at least 2-fold, at least 2.5-fold, or at least 3-fold.

Example 8

5 SHIV challenge after the passive immunization with the Mutant mAbs

This example describes methods used to compare the protection immunity against SHIV challenge after the passive immunization with the mutant mAbs.

0.3 mg/Kg of each mAb (native VRC01, DE, LS, or DE-LS) was injected into rhesus macaques by i.v. The passively immunized monkeys were challenged
10 with repetitive, intrarectal SHIV BaL two days later. The number of challenges required to establish infection and plasma viral load over time was examined.

Example 9

In vivo ADCC activity

15 This example describes methods that can be used to measure ADCC activity of the antibody mutants as compared to the native antibody *in vivo*. It is expected that the ADCC activity of the mutant antibodies (*e.g.*, mutant VRC01 antibodies) will be equal to or better than the native antibody (such as native VRC01).

For example, hFcRn transgenic SCID mouse and monkey infected with SIV
20 can be administered the disclosed hmAbs, and ADCC activity measured (for example as in Dall'Acqua *et al.* (*J. Biol. Chem.* 281:23514-24, 2006, herein incorporated by reference). Mutant and native hmAb ADCC activity can be compared. In one example mice are administered 200 µg Ab or 2 mg/kg and monkeys receive 20 mg/kg via i.v. administration.

25

Example 10

Fc mutant HIV-1 monoclonal neutralizing antibodies specific to gp120 for detecting HIV-1 in a subject

This example describes the use of the disclosed HIV-1 monoclonal
30 neutralizing antibodies specific to gp120 having one or more Fc mutations for the detection of HIV-1 in a subject. This example further describes the use of these antibodies to confirm the diagnosis of HIV-1 in a subject.

A biological sample, such as a blood sample is obtained from the patient diagnosed with, or suspected of having an HIV-1 infection. A blood sample taken from a patient who is not infected can be used as a negative control. An ELISA is performed to detect the presence of HIV-1 in the blood sample. Proteins present in the blood samples (the patient sample and control sample) are immobilized on a solid support, such as a 96-well plate, according to methods well known in the art (see, for example, Robinson *et al.*, *Lancet* 362:1612-1616, 2003). Following immobilization, one or more mutant mAbs disclosed herein that is directly labeled with a fluorescent marker or quantum dot is applied to the protein-immobilized plate. The plate is washed in an appropriate buffer, such as PBS, to remove any unbound antibody and to minimize non-specific binding of antibody. Fluorescence can be detected using a fluorometric plate reader according to standard methods. An increase in fluorescence intensity of the patient sample, relative to the negative control sample, indicates the anti-gp120 antibody with an Fc mutation specifically bound proteins from the blood sample, thus detecting the presence of HIV-1 protein in the sample. Detection of HIV-1 protein in the patient sample indicates the patient has HIV-1, or confirms diagnosis of HIV-1 in the subject.

Example 11

Fc mutant HIV-1 monoclonal neutralizing antibodies specific to gp120 for Treating HIV-1

This example describes a particular method that can be used to treat HIV in a human subject by administration of one or more of the disclosed Fc mutant gp120 specific human neutralizing mAbs. Although particular methods, dosages, and modes of administrations are provided, one skilled in the art will appreciate that variations can be made without substantially affecting the treatment.

Based upon the teaching disclosed herein HIV-1 can be treated by administering a therapeutically effective amount of one or more of the neutralizing mAbs described herein, thereby reducing or eliminating HIV infection.

Screening subjects

In particular examples, the subject is first screened to determine if they have an HIV infection. Examples of methods that can be used to screen for HIV include a combination of measuring a subject's CD4+ T cell count and the level of HIV in serum blood levels. Additional methods using the gp120-specific mAbs described herein can also be used to screen for HIV (*e.g.*, see Example 10).

In some examples, HIV testing consists of initial screening with an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV, such as to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate. If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (*e.g.*, Western blot or an immunofluorescence assay (IFA)). Specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by Western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate Western blot result, which may be either an incomplete antibody response to HIV in an infected person, or nonspecific reactions in an uninfected person. IFA can be used to confirm infection in these ambiguous cases. In some instances, a second specimen will be collected more than a month later and retested for subjects with indeterminate Western blot results. In additional examples, nucleic acid testing (*e.g.*, viral RNA or proviral DNA amplification method) can also help diagnosis.

The detection of HIV in a subject's blood is indicative that the subject has HIV and is a candidate for receiving the therapeutic compositions disclosed herein. Moreover, detection of a CD4+ T cell count below 350 per microliter, such as 200 cells per microliter, is also indicative that the subject is likely to have HIV.

Pre-screening is not required prior to administration of the therapeutic compositions disclosed herein

Pre-treatment of subjects

In particular examples, the subject is treated prior to administration of a therapeutic agent that includes one or more antiretroviral therapies known to those of skill in the art. However, such pre-treatment is not required, and can be
5 determined by a skilled clinician.

Administration of therapeutic compositions

Following subject selection, a therapeutically effective dose of a gp120 specific neutralizing mAb described herein is administered to the subject (such as an
10 adult human or a newborn infant either at risk for contracting HIV or known to be infected with HIV). Additional agents, such as anti-viral agents, can also be administered to the subject simultaneously or prior to or following administration of the disclosed agents. Administration can be achieved by any method known in the art, such as oral administration, inhalation, intravenous, intramuscular,
15 intraperitoneal, or subcutaneous.

The amount of the composition administered to prevent, reduce, inhibit, and/or treat HIV or a condition associated with it depends on the subject being treated, the severity of the disorder, and the manner of administration of the therapeutic composition. Ideally, a therapeutically effective amount of an agent is
20 the amount sufficient to prevent, reduce, and/or inhibit, and/or treat the condition (e.g., HIV) in a subject without causing a substantial cytotoxic effect in the subject. An effective amount can be readily determined by one skilled in the art, for example using routine trials establishing dose response curves. As such, these compositions may be formulated with an inert diluent or with a pharmaceutically acceptable
25 carrier.

In one specific example, antibodies are administered at 5 mg per kg every two weeks or 10 mg per kg every two weeks depending upon the particular stage of HIV. In an example, the antibodies are administered continuously. In another example, antibodies or antibody fragments are administered at 50 μ g per kg given
30 twice a week for 2 to 3 weeks.

Administration of the therapeutic compositions can be taken long term (for example over a period of months or years).

Assessment

Following the administration of one or more therapies, subjects having HIV can be monitored for reductions in HIV levels, increases in a subjects CD4+ T cell count, or reductions in one or more clinical symptoms associated with HIV. In particular examples, subjects are analyzed one or more times, starting 7 days following treatment. Subjects can be monitored using any method known in the art . For example, biological samples from the subject, including blood, can be obtained and alterations in HIV or CD4+ T cell levels evaluated.

Additional treatments

In particular examples, if subjects are stable or have a minor, mixed or partial response to treatment, they can be re-treated after re-evaluation with the same schedule and preparation of agents that they previously received for the desired amount of time, including the duration of a subject's lifetime. A partial response is a reduction, such as at least a 10%, at least 20%, at least 30%, at least 40%, at least 50%, or at least 70% in HIV infection, HIV replication or combination thereof. A partial response may also be an increase in CD4+ T cell count such as at least 350 T cells per microliter.

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that illustrated embodiments are only examples of the invention and should not be considered a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. An isolated antibody, wherein a heavy chain of the antibody comprises one or more amino acid substitutions in the Fc region, wherein the one or more amino acid substitutions comprise:

- 5 M428L/N434S;
T250Q/M428L;
N434A;
T307A/E380A/N434A;
M252Y/S254T/T256E;
10 S239D/I332E;
S239D/A330L/I332E;
S239D/I332E + T250Q/M428L;
S239D/I332E + M428L/N434S;
S239D/I332E + N434A;
15 S239D/I332E + T307A/E380A/N434A;
S239D/I332E + M252Y/S254T/T256E;
S239D/A330L/I332E+ T250Q/M428L;
S239D/A330L/I332E+ M428L/N434S;
S239D/A330L/I332E+ N434A;
20 S239D/A330L/I332E+ T307A/E380A/N434A;
S239D/A330L/I332E + M252Y/S254T/T256E; or combinations thereof.

2. The isolated antibody of claim 1, wherein the one or more amino acid
25 substitutions comprise M428L/N434S, S239D/I332E, or S239D/I332E + M428L/N434S.

3. The isolated antibody of claim 2, wherein the one or more amino acid
substitutions further comprise one or more of T250Q/M428L; N434A;
30 T307A/E380A/N434A; and M252Y/S254T/T256E.

4. The isolated antibody of any of claims 1-3, wherein the antibody comprises:

a light chain; and

a heavy chain comprising,

- 5 a sequence having at least 80%, at least 90%, or at least 95% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, or any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, wherein the antibody specifically binds gp120 of HIV-1, and wherein the antibody is neutralizing.

5. The isolated antibody of any of claims 1-4, wherein the antibody comprises a light chain comprising:

- 15 amino acids 27-30 (CDR1), 48-50 (CDR2), and 87-91 (CDR3) of SEQ ID NO: 36, or

a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 36, 40 or 44.

- 20 6. The isolated antibody of any of claims 1-5, wherein the antibody comprises a heavy chain and a light chain, wherein heavy chain of the antibody comprises any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 and the light chain of the antibody comprises SEQ ID NO: 36.

- 25 7. The isolated antibody of claim 4 or 5, wherein the heavy chain of the antibody comprises one or more of the following amino acid substitutions:

M428L/N434S;

T250Q/M428L;

N434A;

- 30 T307A/E380A/N434A;

M252Y/S254T/T256E;

S239D/I332E;

S239D/A330L/I332E;
 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 S239D/I332E + N434A;
 5 S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 S239D/A330L/I332E+ N434A;
 10 S239D/A330L/I332E+ T307A/E380A/N434A
 S239D/A330L/I332E + M252Y/S254T/T256E; or
 combinations thereof.

8 The isolated antibody of any of claims 1-7, wherein the antibody is a
 15 human monoclonal antibody.

9. An isolated human monoclonal antibody, comprising:
 a light chain; and
 a heavy chain, wherein the heavy chain comprises:
 20 a sequence having at least 80%, at least 90%, or at least 95%
 sequence identity to SEQ ID NO: 38 or 42, and
 one or more of the following amino acid substitutions:
 M428L/N434S;
 T250Q/M428L;
 25 N434A;
 T307A/E380A/N434A;
 M252Y/S254T/T256E;
 S239D/I332E;
 S239D/A330L/I332E;
 30 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 S239D/I332E + N434A;

S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 5 S239D/A330L/I332E+ N434A;
 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E,

wherein the antibody specifically binds gp120 of HIV-1, and wherein the antibody is neutralizing.

10

10. The isolated human monoclonal antibody of claim 9, wherein:
 the heavy chain of the antibody comprises SEQ ID NO: 38 and one
 or more of the amino acid substitutions, and the light chain of the antibody
 comprises SEQ ID NO: 40; or

15

the heavy chain of the antibody comprises SEQ ID NO: 42 and one
 or more of the amino acid substitutions and the light chain of the antibody
 comprises SEQ ID NO: 44.

11. The isolated antibody of any of claims 1-10, wherein the antibody is
 20 an IgG, IgM or IgA.

12. An isolated functional fragment of the isolated antibody of any of
 claims 1-11.

13. The isolated antibody of any of claims 1-11, or the functional
 25 fragment of claim 12, wherein the antibody or functional fragment is labeled.

14. A composition comprising the antibody of any of claims 1-11, or the
 functional fragment of claim 12, and a pharmaceutically acceptable carrier.

30

15. An isolated nucleic acid molecule encoding the antibody of any of
 claims 1-11, or the functional fragment of claim 12.

16. The isolated nucleic acid molecule of claim 15, wherein the molecule comprises a nucleic acid sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29,
5 31, 33, 37 or 41.

17. The isolated nucleic acid molecule of claim 16, encoding a heavy chain and a light chain of an antibody, wherein

(a) the heavy chain of the antibody comprises a sequence having at least
10 80%, at least 90%, or at least 95% sequence identity to any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, and comprising one or more of the following mutations:

M428L/N434S;
T250Q/M428L;
15 N434A;
T307A/E380A/N434A;
M252Y/S254T/T256E;
S239D/I332E;
S239D/A330L/I332E;
20 S239D/I332E + T250Q/M428L;
S239D/I332E + M428L/N434S;
S239D/I332E + N434A;
S239D/I332E + T307A/E380A/N434A;
S239D/I332E + M252Y/S254T/T256E;
25 S239D/A330L/I332E+ T250Q/M428L;
S239D/A330L/I332E+ M428L/N434S;
S239D/A330L/I332E+ N434A;
S239D/A330L/I332E+ T307A/E380A/N434A; or
S239D/A330L/I332E + M252Y/S254T/T256E,

30 and wherein the light chain of the antibody comprises a sequence having at least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 36;

(b) the heavy chain of the antibody comprises the heavy chain of the antibody comprises any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34, and wherein the light chain of the antibody comprises SEQ ID NO: 36;

5 (c) the heavy chain of the antibody comprises SEQ ID NO: 36 or 42 comprising one or more of the following mutations:

T250Q/M428L;
M428L/N434S;
N434A;
10 T307A/E380A/N434A;
M252Y/S254T/T256E;
S239D/I332E;
S239D/A330L/I332E;
S239D/I332E + T250Q/M428L;
15 S239D/I332E + M428L/N434S;
S239D/I332E + N434A;
S239D/I332E + T307A/E380A/N434A;
S239D/I332E + M252Y/S254T/T256E;
S239D/A330L/I332E+ T250Q/M428L;
20 S239D/A330L/I332E+ M428L/N434S;
S239D/A330L/I332E+ N434A;
S239D/A330L/I332E+ T307A/E380A/N434A; or
S239D/A330L/I332E + M252Y/S254T/T256E,

and wherein the light chain of the antibody comprises a sequence having at
25 least 80%, at least 90%, or at least 95% sequence identity to SEQ ID NO: 40 or 44;

(d) the heavy chain of the antibody comprises SEQ ID NO: 36 having one or more of the following mutations:

T250Q/M428L;
M428L/N434S;
30 N434A;
T307A/E380A/N434A;
M252Y/S254T/T256E;

- S239D/I332E;
 S239D/A330L/I332E;
 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 5 S239D/I332E + N434A;
 S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 10 S239D/A330L/I332E+ N434A;
 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E,
 and wherein the light chain of the antibody comprises SEQ ID NO: 40;
 (e) the heavy chain of the antibody comprises SEQ ID NO: 42 having one or
 15 more of the following mutations:
 T250Q/M428L;
 M428L/N434S;
 N434A;
 T307A/E380A/N434A;
 20 M252Y/S254T/T256E;
 S239D/I332E;
 S239D/A330L/I332E;
 S239D/I332E + T250Q/M428L;
 S239D/I332E + M428L/N434S;
 25 S239D/I332E + N434A;
 S239D/I332E + T307A/E380A/N434A;
 S239D/I332E + M252Y/S254T/T256E;
 S239D/A330L/I332E+ T250Q/M428L;
 S239D/A330L/I332E+ M428L/N434S;
 30 S239D/A330L/I332E+ N434A;
 S239D/A330L/I332E+ T307A/E380A/N434A; or
 S239D/A330L/I332E + M252Y/S254T/T256E,

and wherein the light chain of the antibody comprises SEQ ID NO: 44,
wherein the encoded antibody specifically binds gp120 of HIV-1, and
wherein the encoded antibody is neutralizing.

5 18. The isolated nucleic acid molecule of any of claims 15-17, operably
linked to a promoter.

 19. An expression vector comprising the isolated nucleic acid molecule
of any one of claims 15-18.

10

 20. The expression vector of claim 19, wherein the antibody is an IgA or
an IgG.

 21. The expression vector of claim 19 or 20 comprising RNA splicing
15 donor sites, RNA splicing acceptor sites and/or internal ribosomal binding
sequences.

 22. The expression vector of any of claims 19-21, wherein the heavy
chain of the antibody and the light chain of the antibody are expressed as a fusion
20 polypeptide following the introduction of the expression vector in a host cell.

 23. The expression vector of claim 22, comprising a nucleic acid
sequence encoding a furin cleavage site between the heavy chain and the light chain
of the antibody.

25

 24. The expression vector of any of claims 19-22, encoding a selectable
marker.

 25. An isolated host cell transformed with the nucleic acid molecule of
30 any of claims 15-218 or the vector of any of claims 29-24.

26. A method of detecting a human immunodeficiency virus (HIV)-1 infection in a subject comprising:

contacting a biological sample from the subject with at least one isolated antibody of any claims 1-11, or 13 or the functional fragment of claim 12;

5 detecting antibody bound to the sample; and

determining that the subject has an HIV-1 infection when the antibody is detected bound to the sample.

27. The method of claim 26, wherein the isolated antibody is directly
10 labeled.

28. The method of claim 26 or 27, further comprising:

contacting the sample with a second antibody that specifically binds the isolated antibody;

15 detecting the binding of the second antibody; and

determining the presence of an HIV-1 infection in the subject when an increase in binding of the second antibody to the sample is detected as compared to binding of the second antibody to a control sample.

29. A method for treating an human immunodeficiency virus (HIV)-1 infection in a subject, comprising:

administering to the subject a therapeutically effective amount of at least one antibody of any one of claims 1-11, or 13 or the functional fragment of claim 12, thereby preventing or treating the HIV-1 infection.

25

30. The method of claim 29, wherein the method is a method for treating an HIV-1 infection, and wherein the subject has acquired immune deficiency syndrome (AIDS).

31. The method of claim 29 or 30, further comprising administering to the subject an anti-viral agent.

32. The method of any one of claims 29-31, further comprising measuring HIV-1 viral titer in the subject.

33. A method for testing a potential vaccine, comprising:
5 contacting the potential vaccine with an antibody of any one of claims 1-11, or 15 or the functional fragment of claim 13; and
detecting the binding of the antibody to an immunogen in the potential vaccine.

FIG. 1**Mutations to increase half-life**

Code	Mutation sites
QL	T250Q / M428L
LS	M428L / N434S
A	N434A
AAA	T307A / E380A / N434A
YTE	M252Y / S254T / T256E

Mutations to increase ADCC activity

Code	Mutation sites
DE	S239D / I332E
DLE	S239D / A330L / I332E

Mutations to increase both half-life and ADCC activity

Code	Mutation sites
DE - QL	S239D / I332E + T250Q / M428L
DE - LS	S239D / I332E + M428L / N434S
DE - A	S239D / I332E + N434A
DE - AAA	S239D / I332E + T307A / E380A / N434A
DE - YTE	S239D / I332E + M252Y / S254T / T256E
DLE-QL	S239D / A330L / I332E + T250Q / M428L
DLE-LS	S239D / A330L / I332E + M428L / N434S
DLE-A	S239D / A330L / I332E + N434A
DLE - AAA	S239D / A330L / I332E + T307A / E380A / N434A
DLE - YTE	S239D / A330L / I332E + M252Y / S254T / T256E

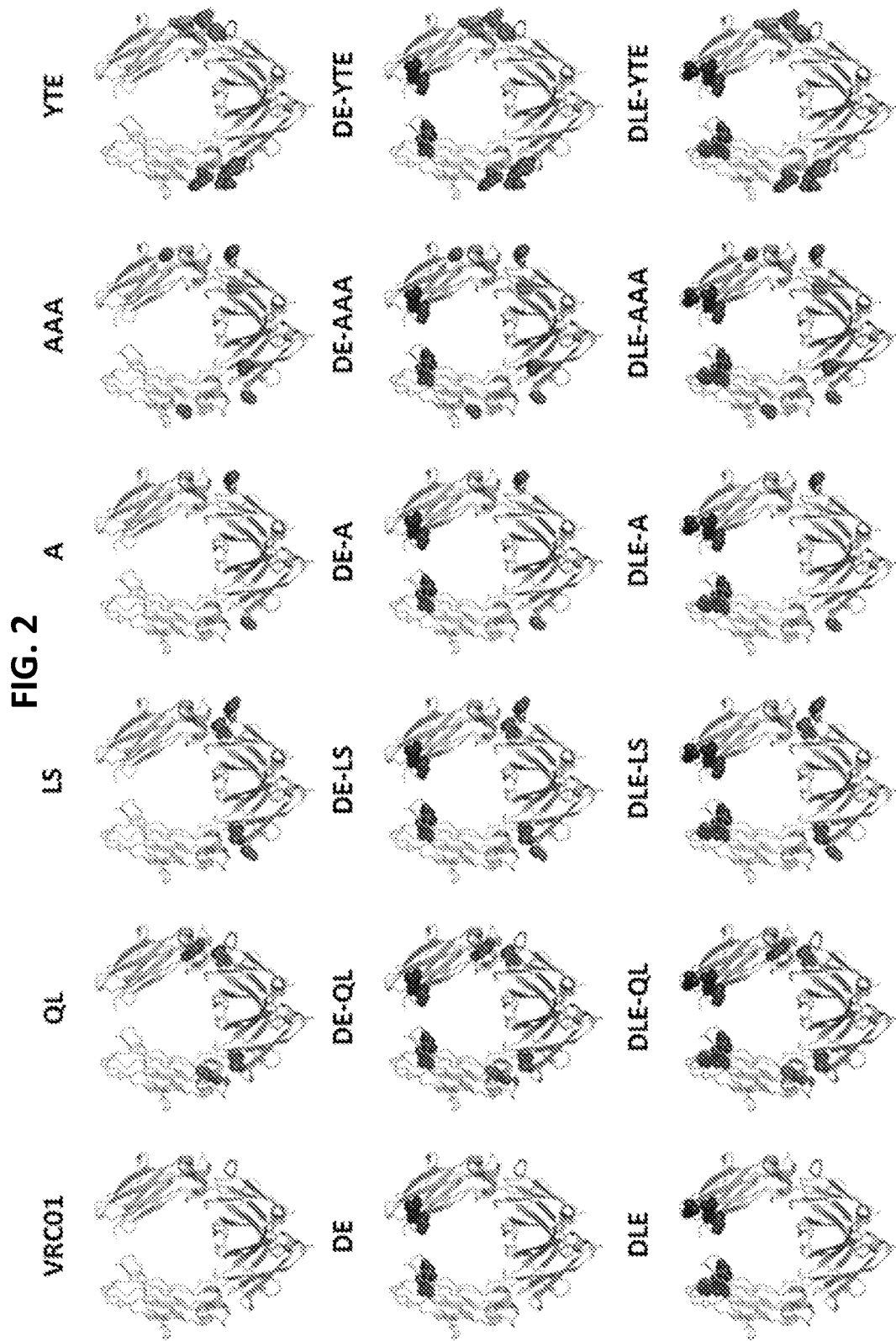


FIG. 3

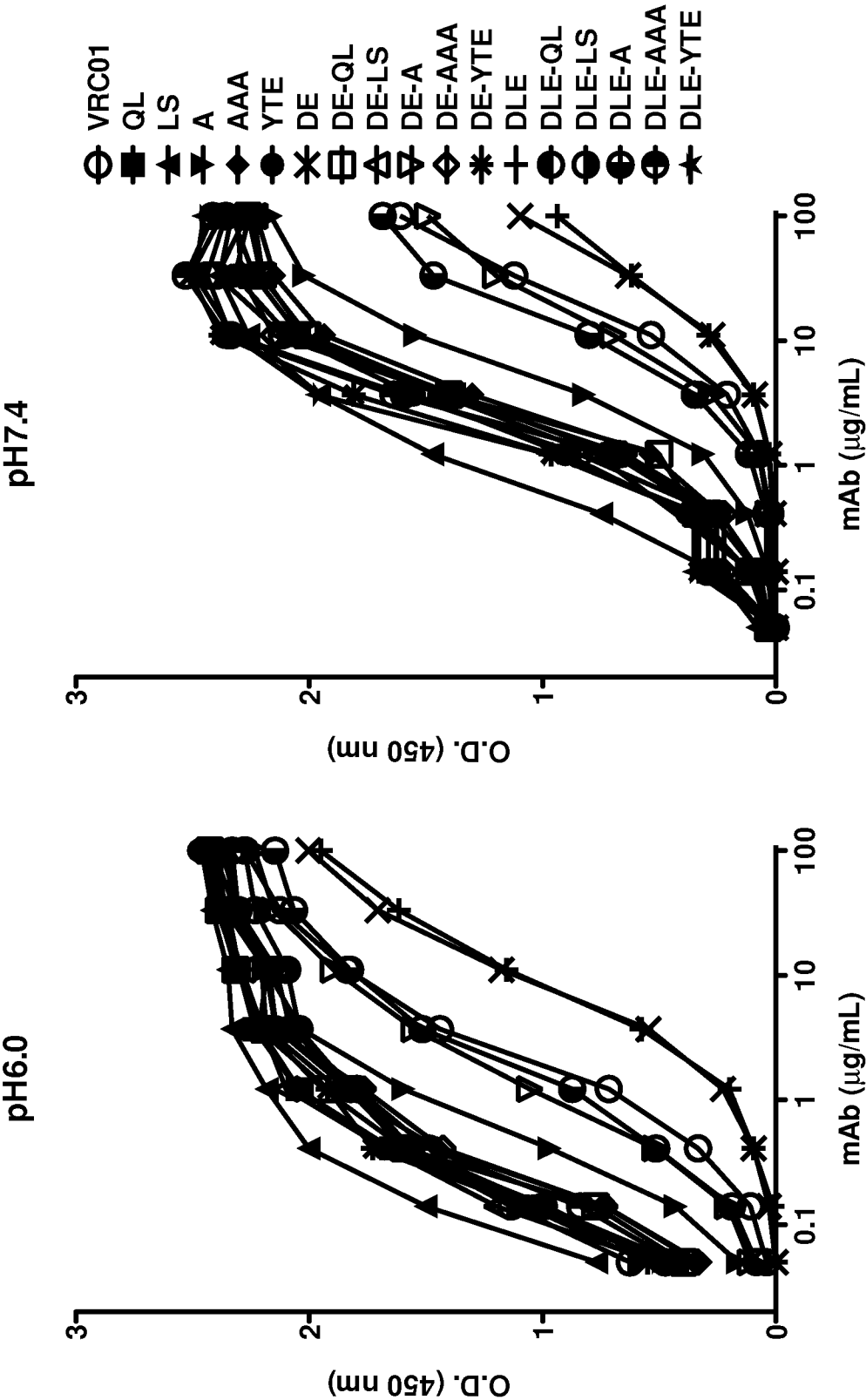


FIG. 4

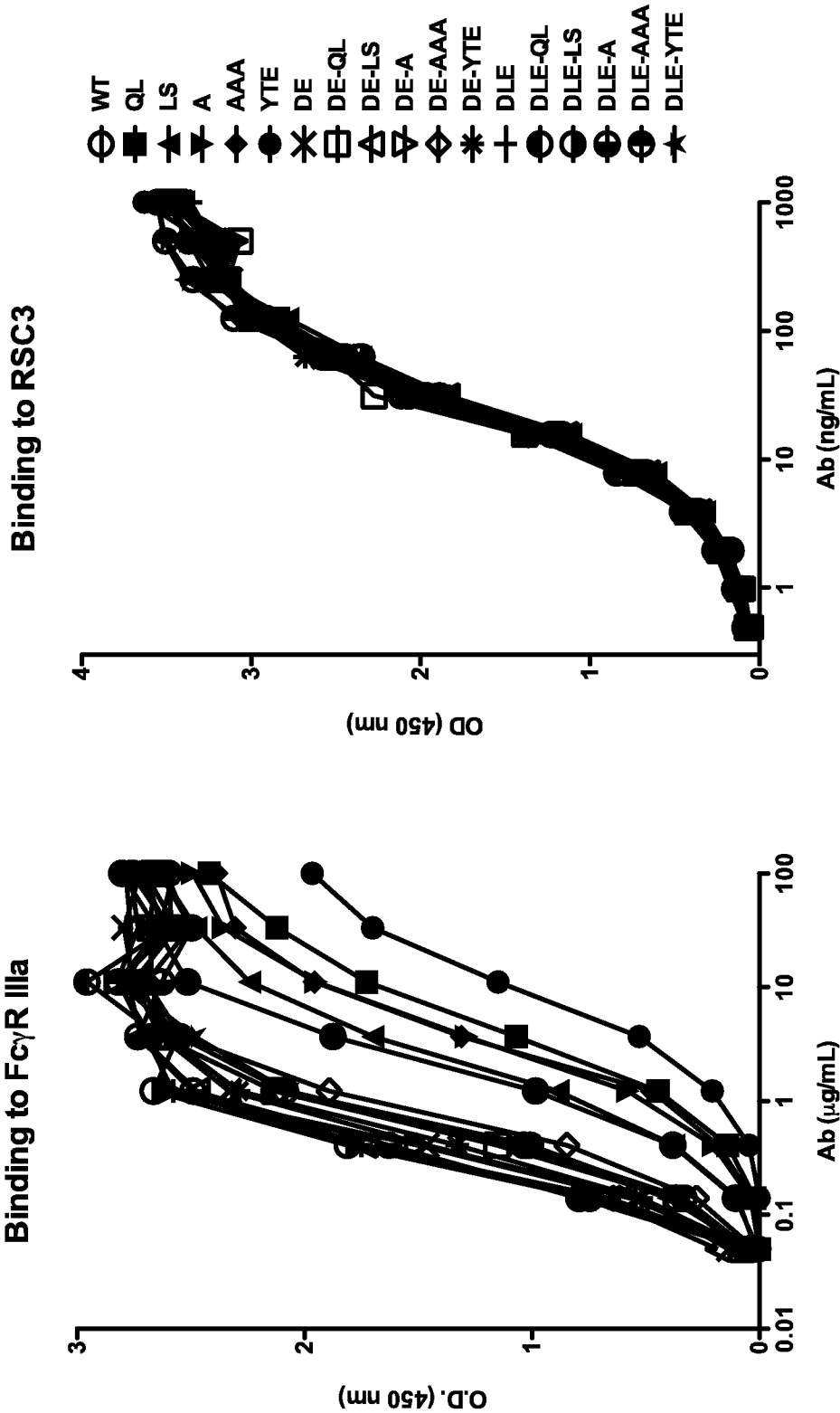


FIG. 5

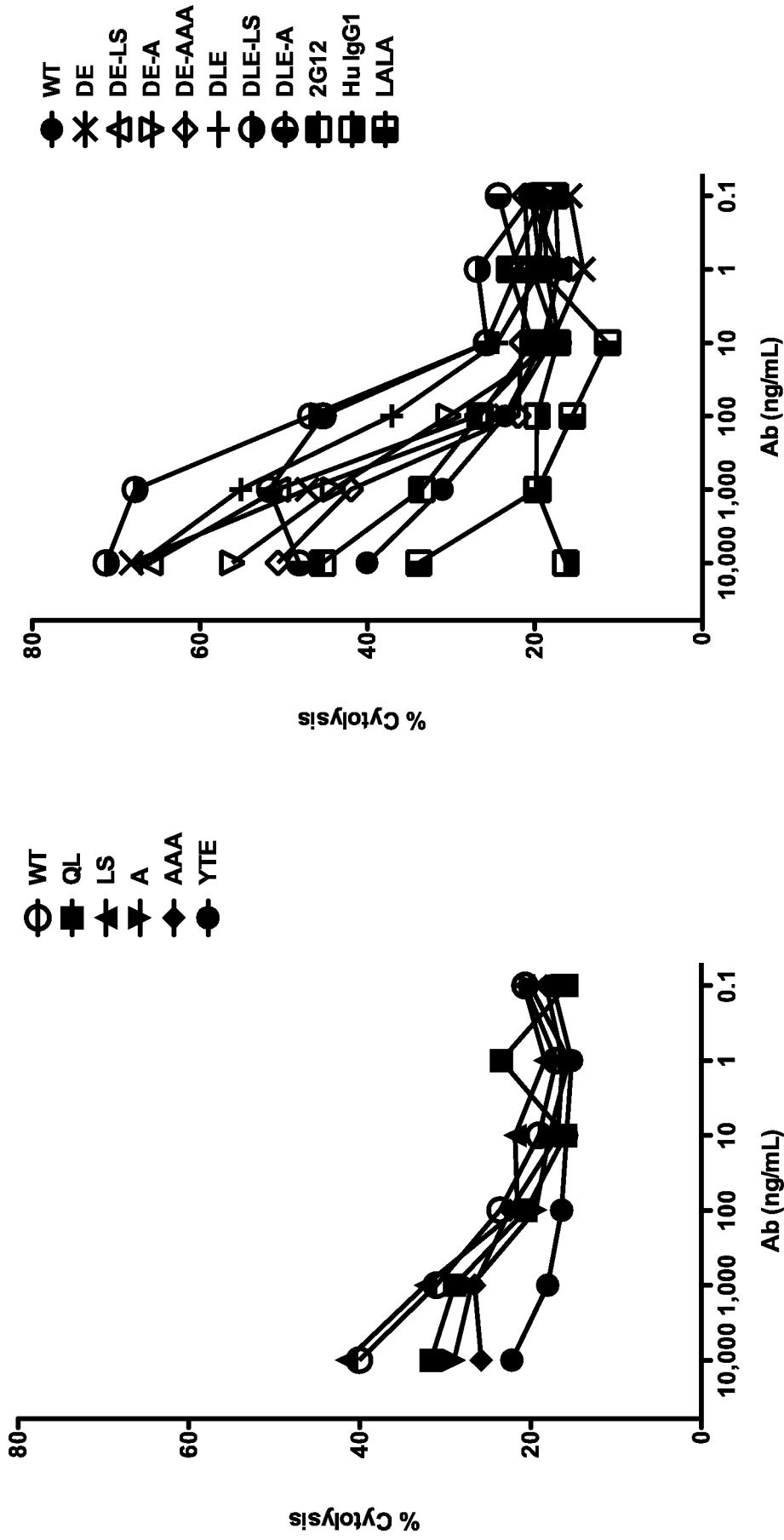
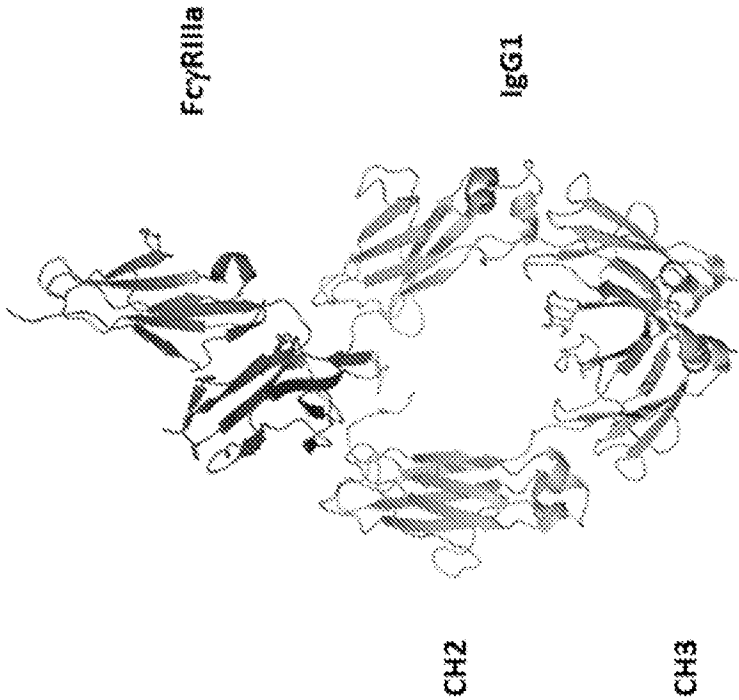
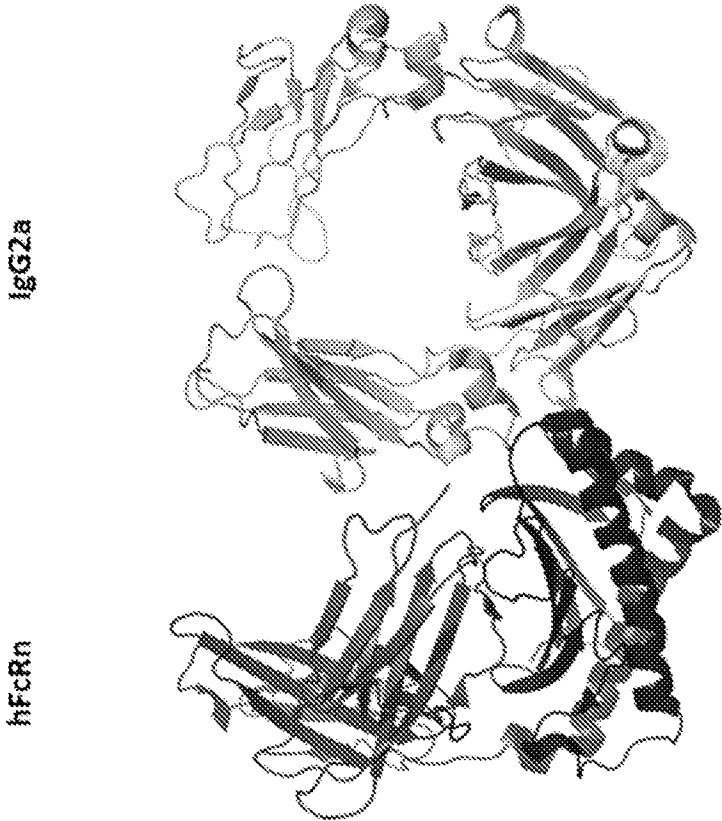


FIG. 6



FcγRIIIa binds to hinge and CH2 regions of IgG



hFcRn binds to CH2 and CH3 regions of IgG

FIG. 7

Section 1									
(1)	1	10	20	30	40	50	60	70	82
VR001	(1)	QVQLVQSGQMKPKGEGSMRIS	CRASGYEFIDCTLNWIRLAFGR	RENNMGLKPRGCAVNIARPLQGR	VTMTIRDVYS	-----			
VR002	(1)	QVQLVQSGQMKPKGEGSMRIS	CQASGYEFIDCTLNWIRLAFGR	RENNMGLKPRGCAVNIARPLQGR	VTMTIRDVYS	-----			
VR003	(1)	QVQLVQSGAVINTPGSSVKIS	CRASGYNFROYSIHWRLIPDKGE	NIWIKIPFLWGAVSIARQLQGR	VSMTIRQLSQDPDPO				
Consensus	(1)	QVQLVQSGQMKPKGEGSMRIS	CRASGYEFIDCTLNWIRLAFGR	RENNMGLKPRGCAVNIARPLQGR	VTMTIRDVYS				
Section 2									
(83)	83	90	100	110	120	130	140	150	164
VR001	(78)	---TAFLELRSLTVD	DTAVYFCIRGKNC	DYNWDF	---EHWGRGIP	VIIVSSASTKGPS	VFPLAPSSKSTSGGTAALGCLVKDYF		
VR002	(78)	---TAFLELRSLTAD	DTAVYFCIRGKNC	DYNWDF	---EHWGRGIP	VIIVSSASTKGPS	VFPLAPSSKSTSGGTAALGCLVKDYF		
VR003	(83)	WGVAYMEFSGLT	PADTAIEYFCV	RRGSCDYCGDEPWQ	YWGQGVVSSASTKGPS	VFPLAPSSKSTSGGTAALGCLVKDYF			
Consensus	(83)	TAFLELRSLT	DTAVYFCIRGKNC	DYNWDF	EHWGRGIP	VIIVSSASTKGPS	VFPLAPSSKSTSGGTAALGCLVKDYF		
Section 3									
(165)	165	170	180	190	200	210	220	230	246
VR001	(156)	EPVIVSNNSGALT	SGVHTFP	PAVLQSSGLYSLS	SVTVPS	SSSLG	IQTYICNVNHNKPS	NTKVDKNVEPKSCDK	THTCPPCPAPE
VR002	(156)	EPVIVSNNSGALT	SGVHTFP	PAVLQSSGLYSLS	SVTVPS	SSSLG	IQTYICNVNHNKPS	NTKVDKNVEPKSCDK	THTCPPCPAPE
VR003	(165)	EPVIVSNNSGALT	SGVHTFP	PAVLQSSGLYSLS	SVTVPS	SSSLG	IQTYICNVNHNKPS	NTKVDKNVEPKSCDK	THTCPPCPAPE
Consensus	(165)	EPVIVSNNSGALT	SGVHTFP	PAVLQSSGLYSLS	SVTVPS	SSSLG	IQTYICNVNHNKPS	NTKVDKNVEPKSCDK	THTCPPCPAPE
Section 4									
(247)	247	260	270	280	290	300	310	320	
VR001	(238)	LLGSPSVLEFP	PKPKDILMISRI	PEVTCVWVDVSH	EDPEVKTNWYVDG	VEVHNAKTKPREEQ	YNSTYRVVSVLT	VLHQDWLN	
VR002	(238)	LLGSPSVLEFP	PKPKDILMISRI	PEVTCVWVDVSH	EDPEVKTNWYVDG	VEVHNAKTKPREEQ	YNSTYRVVSVLT	VLHQDWLN	
VR003	(247)	LLGSPSVLEFP	PKPKDILMISRI	PEVTCVWVDVSH	EDPEVKTNWYVDG	VEVHNAKTKPREEQ	YNSTYRVVSVLT	VLHQDWLN	
Consensus	(247)	LLGSPSVLEFP	PKPKDILMISRI	PEVTCVWVDVSH	EDPEVKTNWYVDG	VEVHNAKTKPREEQ	YNSTYRVVSVLT	VLHQDWLN	
Section 5									
(329)	329	340	350	360	370	380	390	400	410
VR001	(320)	GKEYCKVSNKALP	APIERTISKAKQ	PREPOVYTLPPSR	DELINQVSLTCLV	KGYFSP	SDIAVENESNGQ	PENNYKITPPV	
VR002	(320)	GKEYCKVSNKALP	APIERTISKAKQ	PREPOVYTLPPSR	DELINQVSLTCLV	KGYFSP	SDIAVENESNGQ	PENNYKITPPV	
VR003	(329)	GKEYCKVSNKALP	APIERTISKAKQ	PREPOVYTLPPSR	DELINQVSLTCLV	KGYFSP	SDIAVENESNGQ	PENNYKITPPV	
Consensus	(329)	GKEYCKVSNKALP	APIERTISKAKQ	PREPOVYTLPPSR	DELINQVSLTCLV	KGYFSP	SDIAVENESNGQ	PENNYKITPPV	
Section 6									
(411)	411	420	430	440	450	461			
VR001	(402)	LDSGSEFLYSKL	TVDKSRWQQGNV	SCSVMEALHNHYT	QKSLSPCK	---			
VR002	(402)	LDSGSEFLYSKL	TVDKSRWQQGNV	SCSVMEALHNHYT	QKSLSPCK	---			
VR003	(411)	LDSGSEFLYSKL	TVDKSRWQQGNV	SCSVMEALHNHYT	QKSLSPCK	---			
Consensus	(411)	LDSGSEFLYSKL	TVDKSRWQQGNV	SCSVMEALHNHYT	QKSLSPCK	---			

FIG. 8
Relative transcytosis ability to VRC01 WT

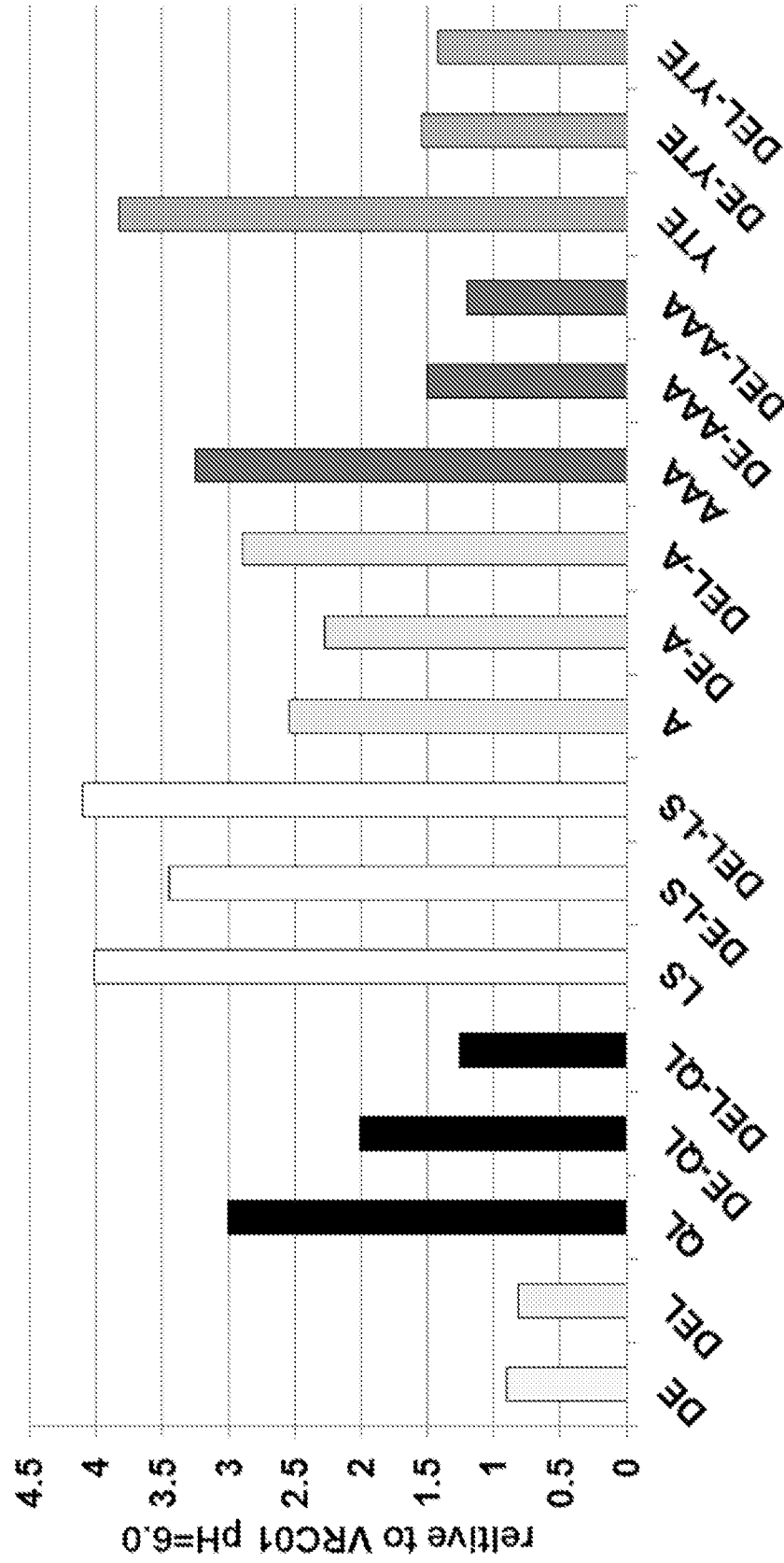


FIG. 9

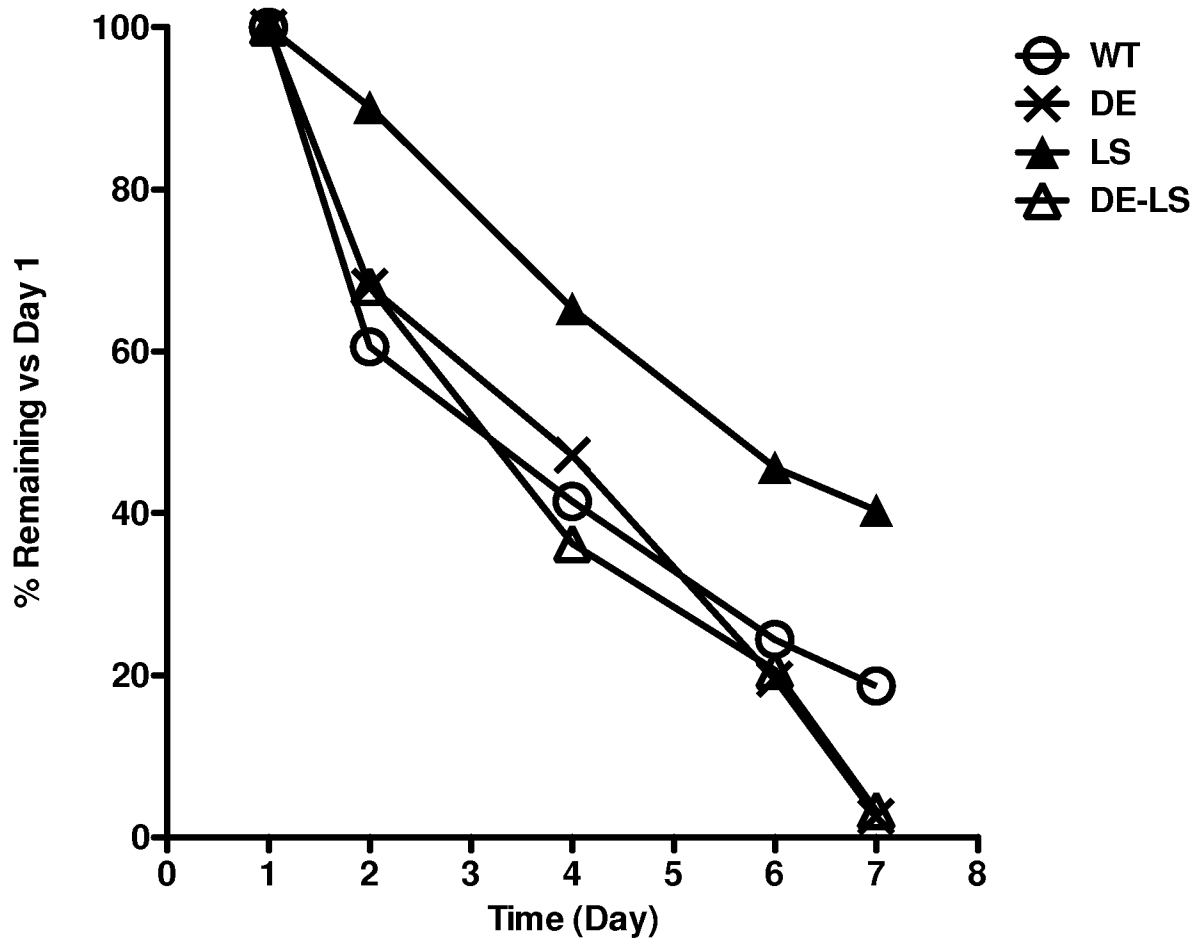
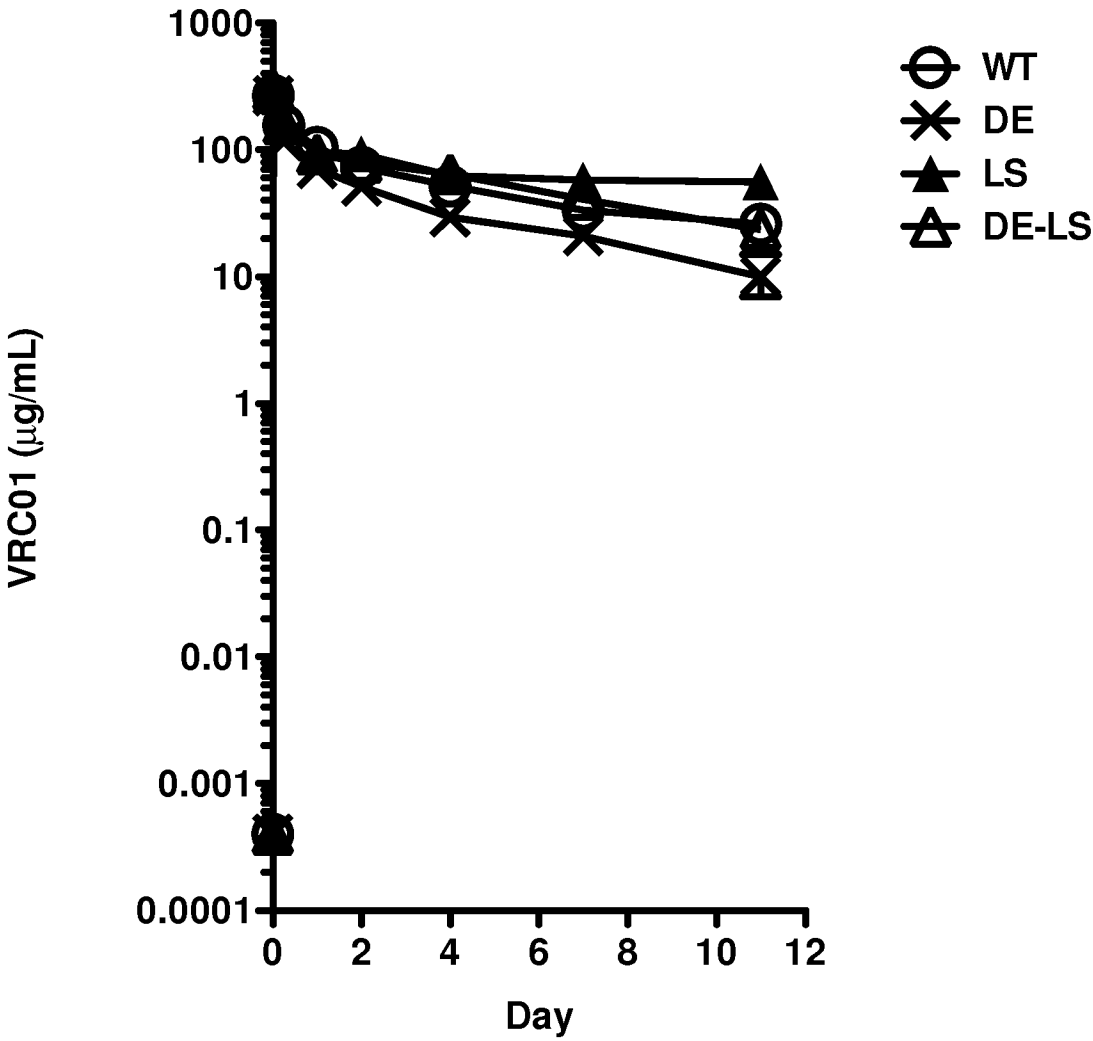


FIG. 10



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/023737

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C07K 16/10 (2006.01)

A61K 39/42 (2006.01)

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)

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)

GenomeQuest: Sequence Search on amino acid SEQ ID NOs: 2-44

WPI, EPODOC, MEDLINE: Immunoglobulin, Antibody, FCRN, Fc Receptor, mutate, modify, substitute, half life, ADCC, HIV, GP120, VRC01, M428L, N434S, N434A, T307A, E380A, M252Y, S254T, T256E, S239D, I332E, A330L

PatentLens: T250Q/M428L, M428L/N434S, N434A, T307A/E380A/N434A, M252Y/S254T/T256E and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KANEKO, E. and NIWA, R. 'Optimizing Therapeutic Antibody Function. Progress with Fc Domain Engineering' Biodrugs, 1 February 2011, vol. 25 (1), pages 1-11	1-3, 8, 11-15, 18-25
Y	Abstract, pages 3, 6.	4-7, 9, 10, 16, 17, 26-33

☒ 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	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
5 April 2012Date of mailing of the international search report
10/04/2012Name and mailing address of the ISA/AU
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/023737

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHIELDS, R.L. <i>et al.</i> 'High Resolution Mapping of the Binding Site on Human IgG1 for FcγRI, FcγRII, FcγRIII and FcRn and Design of IgG1 Variants with Improved Binding to the FcγR.' Journal of Biological Chemistry. 2001, vol. 276, No. 9, pages 6591-6604 Abstract, pages 6595, 6598, tables I, III.	1-3, 8, 11-15, 18-25
Y		4-7, 9, 10, 16, 17, 26-33
X	US 2006/0024298 A1 (LAZAR <i>et al.</i>) 2 February 2006 Paragraphs 3, 24, 25, 98, figures, claim 3.	1-3, 8, 11-15, 18-25
Y		4-7, 9, 10, 16, 17, 26-33
X	US 2009/0041770 A1 (CHAMBERLAIN <i>et al.</i>) 12 February 2009 Abstract, figures 2-5, 13, 14, claims.	1-3, 8, 11-15, 18-25
Y		4-7, 9, 10, 16, 17, 26-33
X	US 2007/0036799 A1 (STAVENHAGEN <i>et al.</i>) 15 February 2007 Abstract, pages 17-21, tables 2, 3	1-3, 8, 11-15, 18-25
Y		4-7, 9, 10, 16, 17, 26-33
Y	TONGQING, Z. <i>et al.</i> , 'Structural Basis for Broad and Potent Neutralization of HIV-1 by Antibody VRC01' Science, 2010, vol. 329, pages 811- 817 Abstract, page 816.	4-7, 9, 10, 16, 17, 26-33
Y	WU, X. <i>et al.</i> , 'Rational Design of Envelope Identifies Broadly Neutralizing Human Monoclonal Antibodies to HIV-1' Science, 2010, vol. 329, pages 856-861 Abstract, pages 859-960.	4-7, 9, 10, 16, 17, 26-33

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/023737

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box I

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.:

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.

Supplemental Box I

(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This Authority has found that there are three different inventions based on the following features that separate the claims into three distinct groups:

- Claims 1-33(in part) are directed to isolated antibodies comprising one or more mutations selected from the group consisting of M428L/N434S, T250Q/M4287L, N434A, T307A/E380A/N434A and M252Y/S254T/T256E in the Fc region, functional fragments of these antibodies, compositions comprising these antibodies, isolated nucleic acids encoding such antibodies, vectors containing the nucleic acid encoding the antibodies, host cells transformed with the nucleic acid or vector, and methods of detecting/treating/testing a vaccine for HIV-1 infection by administering the antibodies. These mutations share the property that they increase the half life of the antibodies. The feature of mutations in the Fc region of an antibody that increase the half life of the antibody is specific to this group of claims.
- Claims 1-33(in part) are directed to isolated antibodies comprising one or more mutations selected from the group consisting of S239D/I332E and S239D/A330L/I332E in the Fc region, functional fragments of these antibodies, compositions comprising these antibodies, isolated nucleic acids encoding such antibodies, vectors containing the nucleic acid encoding the antibodies, host cells transformed with the nucleic acid or vector, and methods of detecting/treating/testing a vaccine for HIV-1 infection by administering the antibodies. These mutations share the property that they increase the ADCC activity of the antibodies. The feature of mutations in the Fc region of an antibody that increase the ADCC of the antibody is specific to this group of claims.
- Claims 1-33(in part) are directed to isolated antibodies comprising the combination that consists of a mutation in the Fc region that increases the half life of the antibody (namely M428L/N434S, T250Q/M4287L, N434A, T307A/E380A/N434A or M252Y/S254T/T256E) with a mutation that increases the ADCC activity of the antibody (namely S239D/I332E or S239D/A330L/I332E), functional fragments of these antibodies, compositions comprising these antibodies, isolated nucleic acids encoding such antibodies, vectors containing the nucleic acid encoding the antibodies, host cells transformed with the nucleic acid or vector, and methods of detecting/treating/testing a vaccine for HIV-1 infection by administering the antibodies. These combination of mutations share the property that they increase the half life and ADCC activity of the antibodies. The feature of the combination of mutations in the Fc region of an antibody that increase half life and ADCC activity of the antibody is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the amino acid substitution in the Fc region. However this feature does not make a contribution over the prior art because it is disclosed in:

Continued in Supplemental Box II

Supplemental Box II

(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of : Supplemental Box I

D1: KANEKO, E. and NIWA, R. 'Optimizing Therapeutic Antibody Function: Progress with Fc Domain Engineering' Biodrugs. 2011, Feb 1: Vol. 25(1): pages 1-11 and references within.

D2: SHIELDS, R.L. *et al.*, Journal of Biological Chemistry. 2001, vol 276, no. 9, pages 6591-6604.

(See Box V and Supplemental Box I of the Written Opinion of the International Searching Authority for a discussion of D1 and D2).

Therefore in the light of these documents this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2012/023737

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
US	2006024298	US	2009053211	AU	2003217912
		AU	2004236160	AU	2004297616
		AU	2005272993	AU	2006230413
		AU	2006299429	AU	2006302254
		AU	2007299843	AU	2008207898
		AU	2008260498	AU	2008261120
		AU	2011202115	BR	0314814
		BR	PI0510674	CA	2499816
		CA	2548817	CA	2561264
		CA	2602663	CA	2624189
		CA	2652434	CA	2658557
		CA	2669412	CA	2693053
		CN	1705491	CN	1867583
		CN	101987870	CN	101987871
		EP	1620467	EP	1697520
		EP	1737890	EP	1871808
		EP	1931709	EP	1951757
		EP	2042517	EP	2053062
		EP	2064240	EP	2125893
		EP	2221315	EP	2298805
		EP	2364996	EP	2368911
		IL	167685	JP	2007525443
		JP	2007512846	JP	2008505174
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		KR	20050116400	KR	20070116176
		KR	20090059170	KR	20080106371
		RU	2005137578	RU	2008104038
		US	2005244403	US	7276585
		US	2006160996	US	7317091
		US	7557190	US	2006008883
				AU	2003279719
				AU	2005227326
				AU	2006268227
				AU	2007285976
				AU	2008229860
				AU	2009200518
				BR	PI0410031
				CA	2524399
				CA	2565961
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				EP	1553975
				EP	1697741
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				EP	2383297
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				US	2005054832
				US	2007161783
				US	7657380

Information on patent family members

PCT/US2012/023737

Form PCT/ISA/210 (patent family annex) (July 2009)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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EP	2212354	EP	2235059	EP	2314618
EP	2325206	EP	2325207	EP	2332985
JP	2008519860	JP	2011502126	JP	2011507963
KR	20080113301	KR	20070086086	KR	20100090266
KR	20100099179	RU	2010121898	RU	2010130851
US	2011110928	US	8088376	US	2006173170
US	2007135620	US	2009163699	US	2010204454
US	2010234571	US	2010234572	US	2010234573
US	2010234574	US	2010234575	WO	2006053301
WO	2009058492	WO	2009086320		
US	2007036799	AU	2006279945	CA	2618681
		JP	2009507470	EP	1919503
			WO	2007021841	

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX