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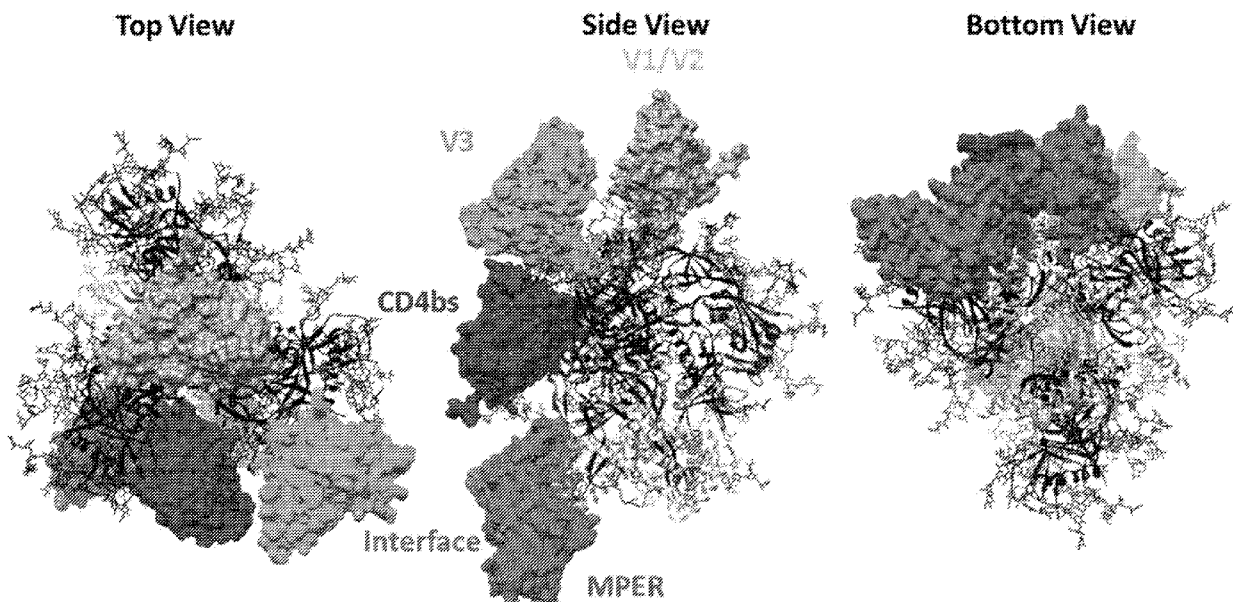
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FIG. 1



(57) Abstract: The present invention provides a multispecific anti-HIV antibody that binds to multiple epitopes on HIV envelope protein, wherein the antibody comprises: i. an amino acid sequence that binds to a V1/V2 apex glycan epitope; ii. an amino acid sequence that binds to a V3-base glycan region epitope; iii. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope; iv. an amino acid sequence that binds to a gp120/gp41 interface epitope; and v. an amino acid sequence that binds to a membrane proximal external region (MPER) epitope.

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MULTISPECIFIC ANTI-HIV ANTIBODIES

This application claims priority to U.S. Provisional Application No. 62/749,510, filed 10/23/2018 and U.S. Provisional Application No. 62/748,228 filed October 19, 2018. The entire disclosures of all of the foregoing applications are incorporated by
5 reference herein.

This invention was made with government support under 5R01 AI136756 awarded by NIH. The government has certain rights in the invention.

FIELD OF THE INVENTION

The field of the invention relates to medicine, infectious disease and in particular
10 antibodies which can neutralize HIV-1 virus strains.

BACKGROUND OF THE INVENTION

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus type 1 (HIV-1) (Gallo *et al.*, Science 224, 500-503. (1984); Barre-Sinoussi *et al.*, Science 220, 868-871. (1983)), and results from infection and
15 depletion of human CD4⁺ lymphocytes. HIV-1 infection of CD4⁺ host cells is mediated by the viral envelope glycoproteins (Env), which are displayed as trimeric spikes that sparsely coat the surface of the HIV virion. Each trimeric Env complex is composed of three of each exterior envelope glycoprotein, gp120, and the gp41 transmembrane
20 envelope glycoprotein (Kowalski *et al.*, Science 237, 1351-1355. (1987); Lu *et al.*, Nat Struct Biol 2, 1075-1082 (1995). This trimeric Env complex is the main target of neutralizing antibody responses [reviewed by (Wyatt *et al.*, Science 280, 1884-1888. (1998))].

Over the past two decades, advances in the treatment of human immunodeficiency virus (HIV) infection have led to dramatic improvements in health outcomes of infected
25 individuals receiving antiretroviral therapy (ART). Nonetheless, the vast majority of HIV-infected individuals must remain on continuous, life-long ART in order to maintain suppression of HIV replication and prevent progression to AIDS. While effective at suppressing HIV replication, current ART requires life-long adherence to daily medication regimens and is associated with significant costs, cumulative toxicities, and
30 the potential for emergence of drug-resistant virus.

Consequently, there has been considerable interest in strategies that would allow for discontinuation of ART while maintaining suppression of plasma viremia for prolonged periods. In this regard, recent advances in immunogen and antibody cloning technologies have led to the isolation of several highly potent and broadly neutralizing HIV-specific antibodies (bNAb) from B cells of infected individuals. Some bNAbs have exhibited strong activities against HIV and SIV in vitro, in infected animals, and in infected individuals who were not receiving ART.

Recently, broadly neutralizing antibodies (bNAbs) have been explored as prevention and therapy agents for the treatment and management of HIV-1, as they can i) inhibit virus entry by neutralizing the free infectious virus particles, ii) prevent virus cell-to-cell spread, and iii) eliminate virus-infected cells by binding the Env molecules expressed on the surface of the infected cells and triggering antibody-dependent cell-mediated cytotoxicity (ADCC) through interactions between the IgG Fc region and the Fc receptors on effector cells (primarily NK cells) [reviewed in Burton *et al.*, Nat Immunol 16, 571-576 (2015)]. Furthermore, the repertoire of isolated bNAbs has drastically increased recently as the process for bNAb isolation and characterization has accelerated with the integration of emerging functional and structural information and new technologies of single B cell sorting and cloning (Burton *et al.*, Proc Natl Acad Sci U S A 88, 10134-10137 (1991); Buchacher *et al.*, AIDS Res Hum Retroviruses 10, 359-369 (1994); Huang *et al.*, Nature 491, 406-412 (2012); Scheid *et al.*, Science 333, 1633-1637 (2011); Wu *et al.*, Science 329, 856-861 (2010); Walker *et al.*, Nature 477, 466-470 (2011)). The characterization of HIV-1 bNAbs and their cognate epitopes on the Env spikes has identified five conserved Env sites of vulnerability including, in the order from the apex to the stem of Env: the V1/V2-glycan region, the V3- glycan region, the CD4-binding site (CD4bs), the gp120-gp41 interface (Burton *et al.*, Nat Immunol 16, 571-576 (2015)), and the gp41 membrane proximal external region (MPER) (Burton *et al.*, Nat Immunol 16, 571-576 (2015); Kwong *et al.*, Nat Rev Immunol 13, 693-701 (2013)) (Fig 1).

Administration of a single bNAb as a therapeutic agent has successfully cleared phase I safety clinical trials, demonstrating temporary HIV-1 viremia suppression in the majority of patients (Caskey *et al.*, Nature 522, 487-491 (2015); Ledgerwood *et al.*, Clin Exp Immunol 182, 289-301 (2015)). Unfortunately, the HIV virus rapidly develops

resistance mutations under the selective pressure of single bNAb, suggesting that passive treatment with a single bNAb is unlikely to result in long-term viremia suppression (Caskey *et al.*, Nature 522, 487-491 (2015); Lynch *et al.*, Sci Transl Med 7, 319ra206 (2015); Bar *et al.*, N Engl J Med 375, 2037-2050 (2016); Caskey *et al.*, Nat Med 23, 185-191 (2017)). Fortunately, some Env mutations associated with bNAb resistance can reduce viral fitness, suggesting that simultaneously targeting different Env epitopes may compromise viral replication (Tebit *et al.*, AIDS Rev 9, 75-87 (2007); Sather *et al.*, J Virol 86, 12676-12685 (2012); Lynch *et al.*, J Virol 89, 4201-4213 (2015); Pietzsch *et al.*, J Exp Med 207, 1995-2002 (2010)). The quick onset of escaping virus quasi species in these clinical trials with single bNAb agent strongly highlights the need to develop combinational therapy regimens to control virus rebound by preventing emergence of resistant virus. Additionally, in vitro data from previous studies have demonstrated that combinatorial bNAb therapies display a substantial gain of neutralization potency and breadth when two or more bNAbs targeting independent epitopes are combined (Kong *et al.*, J Virol 89, 2659-2671 (2015); Doria-Rose *et al.*, J Virol 86, 3393-3397 (2012)). This in vitro data is further supported by a number of in vivo animal studies in which dual-, triple-, and penta- combinations of bNAbs resulted in improved protective efficacy compared to mono bNAb therapy (Shingai *et al.*, Nature 503, 277-280 (2013); Klein *et al.*, Nature 492, 118-122 (2012)).

Collectively, these findings suggest that passive immunotherapy with a bNAb(s) could potentially prevent plasma viral rebound in HIV-infected individuals following cessation of ART. For this reason, we recently conducted a clinical trial to investigate whether VRC01 could prevent plasma viral rebound upon discontinuation of ART. While multiple infusions of VRC01 were safe and well-tolerated, the majority of patients experienced plasma viral rebound due to pre-existing and emergent VRC01-resistant HIV despite adequate levels of antibody in plasma (Lynch, Sci Transl Med 7, 319ra206 (2015); Bar *et al.*, N Engl J Med 375, 2037-2050 (2016)). Therefore, therapeutic strategies involving passive transfer of bNAbs may require a combination of bNAbs that targets multiple regions on HIV Env protein in order to achieve sustained virologic control upon withdrawal of ART.

Of note, the potential significance of the above concept was recently highlighted in an animal experiment in which a trimeric antibody consisting of VRC01, PGDM1400,

and 10E8 showed profound potency and breadth against a mixture of SHIV (Xu *et al.*, Science 358, 85-90 (2017)). Building upon these findings, it is of great importance to explore the effect of multimeric bNAbs (or bNAb-like molecules) that possess broad and potent neutralization capacity against highly heterogeneous infectious viral isolates.

5 While antibody cocktails demonstrated improved efficacy in preclinical studies, multispecific “single agents” are desirable for manufacturing purposes (Hu *et al.*, Adv Drug Deliv Rev 98, 19-34 (2016) as well as for improved avidity that may result in enhanced neutralization breadth and potency (Galimidi *et al.*, Cell 160, 433-446 (2015)). Previous bispecific bNAb designs utilizing CrossMab technology to combine two bNAb
10 Fabs first proved the concept that empirical combinations of bNAb functional moieties in bi-valence format could achieve breadth and coverage (94-97%) superior to individual parental bNAbs (70-90%) (Asokan *et al.*, J Virol 89, 12501-12512 (2015)). These empirical combinations were recently improved upon with cross-over dual variable (CODV) technology to develop Tri-NAbs with improved potency and breadth (Xu *et al.*,
15 Science 358, 85-90 (2017)). In addition, using tandem ScFvs format, we engineered a trispecific HIV-1 neutralizing antibody and two tetra-specific antibodies, consisting of functional moieties of three and four HIV bNAbs, respectively (Steinhardt *et al.*, Nat Commun 9, 877 (2018)) (PCT/US2017/057053), which displays elevated neutralization breadth and potency compared to the parental bNAbs.

20 This background information is provided for informational purposes only. No admission is necessarily intended, nor should it be construed, that any of the preceding information constitutes prior art against the present invention.

SUMMARY OF THE INVENTION

25 It is to be understood that both the foregoing general description of the embodiments and the following detailed description are exemplary, and thus do not restrict the scope of the embodiments.

30 Provided herein are multispecific neutralizing antibodies against HIV which in some embodiments utilize a tandem ScFv format to combine five HIV Env bNAb moieties via structure-based rational design. In some embodiments, these antibodies can achieve simultaneous engagement of five separate epitopes by each respective individual

bNAb functional moiety to achieve superior binding avidity, profoundly enhanced viral inhibition breadth and potency, and ADCC functions.

By targeting >3 Env epitopes, these multi-NAbs display near pan-isolate neutralization breadth (99.6% coverage), high potency (GMT IC₅₀ = 0.006 µg/mL) as assessed by a 208 virus panel and effectively neutralize viral quasi-species isolated from VRC01 clinical trials that are frequently resistant to VRC01. Furthermore, the data herein suggest that these multi-NAbs possess substantially higher antibody-dependent cell-mediated cytotoxicity (ADCC) capacity than their parental bNAbs. Taken together, the data suggest that multi-NAbs may be used as a novel candidate format for the treatment and management of persistent HIV-1 infection.

In some embodiments, the present disclosure describes synergistically combined epitope-binding moieties from five bNAbs that target the CD4 binding site, V2 and V3 conserved glycans, gp120/gp41 interface, as well as the membrane exterior proximal region into a 'single' penta-specific antibody (penta-NAb). The penta-Nabs exhibited superior degrees of inhibition against pseudo-typed viruses, exceptional neutralization capacity over individual bNAbs, and the capacity to neutralize replication-competent HIV isolates from infected individuals.

In one aspect, the invention provides a multispecific anti-HIV antibody that binds to multiple epitopes on HIV envelope protein, wherein the antibody comprises:

- i. an amino acid sequence that binds to a V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to a V3-base glycan region epitope;
- iii. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to a gp120/gp41 interface epitope; and
- v. an amino acid sequence that binds to a membrane proximal external region (MPER) epitope.

In some embodiments, the amino acid sequences of parts i-v) comprise amino acid sequences of single chain fragment variable (ScFv) moieties, wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody. In some
 5 embodiments, the VL and VH sequences are separated by one or more linking amino acids. In some embodiments, the linking amino acids comprise one or more tetra-glycine serine (G4S) linkers. In some embodiments, the antibody further comprises an Fc region of an immunoglobulin or a variant thereof. In some embodiments, the antibody comprises a first and second polypeptide chain, wherein the first and second polypeptide
 10 chains each comprise five ScFv moieties, wherein each ScFv moiety on a single chain recognizes an individual epitope, wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody and an Fc region of an immunoglobulin or a variant thereof.

In some embodiments, the amino acid sequence that binds to the epitope of the V1/V2-glycan region comprises an amino acid sequence from an antibody selected from the group consisting of VRC26.25 and PGDM1400; the amino acid sequence that binds to the epitope of the V3-glycan region comprises an amino acid sequence from antibody PGT121; the amino acid sequence that binds to the epitope of the CD4-binding site
 20 (CD4bs) comprises an amino acid sequence from antibody N6; the amino acid sequence that binds to the epitope of the gp120/gp41 interface comprises an amino acid sequence from antibody 35O22; and the amino acid sequence that binds to the epitope of the membrane proximal external region (MPER) comprises an amino acid sequence from an antibody selected from the group consisting of 10E8v4, 10E8v4_S100cF, and
 25 10E8v4_V5R_S100cF.

In some embodiments, the amino acid sequence from the antibody VRC26.25 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises QFRFDGYG, CDR H2 comprises ISHDGIKK and CDR H3 comprises
 30 AKDLREDECEEWSDDFGKQLPCA KSRGGLVGIADN; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR

L1 comprises TSNIGNNF, CDR L2 comprises ETD and CDR L3 comprises ATWAASLSSARV;

the amino acid sequence from the antibody PGDM1400 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein
5 CDR H1 comprises GNTLKTYD, CDR H2 comprises ISHEGDKK and CDR H3 comprises AKGSKHRLRDYALDDD GALNWAVD VDYLSNLEF; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises HSLIHGDRNNY, CDR L2 comprises LAS and CDR L3 comprises MQGRES PWT;

10 the amino acid sequence from the antibody PGT121 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GASISDSY, CDR H2 comprises VHKS GDT and CDR H3 comprises ARTLHGRR IYGIVAFNEWFTYFYMDV; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises
15 SLGSRA, CDR L2 comprises NNQ and CDR L3 comprises HIWDSRVPTKWV;

the amino acid sequence from the antibody N6 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYTFTAHI, CDR H2 comprises IKPQYGAV and CDR H3 comprises AR; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR
20 L3, wherein CDR L1 comprises QGVGSD, CDR L2 comprises HTS and CDR L3 comprises QVLQF;

the amino acid sequence from the antibody 35O22 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYRFNFYH, CDR H2 comprises ISPYS GDK and CDR H3
25 comprises DDTGTYFCAKGLLRDGSSTWLPYL; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises NSVCCSHKS, CDR L2 comprises EDN and CDR L3 comprises CSYTHNSGCV;

the amino acid sequence from the antibody 10E8v4 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein
30 CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3

comprises TGYFFCARTGKYYDFWSGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV;

5 the amino acid sequence from the antibody 10E8v4_S100cF comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEWWSV and CDR H3 comprises TGYFFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1
10 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV;

the amino acid sequence from the antibody 10E8v4_V5R_S100cF comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEWWSV and
15 CDR H3 comprises TGYFFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV.

In another aspect, the invention provides one or more vectors comprising a nucleic
20 acid encoding the antibody or polypeptide of the invention.

In another aspect, the invention provides a cell comprising the one or more vectors of the invention.

In another aspect, the invention provides an engineered cell that expresses an antibody of the invention. In some embodiments, the cell is an immune cell. In some
25 embodiments, the cell is a B cell.

In another aspect, the invention provides a pharmaceutical composition comprising an antibody of the invention and/or cells of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a method for treating or preventing HIV
30 infection in a subject, comprising administering to the subject an effective amount of a

composition comprising an effective amount of a multispecific antibody as described herein.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE FIGURES

10 The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

FIG. 1. HIV-1 Env major epitope clusters and their spatial relationships.

FIG. 2. Schematic of bNAb entities linked together in tandem to form PentaNAb 2.0. (A) Topology of PentaNAb design: from the Env MPER to the Env apex, with the shortest distance between bNAb entities marked. Modeling of Env trimer BG505 SOSIP.664 binding to five bNAbs targeting the five conserved epitopes including the V1V2 glycan, V3 base glycan, CD4 binding site (CD4bs), gp120/gp41 interface, and membrane proximate exterior region (MPER) simultaneously by superimposing individual bNAb Fab-Env complex structure, only VH-VL regions of Fab are shown. Env trimer in grey, and the bNAb moieties in color; (B) Scheme of the PentaNAb design, with MPER bNAb at the N terminus and V1V2 bNAb connected with CH2-CH3 domains of IgG Fc. Strategies of engineering penta-ScFv by joining the termini of VHs and VLs by linkers in dash lines optimized to accommodate individual VHs and VLs to achieve simultaneous epitope engagement; (C) Linear presentation of PentaNAb 2.0 design.

FIG. 3. Summary of neutralization breadth and potency of PentaNAb2.0 in a comprehensive panel of viruses, in comparison with other multi-specific antibody designs. (A) Comparison of potency-breadth curves and epitope specificities of Tri- and Penta-NAb against a panel of 208 circulating primary HIV-1 strains; (B) Heat maps of IC50, breadth and potency are shown as in (A) and Fig. 4. GMT, geometric mean of titer

(IC₅₀). The IC₅₀ was adjusted to account for the molarity difference between the antibodies.

FIG. 4. (A) IC₅₀ values (μg/ml) for PentaNAb2.0 and other antibodies tested in a 208 virus panel. The IC₅₀ was adjusted to account for the molarity difference between the antibodies. (B) Summary of IC₅₀ values for PentaNAb2.0 and other antibodies.

FIG. 5. Neutralization capacity of PentaNAbs tested against primary HIV-1 viruses isolated from patients involved in VRC01 therapy treatment. Neutralization of virus from patient V07-RD (A), and V09-RD (B), tested with antibodies at 2.5 μg/ml (left) and 10 μg/ml (right), respectively. (C) Summary of IC₅₀ / IC₈₀ (μg/ml) for PentaNAbs tested against primary VRC01 resistant isolates. Neutralization IC₅₀ and IC₈₀ titers (μg/ml) of Penta-scFv and Penta-NAb IgG against HIV isolated from VRC01 clinical trial. The IC₅₀ and IC₈₀ values were adjusted to account for the molarity difference between the antibodies.

FIG. 6. Schemes of various iterations in which bNAb entities can be linked together in tandem to form PentaNAbs in the “Reverse” or “Forward/Reverse” orientation. (A) The “reverse” orientation of the PentaNAb design, in contrast to that in Fig. 2; (B) The PentaNAb “Forward/Reverse” orientation configuration conferred by IgG Fc bearing “knob-into-hole” mutations to form heterodimer.

FIG. 7. Comparison of PentaNAbs in both the Forward, Reverse and Forward/Reverse orders. (A) SDS PAGE gel analysis for PentaNAbs; (B) Summary of neutralization breadth and potency of PentaNAbs against a 26-virus panel of high stringency; (C) Neutralization IC₅₀ values of PentaNAbs against the 26-virus panel. The IC₅₀ was adjusted to account for the molarity difference between the antibodies.

FIG. 8. (A-C) Spatial distance of bNAb functional moieties.

FIG. 9. Bi-ScFv and bi-NAb of VRC01-PGT121 design and EM analysis. (A) Schematic presentation of VRC01-PGT121 ScFv and full length IgG. (B) EM analysis of Env trimer BG505 SOSIP.664 and VRC01-PGT121 ScFv complex suggests bi-specific binding mode. Left, representative EM image of Env trimer BG505 SOSIP.664 and VRC01-PGT121 bi-ScFv complex. Arrows indicate ScFv molecules. Right, EM image of PGV04 (green) and PGT122 (blue) Fab complex with BG505 SOSIP.664 simultaneously.

FIG. 11A-B. (A) Design of PentaNAb4.0 Forward ScFV (FIG. 11A) and PentaNAb 4.0 Reverse ScFv (FIG. 11B). FIG. 12A-B. Design of a multi-specific antibody HexaNAb 1.0 ScFv which contains PentaNAb4.0 Reverse ScFv with variable domains of dB4C7/UB-421, an anti-CD4 antibody.

FIG. 14A-B depicts the neutralization profiles of PentaNAb2.0 Reverse ScFv, PentaNAb4.0 Forward ScFv, PentaNAb4.0 Reverse ScFv, HexaNAb 1.0 ScFv and PentaNAb4.0 Stem HC LS v2 IgG tested with a 208-virus panel (Fig. 14A & B). The PentaNAb2.0 Reverse ScFv has incremental improvement in potency (IC 50 geometric mean) compared to the initial design, PentaNAb2.0 ScFv. The HexaNAb 1.0 ScFv (FIG. 12) shows 100% virus neutralization breadth and promising potency (IC50 geometric mean ~0.005 ug/mL) (Fig. 14 A & B). The PentaNAb4.0 Stem HC LS v2 IgG demonstrates 99.5% virus coverage (207/208 viruses tested) and high potency (IC50 geometric mean ~0.02 ug/mL) (Fig. 14A & B).

25 DETAILED DESCRIPTION OF THE INVENTION

- 11 -

HIV-1 envelope protomers and demonstrates superior neutralization breadth over its parental bNAbs. The epitopes recognized are the V1/V2 glycan region, the V3-glycan region, the CD4-binding site (CD4bs), the gp120-gp41 interface, and the gp41 membrane proximal external region (MPER). Furthermore, the present disclosure shows that using an Fc moiety to combine two penta-ScFv molecules that recognize the same series of HIV-1 epitopes in either the forward or reverse orientation resulted in a penta-specific bNAb, which displays near-pan neutralization breadth potently. Thus, penta-specific antibodies combining functional moieties of Env bNAbs could achieve exceptional neutralization capacity with profoundly augmented avidity. The penta-specific antibodies described herein can be used in studies aimed at preventing HIV disease progression or mother to child transmission, and curing HIV. Furthermore, the approach described herein, that combines multi-functional moieties of individual bNAbs with profoundly elevated avidity and cooperative effect of multivalence interactions, may be applied to generate superior antibody-based anti-viral therapeutics against other infectious agents.

For the purpose of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with the usage of that word in any other document, including any document incorporated herein by reference, the definition set forth below shall always control for purposes of interpreting this specification and its associated claims unless a contrary meaning is clearly intended (for example in the document where the term is originally used). The use of "or" means "and/or" unless stated otherwise. As used in the specification and claims, the singular form "a," "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof. The use of "comprise," "comprises," "comprising," "include," "includes," and "including" are interchangeable and not intended to be limiting. Furthermore, where the description of one or more embodiments uses the term "comprising," those skilled in the art would understand that, in some specific instances, the embodiment or embodiments can be alternatively described using the language "consisting essentially of" and/or "consisting of."

Abbreviations for amino acids are used throughout this disclosure and follow the standard nomenclature known in the art. For example, as would be understood by those of

ordinary skill in the art, Alanine is Ala or A; Arginine is Arg or R; Asparagine is Asn or N; Aspartic Acid is Asp or D; Cysteine is Cys or C; Glutamic acid is Glu or E; Glutamine is Gln or Q; Glycine is Gly or G; Histidine is His or H; Isoleucine is Ile or I; Leucine is Leu or L; Lysine is Lys or K; Methionine is Met or M; Phenylalanine is Phe or F; Proline is Pro or P; Serine is Ser or S; Threonine is Thr or T; Tryptophan is Trp or W; Tyrosine is Tyr or Y; and Valine is Val or V.

As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used.

The term "broad neutralizing antibody" refers to an antibody which inhibits HIV-1 infection, as defined by at least about 50% inhibition of infection in vitro, in more than 50%, 60%, 70%, 80%, 90%, 95%, 99% or greater, of a large panel of (greater than 100) HIV-1 envelope pseudotyped viruses and/or viral isolates. In some embodiments, the broad neutralizing antibody is an antibody that inhibits HIV-1 infection as defined by at least about 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% inhibition of infection in vitro in more than about 50%, 60%, 70%, 80%, 90%, 95%, 99% or greater, of a large panel of (greater than 100) HIV-1 envelope pseudotyped viruses and/or viral isolates. In some embodiments, the disclosure relates to a composition comprising one or a plurality of broad neutralizing antibodies.

As used herein, the term "in combination with," is not intended to imply that the therapy or the therapeutic agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope described herein.

The therapeutic agents can be administered concurrently with, prior to, or subsequent to, one or more other additional therapies or therapeutic agents.

The term "antibody" means a molecule that recognizes and specifically binds to a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within a variable region of the molecule, or any functional fragment, mutant, variant, or derivative thereof which retains the epitope binding features of an immunoglobulin molecule. As used herein, the term "antibody" encompasses intact polyclonal antibodies, intact

monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')₂, and Fv fragments, dual affinity retargeting antibodies (DART)), single chain Fv (scFv) mutants, multispecific antibodies such as pentaspecific antibodies generated from at least five intact immunoglobulins, chimeric antibodies, humanized antibodies, human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity.

In some embodiments, the antibody can comprise a sequence from any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. The different classes of immunoglobulins have different and well known subunit structures and three-dimensional configurations. Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, etc.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is generally composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g.,

threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the invention do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen(s), i.e., the envelope protein to which the polypeptide or antibody binds. Methods of identifying nucleotide and amino acid conservative substitutions which do not eliminate antigen binding are well-known in the art (see, e.g., Brummell *et al.*, Biochem. 32: 1180-1187 (1993); Kobayashi *et al.*, Protein Eng. 12(10):879-884 (1999); and Burks *et al.*, Proc. Natl. Acad. Sci. USA 94:412-417 (1997)).

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. In some embodiments, there are three CDRs in each of the variable regions of the heavy chain and the light chain of an antibody, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987) and Chothia *et al.*, Nature 342:877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as LI, L2 and L3 or HI, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or

experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

5 As used herein, the term "fragment" is defined as a physically contiguous portion of the primary structure of a biomolecule. In the case of polypeptides, a fragment may be defined by a contiguous portion of the amino acid sequence of a protein and may be at least 3-5 amino acids, at least 6-10 amino acids, at least 11-15 amino acids, at least 16-24 amino acids, at least 25-30 amino acids, at least 30-45 amino acids and up to the full
10 length of the protein minus a few amino acids. In the case of polynucleotides, a fragment is defined by a contiguous portion of the nucleic acid sequence of a polynucleotide and may be at least 9-15 nucleotides, at least 15-30 nucleotides, at least 31-45 nucleotides, at least 46-74 nucleotides, at least 75-90 nucleotides, and at least 90-130 nucleotides. In some embodiments, fragments of bio molecules are immunogenic fragments.

15 In some embodiments, the term "functional fragment" means any portion of a polypeptide or amino acid sequence that is of a sufficient length to retain at least partial biological function that is similar to or substantially similar to the parental polypeptide or amino acid sequence upon which the fragment is based. If the fragment is a functional fragment of an antibody or antibody- like molecule, the fragment can possess a binding
20 avidity for one or a plurality of antigens. In some embodiments, a functional fragment is a polypeptide that comprises 80, 85, 90, 95, 96, 97, 98, or 99% sequence identity of any variable region of a polypeptide antibody disclosed herein and has sufficient length to retain at least partial binding affinity to one or a plurality of antigens that bind to the amino acid sequence. In some embodiments, the fragment has a length of at least about
25 10, about 20, about 30, about 40, about 50 , about 60, about 70, about 80, about 90, or about 100 contiguous amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed herein and has a length of at least about 50 amino acids. In some embodiments, the fragment has a length of at least about 100, 150, 200, 250, 300, 350, 400, 450, 500, or 550 amino acids.

30 The term "antigen binding portion" or "antigen binding fragment" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or

more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g. , HIV gp120). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments can be multi-specific formats; specifically binding to two or more different antigens.

5 Examples of binding fragments encompassed within the term "antigen-binding portion" or "antigen binding fragment" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CHI domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CHI domains; (iv) a Fv fragment
10 consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al, (1989) Nature 341 :544-546, Winter *et al.*, PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be
15 joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird *et al.* (1988) Science 242:423-426; and Huston *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding
20 portion" or "antigen binding fragment" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain
25 and creating two antigen binding sites (see e.g., Holliger, P., *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R.J., *et al.* (1994) Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer- Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

The term "multispecific antibody" refers to an antibody or antibody-like molecule,
30 or fragment thereof, capable of binding two or more related or unrelated targets, or antigens. Antibody specificity refers to selective recognition of the antibody for a particular epitope, or amino acid sequence, of an antigen. Natural antibodies, for example,

are monospecific. Pentaspecific antibodies are antibodies which have five different antigen-binding specificities.

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three-dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

The term "antigen" refers to a polypeptide that can stimulate the production of antibodies or a T cell response in an animal, including polypeptides that are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity.

The term "HIV Envelope protein (Env)" refers to the glycoprotein that is found on the surface of HIV. The HIV envelope protein is initially synthesized as a longer precursor protein of 845-870 amino acids in size, designated gp160. gp160 forms a homotrimer and undergoes glycosylation within the Golgi apparatus. In vivo, it is then 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). gp41 contains a transmembrane domain and remains in a trimeric configuration within the membrane of the virus or the membrane of a host cell; it interacts with gp120 in a noncovalent manner. The mature gp120 wildtype polypeptides 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 polypeptide is comprised of five conserved regions (C1-05) and five regions of high variability (V1-V5). Exemplary sequence of wt gp120 polypeptides are shown on GENBANK, for example accession numbers AAB05604 and AAD12142 (as available on Oct. 16, 2009), 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 gp120 core has a molecular structure, which includes two domains: an "inner" domain (which faces gp41) and an

"outer" domain (which is mostly exposed on the surface of the oligomeric envelope glycoprotein complex). The two gp120 domains are separated by a "bridging sheet" that is not part of either domain. The gp120 core includes 25 beta strands, 5 alpha helices, and 10 defined loop segments.

5 The term "CD4 binding site (CD4BS) antibodies" refers to antibodies that bind to the CD4 binding surface of a gp120 polypeptide. The antibodies interfere with or prevent CD4 from binding to a gp120 polypeptide.

 The term "V3 loops" refers to a loop of about 35 amino acids critical for the binding of the co-receptor and determination of which of the co-receptors will bind. In
10 certain examples the V3 loop includes residues 296-331.

 The term "membrane-proximal external region or MPER" refers to a highly conserved region of the gp41 envelope protein. The MPER comprises the last 24 C-terminal amino acids of the gp41 ectodomain, LLELDKWASLWNWF(N/D)ITNWLWYIK (aa 660 to 683) (Zwick *et al.* J Virol. 2005
15 Jan; 79(2): 1252-61).

 The term "V1V2 glycan antibody" refers to antibodies that bind to the V1V2 apex and associated conserved glycans N160 and/or N156.

 The term "gp120/gp41 interface antibody" refers to antibodies that bind to a conserved epitope that stretches across gp120 and gp41.

20 The term "vector," as used herein, is intended to refer to a nucleic acid molecule. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are
25 introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

 Moreover, certain vectors are capable of directing the expression of genes to
30 which they are operatively linked. Such vectors are referred to herein as "recombinant

expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the disclosure is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

"Polynucleotide" or "nucleic acid" as used interchangeably herein, refers to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. A sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-

5 methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, .alpha.-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative
10 linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(0)S ("thioate"), P(S)S ("dithioate"), (0)NR₂ ("amidate"), P(0)R, P(0)OR, CO, or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20C) optionally containing an ether (—O—) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in
15 a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

The term "host cell" as used herein is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell.
20 Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. The host cells can include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. In some embodiments, eukaryotic cells include protist, fungal, plant
25 and animal cells. In some embodiments, host cells include but are not limited to the prokaryotic cell line *E. coli*; mammalian cell lines CHO, HEK 293 and COS; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*. In some embodiments, the host cell is an immune cell.

Standard techniques may be used for recombinant DNA, oligonucleotide
25 synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and
30 more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

5 The term "inhibit" and its various grammatical forms is used to refer to a restraining, blocking, or limiting of the range or extent of a certain biological event or effect.

10 The term "effective amount," is used herein to include the amount of an agent (e.g. a multispecific antibody) that, when administered to a patient for treating an infection, is sufficient to effect treatment of the disease (e.g. , by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease or its related comorbidities). The "effective amount" may vary depending on the agent, how it is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated. An effective amount includes an amount that results in a clinically relevant change or
15 stabilization, as appropriate, of an indicator of a disease or condition. "Effective amount" refers to an amount of a compound, material, or composition, as described herein effective to achieve a particular biological result such as, but not limited to, biological results disclosed, described, or exemplified herein. Such results may include, but are not limited to, the effective reduction of symptoms associated with any of the disease states
20 mentioned herein, as determined by any means suitable in the art. The effective amount of the composition may be dependent on any number of variables, including without limitation, the species, breed, size, height, weight, age, overall health of the subject, the type of formulation, the mode or manner of administration, the type and/or severity of the particular condition being treated, or the need to modulate the activity of the molecular
25 pathway induced by association of the analog to its receptor. The appropriate effective amount can be routinely determined by those of skill in the art using routine optimization techniques and the skilled and informed judgment of the practitioner and other factors evident to those skilled in the art. An effective dose of the antibodies or mutants or variants described herein may provide partial or complete biological activity as compared
30 to the biological activity induced by the wild-type or naturally occurring polypeptides upon which the antibodies or mutants or variants are derived. A therapeutically effective dose of the antibodies or mutants or variants described herein may provide a sustained

biochemical or biological effect and/or an increased resistance to degradation when placed in solution as compared with the normal affect observed when the naturally occurring and fully processed translated protein is administered to the same subject.

5 An "immunoconjugate" is an antibody or multispecific antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small
10 molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

The term "administer" as used herein means to give or to apply. The term "administering" as used herein includes in vivo administration.

The term "linker" refers to a chemical moiety that connects one peptide sequence
15 to another, e.g., one antibody or antigen binding fragment to another. Linkers can also be used to attach antibodies to labels or solid substrates. A linker can include amino acids. Linkers can be straight or branched, saturated or unsaturated carbon chains. They can also include one or more heteroatoms within the chain.

The term "pharmaceutical composition" refers to a preparation which is in such
20 form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition would be administered. A pharmaceutical composition of the present disclosure can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will
25 vary depending upon the desired results. To administer an antibody according to the disclosure by certain routes of administration, it may be necessary to coat the antibody with, or coadminister the antibody with, a material to prevent its inactivation. For example, the antibody may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and
30 aqueous buffer solutions.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. Pharmaceutically acceptable carriers includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion).

The pharmaceutical compositions according to the disclosure can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

The term "subject" is used throughout the specification to describe an animal to which one or more compositions comprising the antibody or antibodies disclosed herein. In some embodiment, the subject is a human. The term "subject" and "patient" may be interchangeably used. In some instances in the description of the present disclosure, the term "patient" will refer to human patients suffering from a particular disease or disorder. In some embodiments, the subject may be a human suspected of having or being identified as at risk to develop HIV infection. In some embodiments, the subject is suspected of having or has been diagnosed with HIV or HIV-1 infection or AIDS. In some embodiments, the subject may be a human suspected of having or being identified as at risk to develop AIDS or an AIDS-associated disorder. In some embodiments, the subject may be a mammal. In some embodiments, the subject may be a non-human animal. In one embodiment, the subject is a human, such as a human being treated or assessed for an HIV infection; or a human having an HIV infection that would benefit from a multispecific antibody as described herein. In some embodiments, the subject is in need of the treatment being administered.

In some embodiments, the compositions or pharmaceutical compositions comprise crystalline forms or lyophilized forms of the antibodies, antibody- like molecules or salts thereof.

5 The term "treat" or "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a disease, condition or disorder, substantially ameliorating clinical or esthetical symptoms of a condition, substantially preventing the appearance of clinical or esthetical symptoms of a disease, condition, or disorder, and protecting from harmful or annoying symptoms. The term "treat" or "treating" as used herein further refers to accomplishing one or more of the following: (a) reducing the
10 severity of the disorder; (b) limiting development of symptoms characteristic of the disorder(s) being treated; (c) limiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting recurrence of symptoms in patients that were previously symptomatic for the disorder(s).

15 The term "potency" as used herein refers to the neutralization capacity, i.e. the IC_{50} or IC_{80} of the antibody, or fragment thereof. The term " IC_{50} " as used herein refers to the concentration of an inhibitor, such as a multispecific antibody, where the response or biological activity is reduced by half. The term " IC_{80} " as used herein refers to the concentration of an inhibitor (e.g. a multispecific antibody) where the response or
20 biological activity is reduced by eighty percent.

Humanization and primatization refer to in cases where the multi-specific antibodies or the five antibodies forming the multi-specific fusion antibody are non-human antibodies, the antibody can be "humanized" to reduce immunogenicity to a human recipient. Methods for humanizing non-human antibodies have been described in
25 the art. See, e.g., Jones et al., Nature 321 :522-525 (1986); Riechmann et al, Nature 332:323-327 (1988); Verhoeyen et al., Science 239: 1534-1536 (1988), and U.S. Pat. No. 4,816,567. Generally, residues from the variable domain of a non-human antibody are "imported" into a human immunoglobulin molecule, resulting in antibodies in which some hypervariable region residues and possibly some FR residues of a human antibody
30 are substituted by residues from analogous sites of non-human antibodies. It is important to humanize a non-human antibody while retaining high affinity for the antigen. To this

end, three dimensional immunoglobulin models are commonly available and suitable for use in analyzing proposed humanized sequences in comparison to the parental non-human antibodies. Such analysis permits identification of residues likely involved in recognition and binding of the antigen, and therefore rational design of humanized sequences that
5 retain the specificity and affinity for the antigen.

In some embodiments, multispecific antibodies are formed from anti-HIV human or humanized antibodies. Similarly, a penta-specific fusion antibody or the five antibodies forming the fusion can be "primatized" to reduce immunogenicity to another primate, non-human recipient, e.g., a rhesus recipient. Residues from the variable domain
10 of a donor antibody (such as a non-primate antibody or an antibody of a primate species different from the recipient primate) are "imported" into a nonhuman primate recipient immunoglobulin molecule, resulting in antibodies in which some hypervariable region residues and possibly some FR residues of a nonhuman primate antibody are substituted by residues from analogous sites of donor antibodies. Alternatively, primatized antibodies
15 can be made for use in a desirable primate species by using a recipient immunoglobulin having non-primate sequences or sequences from a different primate species by introducing the Fc fragment, and/or residues, including particularly framework region residues, from the desirable primate, into the recipient immunoglobulin.

By "affinity maturation" is meant when one or more hypervariable region residues
20 of an antibody can be substituted to select for variants that have improved biological properties relative to the parent antibody by employing, e.g., affinity maturation using phage or yeast display. For example, the Fab region of an anti-HIV antibody can be mutated at several sites selected based on available structural information to generate all possible amino substitutions at each site. The antibody variants thus generated are
25 displayed in a monovalent fashion from phage particles or on the surface of yeast cells. The displayed variants are then screened for their biological activity (e.g. binding affinity).

Anti-HIV Antibodies

In some embodiments, the invention provides multispecific antibodies that are
30 broadly neutralizing antibodies against HIV.

HIV-1 is among the most genetically diverse viral pathogens. Of the three main branches of the HIV-1 phylogenetic tree, the M (main), N (new), and O (outlier) groups, group M viruses are the most widespread, accounting for over 99% of global infections. This group is presently divided into nine distinct genetic subtypes, or clades (A through K), based on full-length sequences. Env is the most variable HIV-1 gene, with up to 35% sequence diversity between clades, 20% sequence diversity within clades, and up to 10% sequence diversity in a single infected person (Shankarappa, R. *et al.* 1999. *J. Virol.* 73:10489-10502). Clade B is dominant in Europe, the Americas, and Australia. Clade C is common in southern Africa, China, and India and presently infects more people worldwide than any other clade (McCutchan, F E. 2000. Understanding the genetic diversity of HIV-1. *AIDS* 14(Suppl. 3):S31-S44). Clades A and D are prominent in central and eastern Africa.

In some embodiments, the invention provides multispecific antibodies that are broadly neutralizing against HIV. In some embodiments, the antibody has a particularly high potency in neutralizing HIV infection *in vitro* across multiple clades as shown in the Table 1 and Figure 4A herein. In some embodiments, only low concentrations are required in order to neutralize a given amount of virus. This facilitates higher levels of protection while administering lower amounts of antibody.

In some embodiments, the invention provides a multispecific anti-HIV antibody that binds to multiple epitopes on HIV envelope protein, wherein the antibody comprises:

- i. an amino acid sequence that binds to a V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to a V3-base glycan region epitope;
- iii. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to a gp120/gp41 interface epitope; and
- v. an amino acid sequence that binds to a membrane proximal external region (MPER) epitope.

The amino acid sequences of parts i-v) of the multispecific anti-HIV antibody can be present on one or more polypeptide chains. In some embodiments, the amino acid sequences of parts i-v) are present on a single polypeptide chain. In some embodiments, the multispecific anti-HIV antibody comprises two polypeptide chains, with each chain comprising amino acid sequences of parts i-v). In some embodiments, the antibody simultaneously binds the multiple epitopes.

In some embodiments, the multispecific anti-HIV antibody is capable of neutralizing at least 99% of the HIV viruses listed in Table 1 with an IC₅₀ value of less than 50 µg/mL. In some embodiments, the multispecific anti-HIV antibody is capable of neutralizing at least 99.5% of the HIV pseudoviruses listed in Table 1 with an IC₅₀ value of less than 50 µg/mL. In some embodiments, the multispecific anti-HIV antibody has an IC₅₀ geomean of less than or equal to 0.10 µg/ml. In some embodiments, the multispecific anti-HIV antibody has an IC₅₀ geomean of less than or equal to 0.072 µg/ml. In some embodiments, the multispecific anti-HIV antibody has an IC₅₀ geomean of less than or equal to 0.006 µg/ml.

Virus ID	Clade
0260.v5.c36	A
0330.v4.c3	A
0439.v5.c1	A
3365.v2.c20	A
3415.v1.c1	A
3718.v3.c11	A
398-F1_F6_20	A
BB201.B42	A
BB539.2B13	A
BG505.W6M.C2	A
BI369.9A	A
BS208.B1	A
KER2008.12	A
KER2018.11	A
KNH1209.18	A
MB201.A1	A
MB539.2B7	A
MI369.A5	A
MS208.A1	A
Q23.17	A
Q259.17	A
Q769.d22	A

Q769.h5	A
Q842.d12	A
QH209.14M.A2	A
RW020.2	A
UG037.8	A
246-F3.C10.2	AC
3301.V1.C24	AC
3589.V1.C4	AC
6540.v4.c1	AC
6545.V4.C1	AC
0815.V3.C3	ACD
6095.V1.C10	ACD
3468.V1.C12	AD
Q168.a2	AD
Q461.e2	AD
620345.c1	AE
BJOX009000.02.4	AE
BJOX010000.06.2	AE
BJOX025000.01.1	AE
BJOX028000.10.3	AE
C1080.c3	AE
C2101.c1	AE
C3347.c11	AE
C4118.09	AE
CM244.ec1	AE
CNE3	AE
CNE5	AE
CNE55	AE
CNE56	AE
CNE59	AE
CNE8	AE
M02138	AE
R1166.c1	AE
R2184.c4	AE
R3265.c6	AE
TH023.6	AE
TH966.8	AE
TH976.17	AE
235-47	AG
242-14	AG
263-8	AG
269-12	AG
271-11	AG
928-28	AG
DJ263.8	AG

T250-4	AG
T251-18	AG
T253-11	AG
T255-34	AG
T257-31	AG
T266-60	AG
T278-50	AG
T280-5	AG
T33-7	AG
3988.25	B
5768.04	B
6101.10	B
6535.3	B
7165.18	B
45_01dG5	B
89.6.DG	B
AC10.29	B
ADA.DG	B
Bal.01	B
BaL.26	B
BG1168.01	B
BL01.DG	B
BR07.DG	B
BX08.16	B
CAAN.A2	B
CNE10	B
CNE12	B
CNE14	B
CNE4	B
CNE57	B
HO86.8	B
HT593.1	B
HXB2.DG	B
JRCSF.JB	B
JRFL.JB	B
MN.3	B
PVO.04	B
QH0515.01	B
QH0692.42	B
REJO.67	B
RHPA.7	B
SC422.8	B
SF162.LS	B
SS1196.01	B
THRO.18	B

TRJO.58	B
TRO.11	B
WITO.33	B
X2278.C2.B6	B
YU2.DG	B
BJOX002000.03.2	BC
CH038.12	BC
CH070.1	BC
CH117.4	BC
CH119.10	BC
CH181.12	BC
CNE15	BC
CNE19	BC
CNE20	BC
CNE21	BC
CNE40	BC
CNE7	BC
286.36	C
288.38	C
0013095-2.11	C
001428-2.42	C
0077_V1.C16	C
00836-2.5	C
0921.V2.C14	C
16055-2.3	C
16845-2.22	C
16936-2.21	C
25710-2.43	C
25711-2.4	C
25925-2.22	C
26191-2.48	C
3168.V4.C10	C
3637.V5.C3	C
3873.V1.C24	C
426c	C
6322.V4.C1	C
6471.V1.C16	C
6631.V3.C10	C
6644.V2.C33	C
6785.V5.C14	C
6838.V1.C35	C
96ZM651.02	C
BR025.9	C
CAP210.E8	C
CAP244.D3	C

CAP256.206.C9	C
CAP45.G3	C
Ce1176.A3	C
CE703010217.B6	C
CNE30	C
CNE31	C
CNE53	C
CNE58	C
DU123.06	C
DU151.02	C
DU156.12	C
DU172.17	C
DU422.01	C
MW965.26	C
SO18.18	C
TV1.29	C
TZA125.17	C
TZBD.02	C
ZA012.29	C
ZM106.9	C
ZM109.4	C
ZM135.10a	C
ZM176.66	C
ZM197.7	C
ZM214.15	C
ZM215.8	C
ZM233.6	C
ZM249.1	C
ZM53.12	C
ZM55.28a	C
3326.V4.C3	CD
3337.V2.C6	CD
3817.v2.c59	CD
191821.E6.1	D
231965.c1	D
247-23	D
3016.v5.c45	D
57128.vrc15	D
6405.v4.c34	D
A03349M1.vrc4a	D
A07412M1.vrc12	D
NKU3006.ec1	D
UG021.16	D
UG024.2	D
P0402.c2.11	G

P1981.C5.3	G
X1193.c1	G
X1254.c3	G
X1632.S2.B10	G
X2088.c9	G
X2131.C1.B5	G

Table 1. List of HIV-1 Pseudovirus Panel: 208 Total Pseudoviruses Included.

In some embodiments, the amino acid sequences of parts i-v) comprise amino acid sequences of single chain fragment variable (ScFv) moieties, wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody. In some embodiments, one or more of the ScFv moieties is organized such that the VL is at the amino terminal end of the ScFv moiety and the VH is at the carboxy terminal end of the ScFv moiety. In some embodiments, one or more of the ScFv moieties is organized such that the VH is at the amino terminal end of the ScFv moiety and the VL is at the carboxy terminal end of the ScFv moiety. In some embodiments, each ScFv moiety is organized such that the VL is at the amino terminal end of the ScFv moiety and the VH is at the carboxy terminal end of the ScFv moiety.

In some embodiments, the amino acid sequences that bind to the various epitope are separated from each other by one more linking amino acids. In some embodiments, the VL and VH sequences are separated by one or more linking amino acids. The linking amino acid sequences are not limiting. In some embodiments, the linking amino acids comprise one or more tetra-glycine serine (G₄S) linkers. In some embodiments, the VL and VH sequences are separated by 1-10 tetra-glycine serine (G₄S) linkers. In some embodiments, the VL and VH sequences are separated by 3 tetra-glycine serine (G₄S) linkers.

In some embodiments, one or more of the polypeptide chains of the multispecific anti-HIV antibody can further comprises an Fc region of an immunoglobulin or a variant thereof. In some embodiments, the immunoglobulin is IgG. In some embodiments, the immunoglobulin is IgG1. In some embodiments, the antibody comprises an IgG1 Fc region variant comprising mutations corresponding to M428L and N434S. In some

embodiments, the antibody comprises an immunoglobulin Fc region that has been modified to facilitate heterodimerization.

In some embodiments, the antibody further comprises a sequence tag that facilitates purification of the antibody. In some embodiments, the sequence tag comprises
5 a polyhistidine tag.

In some embodiments, the multispecific anti-HIV antibody comprises a first and second polypeptide chain, wherein the first and second polypeptide chains each comprise

- 10 a. five ScFv moieties, wherein each ScFv moiety on a single chain recognizes an individual epitope (V1/V2 apex glycan, V3-base glycan region, CD4 binding site (CD4bs), gp120/gp41 interface, membrane proximal external region (MPER), wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody; and
- 15 b. an Fc region of an immunoglobulin or a variant thereof.

In some embodiments, the ScFv moieties are separated on the polypeptide chain by one or more linking amino acids. In some embodiments, the linking amino acids comprise one or more tetra-glycine serine (G₄S) linkers. In some embodiments, each ScFv moiety is separated by 5 tetra-glycine serine (G₄S) linkers.

20 In some embodiments, the amino acid sequences of parts i-v) are organized on a single polypeptide chain in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
- 25 ii. an amino acid sequence that binds to the V3-base glycan region epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the gp120/gp41 interface epitope; and

- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope.

In some embodiments, the amino acid sequences of parts i-v) are organized on a single polypeptide chain in the following order, from its N-terminus to its C-terminus:

- 5 i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
- ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- 10 iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the V3-base glycan region epitope; and
- v. an amino acid sequence that binds to the V1/V2 apex glycan epitope.

15 In some embodiments, the multispecific anti-HIV antibody comprises a first and second polypeptide chain, wherein the amino acid sequences of parts i-v) are organized in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
- 20 ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- 25 iv. an amino acid sequence that binds to the V3-base glycan region epitope;
- v. an amino acid sequence that binds to the V1/V2 apex glycan epitope; and
- vi. an Fc region of an immunoglobulin or a variant thereof.

In some embodiments, the multispecific anti-HIV antibody comprises a first and second polypeptide chain, wherein the amino acid sequences of parts i-v) are organized in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to the V3-base glycan region epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope; and
- vi. an Fc region of an immunoglobulin or a variant thereof.

In some embodiments, the multispecific anti-HIV antibody comprises

- 1) a first polypeptide chain, wherein the amino acid sequences of parts i-v) are organized in the following order, from its N-terminus to its C-terminus:
 - i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
 - ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;
 - iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
 - iv. an amino acid sequence that binds to the V3-base glycan region epitope;
 - v. an amino acid sequence that binds to the V1/V2 apex glycan epitope; and
 - vi. an Fc region of an immunoglobulin or a variant thereof; and
- 2) a second polypeptide chain, wherein the amino acid sequences of parts i-v) are organized in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to the V3-base glycan region epitope;
- 5 iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope; and
- 10 vi. an Fc region of an immunoglobulin or a variant thereof.

In some embodiments, the amino acid sequence that binds to the epitope of the V1/V2-glycan region comprises an amino acid sequence from an antibody selected from the group consisting of VRC26.25 and PGDM1400; the amino acid sequence that binds to the epitope of the V3-glycan region comprises an amino acid sequence from antibody PGT121; the amino acid sequence that binds to the epitope of the CD4-binding site (CD4bs) comprises an amino acid sequence from antibody N6; the amino acid sequence that binds to the epitope of the gp120/gp41 interface comprises an amino acid sequence from antibody 35O22; and the amino acid sequence that binds to the epitope of the membrane proximal external region (MPER) comprises an amino acid sequence from an antibody selected from the group consisting of 10E8v4, 10E8v4_S100cF, and 10E8v4_V5R_S100cF.

In some embodiments, the amino acid sequence from the antibody VRC26.25 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises QFRFDGYG, CDR H2 comprises ISHDGIKK and CDR H3 comprises AKDLREDECEEWSDDFGKQLPCA KSRGGLVGIADN; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises TSNIGNNF, CDR L2 comprises ETD and CDR L3 comprises ATWAASLSSARV;

In some embodiments, the amino acid sequence from the antibody PGDM1400 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GNTLKTYD, CDR H2 comprises ISHEGDKK and CDR H3 comprises
5 AKGSKHRLRDYALDDDGALNWAVDVDYLSNLEF; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises HSLIHGDRNNY, CDR L2 comprises LAS and CDR L3 comprises MQGRESPT.

In some embodiments, the amino acid sequence from the antibody PGT121
10 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GASISDSY, CDR H2 comprises VHKS GDT and CDR H3 comprises ARTLHGRR IYGIVAFNEWFTYFYMDV; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises SLGSRA, CDR L2 comprises NNQ and CDR L3 comprises
15 HIWDSRVPTKWV.

In some embodiments, the amino acid sequence from the antibody N6 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYTFTAH I, CDR H2 comprises IKPQYGAV and CDR H3 comprises AR; and an amino acid sequence from the VL region comprising CDR L1,
20 CDR L2 and CDR L3, wherein CDR L1 comprises QGVGSD, CDR L2 comprises HTS and CDR L3 comprises QVLQF.

In some embodiments, the amino acid sequence from the antibody 35O22 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYRFNFYH, CDR H2 comprises
25 ISPYS GDK and CDR H3 comprises DDTGTYFCAKGLLRDGSSTWLPYL; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises NSVCCSHKS, CDR L2 comprises EDN and CDR L3 comprises CSYTHNSGCV.

In some embodiments, the amino acid sequence from the antibody 10E8v4
30 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises

ITGPGEGWSV and CDR H3 comprises TGY YFCARTGKYYDFWSGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV.

5 In some embodiments, the amino acid sequence from the antibody 10E8v4_S100cF comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3 comprises TGY YFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL
10 region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV.

In some embodiments, the amino acid sequence from the antibody 10E8v4_V5R_S100cF comprises an amino acid sequence from the VH region comprising
15 CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3 comprises TGY YFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises
20 SSRDKSGSRLSV.

In some embodiments, the amino acid sequence from the antibody VRC26.25 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:1; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein
25 the VL region comprises amino acids SEQ ID NO:5.

In some embodiments, the amino acid sequence from the antibody PGDM1400 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:9; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein
30 the VL region comprises amino acids SEQ ID NO:13.

In some embodiments, the amino acid sequence from the antibody PGT121 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:17; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:21.

In some embodiments, the amino acid sequence from the antibody N6 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:25; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:29.

In some embodiments, the amino acid sequence from the antibody 35O22 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:33; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:37.

In some embodiments, the amino acid sequence from the antibody 10E8v4 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:41; and VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45.

In some embodiments, the amino acid sequence from the antibody 10E8v4_S100cF comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:49; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45.

In some embodiments, the amino acid sequence from the antibody 10E8v4_V5R_S100cF comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:51; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45.

Variable regions and CDR sequences of the antibodies described herein are shown in Table 2, below.

SEQ ID NO	DESCRIPTION	AMINO ACID SEQUENCE
1	VRC26.25 VH	QVQLVESGGGVVQPGTSLRLSCAASQFRFDGYGMHWVRQA PGKGLEWVASISHDGIKKYHAEKVWGRFTISRDN SKNTLY LQMNSLRPEDTALYYCAKDLREDECEEWSSDDFGKQLPCA KSRGGLVGIADNWGQGTMTVTVSS
2	VRC26.25 VH CDR1	<u>QFRFDGYG</u>
3	VRC26.25 VH CDR2	<u>ISHDGIKK</u>
4	VRC26.25 VH CDR3	<u>AKDLREDECEEWSSDDFGKQLPCA</u> KSRGGLVGIADN
5	VRC26.25 VL	QSVLTQPPSVSAAPGQKVTISCSGNTSNIGNNFVSWYQQR PGRAPQLLIYETDKRPSGIPDRFSASKSGTSGTLAITGLQ TGDEADYYCATWAASLSSARVFGTGTKVIVLV
6	VRC26.25 VL CDR1	<u>TSNIGNNF</u>
7	VRC26.25 VL CDR2	<u>ETD</u>
8	VRC26.25 VL CDR3	<u>ATWAASLSSARV</u>
9	PGDM1400 VH	QAQLVQSGPEVRKPGTSVKVSCAPGNTLKYDHLHWVRSV PGQGLQWMGWISHEGDKKIVVERFKAKVTIDWDRSTNTAY LQLSGLTSGDTAVYYCAKGSKHRLRDYALDDDGALNWAVD VDYLSNLEFWGQGTAVTVSS
10	PGDM1400 VH CDR1	<u>GNTLKYTD</u>
11	PGDM1400 VH CDR2	<u>ISHEGDKK</u>
12	PGDM1400 VH CDR3	<u>AKGSKHRLRDYALDDDGALNWAVD</u> VDYLSNLEF
13	PGDM1400 VL	DFVLTQSPHSLSVTPGESASISCKSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGVPDRFSGSGDKDFTLKI SRVETEDVGTYYCMQGRESPTWFGQGTKVDIK
14	PGDM1400 VL CDR1	<u>HSLIHGDRNNY</u>
15	PGDM1400 VL CDR2	<u>LAS</u>
16	PGDM1400 VL CDR3	<u>MQGRESPTW</u>
17	PGT121 VH	QMQLQESGPGLVKPSSETLSLTCSVSGASISDSYWSWIRRS PGKGLEWIGYVHKSGDTNYSPLKSRVNLSDTSKNQVSL SLVAATAADSGKYYCARTLHGRIYGIVAFNEWFTYFYMD VWNGTQVTTVSS
18	PGT121 VH CDR1	<u>GASISDSY</u>
19	PGT121 VH CDR2	<u>VHKSGDT</u>
20	PGT121 VH CDR3	<u>ARTLHGRIYGIVAFNEWFTYFYMDV</u>
21	PGT121 VL	SDISVAPGETARISCGEKSLSRAVQWYQHRAGQAPSLII YNNQDRPSGIPERFSGSPDSPFGTTATLTITSVEAGDEAD YYCHIWDNRVPTKWVFGGGTTLTVL
22	PGT121 VL CDR1	<u>SLGSRA</u>
23	PGT121 VL CDR2	<u>NNQ</u>
24	PGT121 VL CDR3	<u>HIWDSRVPTKWV</u>
25	N6 VH	RAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHLFWFRQA PGRGLEWVGWIKPQYGAVNFGGGRDVRTLTTRDVYREIAY MDIRGLKPDDTAVYYCARTDRSYGDSWALDAWGQGTTVV SA
26	N6 VH CDR1	<u>GYTFTAHI</u>

27	N6 VH CDR2	<u>IKPOYGAV</u>
28	N6 VH CDR3	<u>AR</u>
29	N6 VL	YIHVTQSPSSLSVSI GDRVTINCQTSQGVGSDLHWYQHKP GRAPKLLIHHTSSVEDGVPSRFSGSGFHTSFNLTISDLQA DDIATYYCQVLQFFGRGSR LHIK
30	N6 VL CDR1	<u>QGVGSD</u>
31	N6 VL CDR2	<u>HTS</u>
32	N6 VL CDR3	<u>QVLQF</u>
33	35O22 VH	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQT AGRGPEWMGWISPYS GDKNLAPAFQDRVIMTTDTEVPVTS FTSTGAAYMEIRNLKFDDTGTYFCAKGLLRDGSSTWLPYL WGQGTLLTVSS
34	35O22 VH CDR1	<u>GYRFNFYH</u>
35	35O22 VH CDR2	<u>ISPYS GDK</u>
36	35O22 VH CDR3	<u>DDTGTYFCAKGLLRDGSSTWLPYL</u>
37	35O22 VL	QSVLTQSASVSGSLGQSVTISCTGPNVCCSHKSISWYQW PPGRAPTLLIYEDNERAPGISPRFSGYKSYWSAYLTISDL RPEDETTYCCSYTHNSGCVFGTGTKVSVL
38	35O22 VL CDR1	<u>NSVCCSHKS</u>
39	35O22 VL CDR2	<u>EDN</u>
40	35O22 VL CDR3	<u>CSYTHNSGCV</u>
41	10E8v4 VH	EVRLVESGGGLVKPGGSLRLSCSASGFDFD NAWMTWVRQP PGKGLEWVGRITGPGEGWSVDYAESVKGRFTISRDN TKNT LYLEMNNVRTEDTGY YFCARTGKYYDFW SGYPPGEEYFQD WGQGT LIVIVSS
42	10E8v4 VH CDR1	<u>GFDFDNAW</u>
43	10E8v4 VH CDR2	<u>ITGPGEGWSV</u>
44	10E8v4 VH CDR3	<u>TGYYFCARTGKYYDFWSGYPPGEEYFQD</u>
45	10E8v4 VL	SELTQDPAVSVALKQTVTITCRGDSLRSHYASWYQKKPGQ APVLLFYGKNNRPSGIPDRFSGSASGNRASLTITGAQAE EADYYCSSRDKSGSRLSVFGGGT KLTVL
46	10E8v4 VL CDR1	<u>RGDSLRSHYAS</u>
47	10E8v4 VL CDR2	<u>GKNNRPS</u>
48	10E8v4 VL CDR3	<u>SSRDKSGSRLSV</u>
49	10E8v4_S100cF VH	EVRLVESGGGLVKPGGSLRLSCSASGFDFD NAWMTWVRQP PGKGLEWVGRITGPGEGWSVDYAESVKGRFTISRDN TKNT LYLEMNNVRTEDTGY YFCARTGKYYDFW FGYPPGEEYFQD WGQGT LIVIVSS
<i>10E8v4 VH CDR1</i>	10E8v4_S100cF VH CDR1	<u>GFDFDNAW</u>
<i>10E8v4 VH CDR2</i>	10E8v4_S100cF VH CDR2	<u>ITGPGEGWSV</u>
50	10E8v4_S100cF VH CDR3	<u>TGYYFCARTGKYYDFWFGYPPGEEYFQD</u>
51	10E8v4_V5R_S100cF VH	EVRLRESGGGLVKPGGSLRLSCSASGFDFD NAWMTWVRQP PGKGLEWVGRITGPGEGWSVDYAESVKGRFTISRDN TKNT LYLEMNNVRTEDTGY YFCARTGKYYDFW FGYPPGEEYFQD WGQGT LIVIVSS
<i>10E8v4 VH CDR1</i>	10E8v4_V5R_S100cF VH CDR1	<u>GFDFDNAW</u>
<i>10E8v4 VH CDR2</i>	10E8v4_V5R_S100cF VH CDR2	<u>ITGPGEGWSV</u>

10E8v4_S100cF VH CDR3	10E8v4_V5R_S100cF VH CDR3	TGYYFCARTGKYYDFWFGYPPGEEYFQD
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Table 2. Amino Acid Sequences of variable domains and CDR sequences.

In some embodiments, the multispecific anti-HIV antibody is capable of binding five different antigen targets. The disclosure features a multispecific antibody that binds five non-overlapping epitopes. In some embodiments, the multispecific anti-HIV antibody comprises an amino acid sequence selected from any of SEQ ID NOS:72-80, as shown below in Table 3.

SEQ ID NO:	Name	Orientation	Amino Acid Sequence
72	PentaNA b _{1.0}	10E8v4--5X--35O22-- 5X--N6--5X--PGT121-- 5X--VRC26.25--His	MGWSCIIILFLVATATGVHSELTDQPAVSVALKQTVTITCRGD SLRSHYASWYQKKPGQAPVLLFYGKNRPSGIPDRFSGSASGN RASLTITGAQAEDADYCYSSRDKSGSRLSVFGGGTCLTLVLGG GGSGGGGSGGGGSEVRLVESGGGLVKPGGSLRLSCSASGFD NAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAESVKGRFTIS RDNTKNTLYLEMNNVRTEDTGYYFCARTGKYYDFWSGYPPGEE YFQDWGQGTLVIVSSGGGGSGGGGSGGGGSGGGGSGGGGSGQSV LTQSASVSGSLGQSVTISCTGPNSVCCSHKISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETYY CCSYTHNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGQGQLVQS GAELKKPGASVKISCKTSGYRNFYHINWIRQTAGRGPWEMGW ISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG QTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSG FHTSFNLTISDLQADDIATYYCQVLQFFGRGSRHLIKGGGGSG GGGGSGGGGSRHLVQSGTAMKKPGASVRVSCQTSGYTFTAHIL FWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTTLTRDVYRE IAYMDIRGLKPDPTAVYYCARDRSYGDSSWALDAWGQGTTVV SAGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGDISVAPGETARISCG EKSLGSRVAVQWYQHRAGQAPSLIYNNQDRPSGIPERFSGSPD SPFGTTATLTITISVEAGDEADYCYCHIWDNRVPTKWVFGGGTTL TVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GASISDSYWSWIRRSPGKGLEWIGYVHKSQDNTNYSPLKSRVN LSLDTSKNQVSLSLVAATAADSGKYCARTLHGRIYGIYAFN EWFTYFYMDVWNGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGQSVLTQPPSVSAAPGQKVTISCSGNTSNIGNNFVSWYQQR PGRAPQLLIYETDKRPSGIPDRFSASKSGTSGTLAITGLQTGD EADYYCATWAASLSARVFGTGTKVIVLVGGGGSGGGGSGGGGSGGGG SQVQLVESGGGVVQPGTSLRLSCAASQFRFDGYGMHWVRQAPG KGLEWVASISHDGIKKYHAEKVWGRFTISRDNKNTLYLQMNS LRPEDTALYYCAKDLREDECEEWSDYYDFGKQLPCAASRGGL VGIADNWGQGTMTVTVSSSGSGHHHHHH
73	PentaNA b _{2.0}	10E8v4_S100cF-- 5X--35O22--5X--N6-- 5X--PGT121--5X-- VRC26.25--His	MGWSCIIILFLVATATGVHSELTDQPAVSVALKQTVTITCRGD SLRSHYASWYQKKPGQAPVLLFYGKNRPSGIPDRFSGSASGN RASLTITGAQAEDADYCYSSRDKSGSRLSVFGGGTCLTLVLGG GGSGGGGSGGGGSEVRLVESGGGLVKPGGSLRLSCSASGFD NAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAESVKGRFTIS RDNTKNTLYLEMNNVRTEDTGYYFCARTGKYYDFWSGYPPGEE YFQDWGQGTLVIVSSGGGGSGGGGSGGGGSGGGGSGGGGSGQSV LTQSASVSGSLGQSVTISCTGPNSVCCSHKISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETYY CCSYTHNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGQGQLVQS GAELKKPGASVKISCKTSGYRNFYHINWIRQTAGRGPWEMGW ISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG QTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSG FHTSFNLTISDLQADDIATYYCQVLQFFGRGSRHLIKGGGGSG GGGGSGGGGSRHLVQSGTAMKKPGASVRVSCQTSGYTFTAHIL FWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTTLTRDVYRE IAYMDIRGLKPDPTAVYYCARDRSYGDSSWALDAWGQGTTVV SAGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGDISVAPGETARISCG EKSLGSRVAVQWYQHRAGQAPSLIYNNQDRPSGIPERFSGSPD SPFGTTATLTITISVEAGDEADYCYCHIWDNRVPTKWVFGGGTTL TVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GASISDSYWSWIRRSPGKGLEWIGYVHKSQDNTNYSPLKSRVN LSLDTSKNQVSLSLVAATAADSGKYCARTLHGRIYGIYAFN EWFTYFYMDVWNGTQVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGQSVLTQPPSVSAAPGQKVTISCSGNTSNIGNNFVSWYQQR PGRAPQLLIYETDKRPSGIPDRFSASKSGTSGTLAITGLQTGD EADYYCATWAASLSARVFGTGTKVIVLVGGGGSGGGGSGGGGSGGGG SQVQLVESGGGVVQPGTSLRLSCAASQFRFDGYGMHWVRQAPG KGLEWVASISHDGIKKYHAEKVWGRFTISRDNKNTLYLQMNS LRPEDTALYYCAKDLREDECEEWSDYYDFGKQLPCAASRGGL VGIADNWGQGTMTVTVSSSGSGHHHHHH

			NAWMTWVRQPPGKGLEWVGRITGPGEWGSVDYAESVKGRFTTIS RDNTKNTLYLEMNNVRTEDTGYFFCARTGKYDFWFGYPPGEE YFQDWGQGTLLVIVSSGGGSGGGGSGGGGSGGGGSGGGGSSQSV LTQSASVSGSLGQSVTISCTGPNSVCCSHKSI SWYQWPPGRAP TLI IYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTY CCSYTHNSGCVFGTGTKVSVLGGGSGGGGSGGGGSGGGGSLVQS GAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPPEWGMW ISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG QTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSPRFSGS FHTSFNLTISDLQADDIATYYCQVLQFFGRGSRHLIKGGGGSG GGGSGGGGSPRAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHL FWFQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLLTRDVYRE IAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQGTTVV SAGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCG EKSLGSRVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPD SPFGTTATLTITSVEAGDEADYYCHIWDSPVPTKWVFGGGTTL TVLGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG GASISDSYWSWIRRSPPGKLEWIGYVHKSGDTNYSPLKSRVN LSLDTSKNQVSLSLVAATAADSGKYCARTLHGRRYIGIVAFN EWFYFYMDVWNGTQVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGG GGSQSVLTQPPSVSAAPGQKVTISCSGNTSNIGNNFVSWYQQR PGRAPQLLIYETDKRPSGIPDRFSASKSGTSGTLAITGLQTGD EADYYCATWAASLSSARVFGTGTKVIVLVGGGSGGGGSGGGGSGGGG SQVQLVESGGGVVQPGTSLRLSCAASQFRFDGYGMHWVRQAPG KGLEWVASISHDGIKKYHAEKVWGRFTISRDNKNTLYLQMN LRPEDTALYYCAKDLREDECEEWSDYYDFGKQLPCA SRGGL VGIADNWGQGTMTVTVSSSGSGHHHHHH
74	PentaNA b _{2.0} Reverse	VRC26.25-5X- PGT121-5X-N6-5X- 35022-5X- 10E8v4_s100cF- His	MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGTSLRLSCAA SQFRFDGYGMHWVRQAPGKLEWVASISHDGIKKYHAEKVWGR FTISRDNKNTLYLQMNLRPEDTALYYCAKDLREDECEEWSD DYYDFGKQLPCA SRGGLVGIADNWGQGTMTVTVSSGGGSGGGG GSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG YQQRPGRAPQLLIYETDKRPSGIPDRFSASKSGTSGTLAITGL QTGDEADYYCATWAASLSSARVFGTGTKVIVLVGGGSGGGGSGGGG GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG ISDSYWSWIRRSPPGKLEWIGYVHKSGDTNYSPLKSRVNL DTSKNQVSLSLVAATAADSGKYCARTLHGRRYIGIVAFNEWF TYFYMDVWNGTQVTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGG ARI SCGEKSLGSRVQWYQHRAGQAPSLIIYNNQDRPSGIPER FSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSPVPTKWV GGGTTLTVLGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG AMKKPGASVRVSCQTSGYTFTAHLFWFQAPGRGLEWVGWIK PQYGAVNFGGGFRDRVTLLTRDVYREIAYMDIRGLKPDDTAVYY CARDRSYGDSSWALDAWGQGTTVVVSAGGGGSGGGGSGGGGSGGGG IHVTQSPSSLSVSI GDRVTINCQTSQGVGSDLHWYQHKPGRAP KLLIHHTSSVEDGVPSPRFSGSFHTSFNLTISDLQADDIATYY CQVLQFFGRGSRHLIKGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG QLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGP EWMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAY MEIRNLKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG CCSHKSI SWYQWPPGRAPTLLIYEDNERAPGISPRFSGYKSYW SAYLTISDLRPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGG SGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG SASGFDFDNAWMTWVRQPPGKGLEWVGRITGPGEWGSVDYAES VKGRFTISRDNKNTLYLEMNNVRTEDTGYFFCARTGKYDFW FGYPPGEEYFQDWGQGTLLVIVSSGGGSGGGGSGGGGSGGGGSGGGG

			DPAVSVALKQTVTITTCRGDSLRSYASWYQKKPGQAPVLLFYG KNNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDK SGSRLSVFGGGTCLTVLGS GHHHHH
75	PentaNA b _{3.0}	10E8v4_s100cF- 5X-35022-5X-N6- 5X-PGT121-5X- PGDM1400-His	MGWSCIILFLVATATGVHSELTDQDPAVSVALKQTVTITTCRGD SLRSYASWYQKKPGQAPVLLFYGKNNRPSGIPDRFSGSASGN RASLTITGAQAEDEADYYCSSRDKSGSRLSVFGGGTCLTVLGG GGSGGGSGGGGSEVRLVESGGGLVKPGGSLRLSCSASGFDFD NAWMTWVRQPPGKGLEWVGRITGPGEWSDYAESVKGRFTTIS RDNTKNTLYLEMNNVRTEDTGYFECARTGKYDFWFGYPPGEE YFQDWGQGTLLVIVSSGGGGSGGGSGGGSGGGSGGGSGQSV LTQSASVSGSLGQSVTISCTGPNVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETYY CCSYTHNSGCVFGTGTVKSVLGGGGSGGGSGGGSGGGSGQQLVQS GAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPWEMGW ISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG GGGGSGGGSGGGSGGGSGGGSGYHVTQSPSSLVSIGDRVITINC QTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSG FHTSFNLITISDLQADDIATYYCQVLQFFGRGSRHLIKGGGGSG GGSGGGSGRAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHL FWFRQAPGRGLEWVGWIKPQYGAVNFGGGRDRVTLTRDVYRE IAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQGTTVV SAGGGSGGGSGGGSGGGSGGGSGGGSGGGSSDISVAPGETARISCG EKSLGSRVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPD SPFGTTATLTITSVEAGDEADYYCHIWDSDRVPTKWVFGGGTTL TVLGGGGSGGGSGGGSGQMQLQESGPGLVKPSETLSLTCSVS GASISDSYWSWIRRSPGKGLEWIGYVHKS GDTNYSPLKSRVN LSLDTSKNQVLSLVAATAADSGKYCARTLHGRRYIGIVAFN EWFYFYMDVWNGTQVTVSSGGGGSGGGSGGGSGGGSGGGSGG GGSDFVLTQSPHLSVTPGESASISCKSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGVPDRFSGSGSDKDFTLKISR ETEDVGTYTCMQGRESFWTFGQGTQVDIKGGGGSGGGSGGGG SQAQLVQSGPEVRKPGTSVKVSCKAPGNTLKYDHLWVRSVPG QGLQWGMWISHEGDKKIVIVERFKAKVTIDWDRSTNTAYLQLSG LTSGDTAVYYCAKGSKHRLRDYALYDDDGALNWAVDVDYLSNL EFGWQGTAVTVSSSGSGHHHHH
76	PentaNA b _{3.0} Reverse	PGDM1400-5X- PGT121-5X-N6-5X- 35022-5X- 10E8v4s100cF-His	MGWSCIILFLVATATGVHSAQLVQSGPEVRKPGTSVKVSCKA PGNTLKYDHLWVRSVPGQGLQWGMWISHEGDKKIVIVERFKAK VTIDWDRSTNTAYLQLSGLTSGDTAVYYCAKGSKHRLRDYALY DDDGALNWAVDVDYLSNLEFWGQGTAVTVSSGGGGSGGGSGGG GGSDFVLTQSPHLSVTPGESASISCKSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGVPDRFSGSGSDKDFTLKISR ETEDVGTYTCMQGRESFWTFGQGTQVDIKGGGGSGGGSGGGG SGGGSGGGSGQMQLQESGPGLVKPSETLSLTCSVSGASISDS YWSWIRRSPGKGLEWIGYVHKS GDTNYSPLKSRVNLSLDTSK NQVLSLVAATAADSGKYCARTLHGRRYIGIVAFNEWFYFY MDVWNGTQVTVSSGGGGSGGGSGGGSGGGSSDISVAPGETARIS CGEKSLSRAVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGS PDSFGTTATLTITSVEAGDEADYYCHIWDSDRVPTKWVFGGGT TLTVLGGGGSGGGSGGGSGGGSGGGSGGGSGRAHLVQSGTAMKK PGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYG AVNFGGGRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYCARD RSYGDSSWALDAWGQGTTVVVSAGGGSGGGSGGGSGGGSGYHVT QSPSSLVSIGDRVITINCQTSQGVGSDLHWYQHKPGRAPKLLI HHTSSVEDGVPSRFSGSGFHTSFNLITISDLQADDIATYYCQVL QFFGRGSRHLIKGGGGSGGGSGGGSGGGSGGGSGGGSGQQLVQ SGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPWEMG WISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIR NLKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG

			SGGGGSGGGGSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSH KSI SWYQWPPGRAPTLLIYEDNERAPGISPRFSGYKSYWSAYL TISDLRPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGGSGGG GSGGGGSGGGGSGGGGSEVRLVESGGGLVKPGGSLRLSCSASG FDFD NAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAESVKGR FTISRDN TKNTLYLEMNNVRTEDTGYFFCARTGKYDFWFGYP PGEEYFQDWGQGT LVIVSSGGGGSGGGGSGGGGSSSELTQDPAV SVALKQTVTITCRGDSLRSYASWYQKKPGQAPVLLFYGKNNR PSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSR LSVFEGGKTCLTVLGS GHHHHHH
77	PentaNA b _{4.0}	10E8v4 V5R_S100cF-5X- 35022-5X-N6-5X- PGT121-5X- PGDM1400-His	MGWSCIILFLVATATGVHSSSELTQDPAVSVALKQTVTITCRGD SLRSYASWYQKKPGQAPVLLFYGKNNRPSGIPDRFSGSASGN RASLTITGAQAEDEADYYCSSRDKSGSRLSVFEGGKTCLTVLGG GSGGGGSGGGGSEVRLRESGGGLVKPGGSLRLSCSASGDFD NAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAESVKGRFTIS RDNTKNTLYLEMNNVRTEDTGYFFCARTGKYDFWFGYPPGEE YFQDWGQGT LVIVSSGGGGSGGGGSGGGGSGGGGSGGGGSQSV LTQSASVSGSLGQSVTISCTGPNSVCCSHKSI SWYQWPPGRAP TLIYEDNERAPGISPRFSGYKSYWSAYLTI SDLRPEDETTY CCSYTHNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSQGLVQS GAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGP EWMGW ISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGTYFCAKGLLRDGSSTWLPYLGWQGTLLTVSSGGGG GGGGSGGGGSGGGGSGGGGSIHVTSQSPSSLSVSI GDRVTINC QTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSG FHTSFNLTI SIDLQADDIATYYCQVLQFFGRGSR LHIKGGGGSG GGGSGGGGSR AHLVQSGTAMKKPGASVRVSCQTSGYTFTAHL FWFRQAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTLTRDVYRE IAYMDIRGLKPDDTAVYYCARDRSYGDSSWALDAWGQGTTVV SAGGGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCG EKSLSGRAVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPD SPFGTTATLTITTSVEAGDEADYYCHIWD SRVP TKWVFEGGTT TVLGGGGSGGGGSGGGGSMQLQESGPGLVKPSETLSLTCSVS GASISDSYWSWIRRSPGKGLEWIGYVHKSGDTNYSPLKSRVN LSLDTSKNQVSLSLVAATAADSGKYCARTLHGRRIYGI VAFN EWFTYFYMDVWNGTQVTVSSGGGGSGGGGSGGGGSGGGGSGG GGSD FVLTQSPHSLSVTPGESASISCKSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGV PDRFSGSGSDKDFTLKISR ETEDVGTYYCMQGRES PWTFGQGT KVDIKGGGGSGGGGSGGGG SQAQLVQSGPEVRKPGTSVKVSCAPGNTLKYIDLHWVRSVPG QGLQWMGWISHEGDKKIVIVERFKAKVTIDWDRSTNTAYLQLSG LTSGDTAVYYCAKGSKHRLRDYALYDDD GALNWAVD VDYLSNL EFWGQGTAVTVSSSGSGHHHHHH
78	PentaNA b _{4.0} Reverse	PGDM1400-5X- PGT121-5X-N6-5X- 35022-5X-10E8v4 V5R_S100cF-His	MGWSCIILFLVATATGVHSSQAQLVQSGPEVRKPGTSVKVSCA PGNTLKYIDLHWVRSVPGQGLQWMGWISHEGDKKIVIVERFKAK VTIDWDRSTNTAYLQLSGLTSGDTAVYYCAKGSKHRLRDYALY DDD GALNWAVD VDYLSNLEFWGQGTAVTVSSGGGGSGGGGSGG GGSD FVLTQSPHSLSVTPGESASISCKSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGV PDRFSGSGSDKDFTLKISR ETEDVGTYYCMQGRES PWTFGQGT KVDIKGGGGSGGGGSGGGG SGGGGSGGGGSMQLQESGPGLVKPSETLSLTCSVSGASISDS YWSWIRRSPGKGLEWIGYVHKSGDTNYSPLKSRVNLSLDTSK NQVSLSLVAATAADSGKYCARTLHGRRIYGI VAFNEWFTYFY MDVWNGTQVTVSSGGGGSGGGGSGGGGSSDISVAPGETARIS CGEKSLSGRAVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGS PDS PFGTATLTITTSVEAGDEADYYCHIWD SRVP TKWVFEGGT TLTVLGGGGSGGGGSGGGGSGGGGSGGGGSR AHLVQSGTAMKK PGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYG AVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYCARD

			RSYGDSSWALDAWGQGTTVVVSAGGGGSGGGGSGGGGSIYHVT QSPSSLSVSI GDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLI HHTSSVEDGVPSRFSGSGFHTSFNLTISDLQADDIATYYCQVL QFFGRGSRHLHIKGGGSGGGGSGGGGSGGGGSGGGGSGGGGSLVQ SGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPPEWMG WISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIR NLKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG SGGGGSGGGGSGSVLTQSASVSGSLGQSVTISCTGPNNSVCCSH KSISWYQWPPGRAPTLIIYEDNERAPGISPRFSGYKSYWSAYL TISDLRPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGGSGGG GSGGGGSGGGGSGGGGSEVRLRESGGGLVKPGGSLRLSCSASG FDFDNAWMTWVRQPPGKGLEWVGRTGPGEWSVDYAESVKGR FTISRDNKNTLYLEMNNVRTEDTGYYFCARTGKYDFWFGYP PGEEYFQDWGQGTLLVIVSSGGGGSGGGGSGGGGSGGGGSLQDPAV SVALKQTVTITCRGDSLRSYASWYQKKPGQAPVLLFYGKNNR PSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSR LSVFGGGTKLTVLGSCHHHHHH
79	PentaNA b _{4.0} Reverse	PGDM1400-5X- PGT121-5X-N6-5X- 35022-5X-10E8v4 V5R S100cF VH fused to <u>IgG1 LS</u> <u>HC with His Tag</u>	MGWSCIIILFLVATATGVHSAQQLVQSGPEVRKPGTSVKVSCKA PGNTLKTYDLHWVRSVPQGQLQWGMWISHEGDKKIVIVERFKA VTIDWDRSTNTAYLQLSGLTSGDTAVYYCAKSKHRLRDYALY DDDGAALNWAVDVDYLSNLEFWGQGTAVTVSSGGGGSGGGGSGG GGSDFVLTQSPHSLSVTPGESASISCKSSSHSLIHGDRNNYLAW YVQKPGRSFQQLLIYLASSRASGVPDRFSGSGSDKDFTLKISR ETEDVGTYYCMQGRESPTWTFGQGTQVDIKGGGGSGGGGSGGGG SGGGGSGGGGSGMQLQESGPGLVKPSSETLSLTCVSAGASISDS YWSWIRRSFPGKLEWIGYVHKSGDTNYSPLSKSRVNLSDTSK NQVSLSLVAATAADSGKYCARTLHGRRYIGIVAFNEWFTYFY MDVWNGTQVTVSSGGGGSGGGGSGGGGSSDISVAPGETARIS CGEKSLSRAVQWYQHPAGQAPSLIIYNNQDRPSGIPERFSGS PDSFPGTTATLTITSVEAGDEADYYCHIWDNRVPTKWVFGGTT TLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG PGASVRVSCQTSQYTFHTAHILFWFRQAPGRGLEWVGWIKPQYG AVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDPTAVYYCARD RSYGDSSWALDAWGQGTTVVVSAGGGGSGGGGSGGGGSIYHVT QSPSSLSVSI GDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLI HHTSSVEDGVPSRFSGSGFHTSFNLTISDLQADDIATYYCQVL QFFGRGSRHLHIKGGGSGGGGSGGGGSGGGGSGGGGSGGGGSLVQ SGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPPEWMG WISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIR NLKFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGG SGGGGSGGGGSGSVLTQSASVSGSLGQSVTISCTGPNNSVCCSH KSISWYQWPPGRAPTLIIYEDNERAPGISPRFSGYKSYWSAYL TISDLRPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGGSGGG GSGGGGSGGGGSGGGGSEVRLRESGGGLVKPGGSLRLSCSASG FDFDNAWMTWVRQPPGKGLEWVGRTGPGEWSVDYAESVKGR FTISRDNKNTLYLEMNNVRTEDTGYYFCARTGKYDFWFGYP PGEEYFQDWGQGTLLVIVSS<u>ASTKGPSVFPLAPSSKSTSGGTAA</u> <u>LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS</u> <u>VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCTP</u> <u>PCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED</u> <u>PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW</u> <u>LNQKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRE</u> <u>EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD</u> <u>SDGSFFLYSKLTVDKSRWQQGNVFCFVLSHLYSTQKSLS</u> <u>LSPGKSGHHHHHH</u>
80	PentaNA b _{4.0} Reverse	PGDM1400-5X- PGT121-5X-N6-5X- 35022-5X-10E8v4 V5R S100cF VH-	MGWSCIIILFLVATATGVHSAQQLVQSGPEVRKPGTSVKVSCKA PGNTLKTYDLHWVRSVPQGQLQWGMWISHEGDKKIVIVERFKA VTIDWDRSTNTAYLQLSGLTSGDTAVYYCAKSKHRLRDYALY DDDGAALNWAVDVDYLSNLEFWGQGTAVTVSSGGGGSGGGGSGG

		VL-fused to <u>IgG1</u> <u>LS FC with His</u> <u>Tag</u>	GGSDFVLTQSPHSLSVTPGESASISCKSSSHSLIHGDRNNYLAW YVQKPGRSPQLLIYLASSRASGVDPDRFSGSGSDKDFTLKISR ETEDVGTYYCMQGRESFWTFGQGTQVDIKGGGGSGGGGSGGGG SGGGGSGGGGSGMQLQESGPGLVKPSSETLSLTCVSGASISDS YWSWIRRSPPGKLEWIGYVHKSGDTNYSPLKSRVNLSDTSK NQVSLSLVAATAADSGKYCARTLHGRRYGIYAFNEWFTYFY MDVWNGTQVTVSSGGGGSGGGGSGGGGSSDISVAPGETARIS CGEKSLSRAVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGS PDSPPFGTTATLTITSVEAGDEADYYCHIWDSDRVPTKWVFGGT TLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGRAHLVQSGTAMKK PGASVRVSCQTSGYTFTAHLFWFRQAPGRGLEWVGWIKPQYG AVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDPTAVYYCARG RSYGDSWALDAWQGTQTVVVSAGGGGSGGGGSGGGGSGYIHVT QSPSSLSVSIQDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLI HHTSSVEDGVPSRFSGSGFHTSFNLTISDLQADDIATYYCQVL QFFGRGSRSLHIKGGGGSGGGGSGGGGSGGGGSGGGGSGGQLVQ SGAELKKPGASVKISCKTSGYRFNFIHINWIRQTAGRGPEWMG WISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIR NLKFDDTGTYFCAKGLLRDGSSTWLPYLVWGQGTLLTVSSGGGG SGGGGSGGGGSGSVLTQSASVSGSLGQSVTISCTGPNVCCSH KSISWYQWPPGRAPTLLIYEDNERAPGISPRFSGYKSYWSAYL TISDLRPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGGSGGG GSGGGGSGGGGSGGGGSEVRLRESGGGLVKPGGSLRLSCSASG FDFDNAWMTWVRQPPGKLEWVGRITGPGEWSVDYAESVKGR FTISRDNKNTLYLEMNNVREDTGYIFCARTGKYDFWFGYP PGEEYFQDWGQGTLVIVSSGGGGSGGGGSGGGGSGSELTQDPAV SVALKQTVTITCRGDSLRSHYASWYQKPGQAPVLLFYGKNR PSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSR LSVFGGGTKLTVL <u>gsqPKSCDKTHTCPPCPAPELLGGPSVFLF</u> <u>PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP</u> <u>APIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS</u> <u>RWQQGNVFSCSVLHEALHSHYTQKSLSLSPGKSGHHHHHH</u>
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Table 3. Penta-Nab names, orientations of variable light and variable heavy chains, and amino acid sequences.

In some embodiments, 3D structure information of broadly neutralizing antibodies (bNAbs) in complex with HIV-1 envelope glycoproteins (Env) was used to perform rational design of penta-specific antibodies (PentaNAbs) capable of targeting five major Env neutralizing epitopes. In some embodiments, “PentaNAbs” as referred to herein comprise five functional single chain fragment variable (ScFv) moieties connected in tandem via (G4S)_n linkers, such that penta-valence engagement of five individual epitopes on the HIV-1 Env by each respective moiety is possible. The bNAb functional moieties incorporated in the design target five major HIV neutralization epitopes including the i) V1/V2 apex glycan, ii) V3-base glycan, iii) CD4 binding site, iv) gp120/gp41 interface, and v) the membrane proximal external region (MPER). In

addition, these PentaScFv entities can be fused to the fragment crystallizable region (Fc) region of IgG1 to accommodate effector functions in a Penta-IgG format.

The term “protomer” is the structural unit of an oligomeric protein unit. A protomer may span a single segment of a protein’s primary structure, or may be
5 composed of one or more different protein sequences that spans across a protein’s three-dimensional folded or unfolded protein structure. A protomer may be a repeating structural unit.

In some embodiments, to design the Penta-specific ScFv and bNAbs, the physical distance between the compatible N/C termini of parental bNAb variable heavy (VH) and
10 light chain (VL) domains was estimated to identify the shortest linker combinations allowing optimal VH/VL connection within each bNAb ScFv entity. In some embodiments multiple tandem (G4S)_n linkers were used. In some embodiments, at least three G4S linkers were used between the VH and VL domains of a bNAb in the PentaScFv or Penta-bNAb molecule. The number of G4S linkers between VH and VL
15 regions is not limiting and may not be required. In some embodiments there is no linker between the VH and VL regions. The number of G4S linkers between VH and VL domains of a bNab may include at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, or at least fifteen linkers. In
20 some embodiments multiple tandem (G4S)_n linkers can be used between individual bNab moieties in the PentaScFv and Penta-bNAb molecules. In some embodiments at least five G4S linkers were used between individual bNAbs. The number of G4S linkers between individual bNAbs may include at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at
25 least twelve, at least thirteen, at least fourteen, or at least fifteen linkers.

In some embodiments, five G4S linkers were used to connect moieties of bNAbs targeting the V1V2 (e.g. VRC26.25) and V3 (e.g. PGT121) glycans. A linker of sufficient length (e.g. a linker with five G4S units) can be utilized to connect VRC26.25 or PGDM1400 with PGT121. In some embodiments, epitopes located on adjacent
30 protomers of the HIV-1 trimer are often more proximate to each other than those on the same protomer. Thus, in some embodiments, two functional bNab moieties can be

arranged binding to the same protomer (intra-protomer mode) and in some cases to separate protomers (inter-protomers) of the HIV-1 Env trimer. In some embodiments, the shortest distance between the CD4bs targeting bNAb moiety, N6 (with VRC01 as surrogate), and the gp120/gp41 interface targeting 35O22 entity was determined to be an intra-protomer distance. Using the model in PDB 5FYK, the distance between the 35O22 and N6 functional variable domains were determined to be 57 Å between the C-terminus of 35O22 VH domain and the N-terminus of N6 VL domain with the topology of 35O22(VL-VH)-N6(VL-VH), and 47 Å between the C-terminus of N6 VL and the N-terminus of 35O22 VH with the topology of N6(VH-VL)-35O22(VH-VL), respectively. Thus, five G4S linkers can be used to connect N6 and 35O22. The length of the linkers is not limiting and may be varied.

In some embodiments, five G4S linkers can be used to connect the V3-glycan targeting PGT121 and the CD4bs targeting N6 ScFv entities. The shortest distance between PGT122 (a surrogate for PGT121) and VRC01 (a surrogate for N6) was previously determined between adjacent protomers within an Env trimer (inter-protomer). Using the PDB model 5FYK, the distances between the VRC01 and PGT121 functional variable domains were determined as the follows: 37 Å between the C-terminus of VRC01 VH domain and the N-terminus of PGT121 VL domain with VRC01(VL-VH)-PGT121(VL-VH) topology, and 53 Å between the C-terminus of PGT121 VL and the N-terminus of VRC01 VH with PGT121(VH-VL)-VRC01(VH-VL) topology. Furthermore, negative stain electron microscopy data demonstrated that one of the Bi-ScFvs previously generated, dVRC01(VL-VH)-5X-PGT121(VL-VH), was able to simultaneously engage both the PGT121 and VRC01 epitopes in an inter-protomer manner as predicted (31), which supports rationale for this linker design.

In some embodiments, the shortest distances between the gp120/gp41 interface targeting 35O22 entity and the MPER targeting 10E8v4 entity was determined to be inter-protomer manner. Using PDB 5IQ7 superimposed with PDB 5FYK, a model for Env engaged with both 35O22 and 10E8 was generated. In this model, the distance between the 10E8v4 and 35O22 functional variable domains were determined to be 48 Å between the C-terminus of 10E8v4 VH domain and the N-terminus of 35O22 VL domain with the topology of 10E8v4(VL-VH)-35O22(VL-VH), and 25 Å between the C-terminus of 35O22 VL and the N-terminus of 10E8v4 VH with the topology of 35O22(VH-VL)-

10E8v4(VH-VL) respectively. Therefore, five G4S linkers can be used to connect 35O22 and 10E8v4. The linkers used are not limiting and can be optimized.

The type of linker between VH and VL domains of a bNAb is not limited to the use of G4S linkers. The type of linker between individual bNAbs is not limited to the use of G4S linkers. The length, sequence, and/or identity between the linkers separating the VH and VL regions and the linkers separating the individual bNAb moieties may be the same or may be different. The relationship between the types and characteristics of linkers used is not limiting. Such linkers are typically polypeptide sequences of between about 1 and 200 amino acids. The linkers can be any combination of natural or unnatural amino acids, and the sequence identity of the linkers is not limiting. Linkers can be flexible or rigid amino acid subsequences which are synthesized as part of a recombinant fusion protein.

The linkers may be flexible, the linkers may be rigid, or the linkers may be cleavable. Linkers may offer many other advantages for the production of fusion proteins, such as improving biological activity, increasing expression yield, and achieving desirable pharmacokinetic profiles. As such, the linkers in certain embodiments are not limiting and may be optimized for length, flexibility, rigidity, identity, cleavability, chemical composition, or other physical or chemical properties, for reasons that may or may not affect yield, half-life, biological activity, neutralization activity, pharmacokinetic properties, bioavailability, binding affinity, protein size, or stoichiometric binding ratios.

In some embodiments, the linker can be non-peptide based. A chemical linker may be used to connect protein sequences. Such linkers are known to persons of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc. Huntsville, Ala. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages. In addition to covalent linkage of bNab sequences and linkers, non-covalent methods can be used to produce molecules with bNabs and linkers. Disulfide bonds may be used to form linkers. Chemical conjugation methods are known to persons of skill in the art and may be used to link VH and VL regions, or individual bNab moieties.

In some embodiments, the disclosure relates to an antibody comprising a variable portion and a constant portion, the variable portion comprising any one or plurality of

variable heavy and/or light antibody fragments (such as a scFv fragment) capable of binding to the epitopes disclosed herein, and the constant portion comprising an IgG-like domain.

5 The antibody can be modified to improve certain biological properties of the antibody, e.g., to improve stability, to enhance or reduce effector functions such as antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody, improved or decreased internalization and/or recycling, among others.

10 In certain embodiments, the multispecific antibody as described herein further includes a modification in the Fc region. In certain embodiments, the multispecific antibodies of the disclosure include mutations that increase binding to the neonatal Fc receptor (FcRn), which recycles IgG in intestinal epithelial cells and increases levels in the serum, extended half-life, enhanced mucosal localization, and conferred more efficient protection against lentivirus infection relative to the wild-type antibody.
15 Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. Such mutations are described, for example in Ko et al. (Nature 514, 642-645 (2014)).

For example, the Fc fragment of some antibodies (derived from human Ig4) can be replaced with human IgG1 that increases effector function mediated through FcRs
20 (except FcRn). Such modification may improve the stability of the resulting antibody by about 5 fold. In another example, the IgG1 Fc fragment can be modified to improve the recycling of the antibody via the antibody salvage pathway.

Still another type of modification involves alteration of the glycosylation pattern of a parent antibody, including deletions of one or more carbohydrate moieties found in
25 the parent antibody, or addition of one or more carbohydrates (via addition of one or more glycosylation sites) that are not present in the parent antibody.

In some embodiments, “knob into hole” technology (J. B. Ridgway, L. G. Presta, P. Carter, 'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng 9, 617-621 (1996)) can be utilized to generate a
30 heterodimer of the “Forward” and “Reverse” PentaNAb arms, with LS mutations

(M428L/N434S) in the Fc fragment for elongated antibody in vivo half-lives as well as improved biodistribution into the mucosal compartment. The use of this “knob into hole” technology is not limiting any method of generating PentaNAb dimers known to persons of skill in the art may be used.

5 PentaNAbs fused to a C-terminal fragment crystallizable region (Fc) region of IgG1, which may or may not contain a Hinge, CH2, and CH3 domains, can accommodate effector functions in a Penta-IgG format. The Fc region may or may not be fused to the C-terminus of each pentaNAb. Such recombinant formats are known to persons of skill in the art. The formats may include, but are not limited to the use of diabodies, minibodies,
10 scFab, or scFv-zippers. For example, the types of recombinant formats may be found in the literature (*See Frenzel A, Hust M, and Schirrmann T., Frontiers in Immunology. 2013; 4:217*).

In some embodiments, two Forward PentaNAbs can be combined through the use of the Fc regions of IgG1. In some embodiments, one Forward PentaNAb can be
15 combined with one Reverse PentaNAb through the use of Fc regions of IgG1. The combinations of Forward and Reverse PentaNAbs are advantageous but not limiting.

Polynucleotides

In some embodiments, the invention encompasses polynucleotides comprising polynucleotides that encode a polypeptide as described herein, such as a sequence of an
20 HIV antibody or a fragment of such a polypeptide. For example, in some embodiments, the invention provides a polynucleotide comprising a nucleic acid sequence that encodes a multispecific antibody to HIV Env protein or encodes a fragment of such an antibody. The polynucleotides of the invention can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or
25 single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

In some embodiments, the polynucleotides are isolated. In certain embodiments, the polynucleotides are substantially pure.

In some embodiments, the invention provides isolated nucleic acid molecules that
30 encode multispecific anti-HIV antibodies as described herein. In some embodiments, the

invention provides isolated nucleic acid molecules that encode isolated variable domains of the antibodies described herein. In some embodiments, the nucleotide sequence is selected from any one of SEQ ID NOS:52-71 and SEQ ID NOS:81-89, as shown below in Table 4 and below.

5 In some embodiments, the invention provides a polynucleotide comprising a polynucleotide encoding a polypeptide comprising a sequence selected from the group consisting of SEQ ID NOS:1-51 and 72-80.

In some embodiments, the invention provides a polynucleotide comprising a polynucleotide encoding a polypeptide comprising the heavy or light chain variable
10 region selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17 21, 25, 29, 33, 37, 41, 45, 49 and 51.

Also provided is a polynucleotide encoding a polypeptide comprising the heavy or light chain variable region having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to any of SEQ ID NOS:
15 1, 5, 9, 13, 17 21, 25, 29, 33, 37, 41, 45, 49 and 51.

In some embodiments, the invention provides a polynucleotide comprising a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:72-80 and SEQ ID NOS: 90, 92, 94, 96, 98, 99 and 107.

20 Also provided is a polynucleotide encoding a polypeptide having at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to any of SEQ ID NOS:72-80 and SEQ ID NOS: 90, 92, 94, 96, 98, 99 and 107.

The invention further provides a polynucleotide comprising a sequence selected
25 from the group consisting of SEQ ID NOS:52-71 and SEQ ID NOS:81-89.

Also provided is a polynucleotide having at least 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to any of SEQ ID NOS:52-71, SEQ ID NOS:81-89 and SEQ ID NOS 91, 93, 95, 97 and 106.

In some embodiments the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a polynucleotide which aids, for example, in expression and secretion of a polypeptide from a host cell (e.g. a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell). The polypeptide having a leader sequence is a preprotein and can have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides can also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

In certain embodiments the polynucleotides comprise the coding sequence for the mature polypeptide fused in the same reading frame to a marker sequence that allows, for example, for purification of the encoded polypeptide. For example, the marker sequence can be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or the marker sequence can be a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g. COS-7 cells) is used.

The present invention further relates to variants of the hereinabove described polynucleotides encoding, for example, fragments, analogs, and derivatives.

The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments the polynucleotide variants contain alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. In some embodiments, nucleotide variants are produced by silent substitutions due to the degeneracy of the genetic code. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Vectors and cells comprising the polynucleotides described herein are also provided. The term "vector" means a construct, which is capable of delivering, and expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression

- vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells. "Vector" also includes shuttle and expression vectors. In some embodiments, the vector is a plasmid construct and also includes an origin of replication (e.g., the ColE1 origin of replication) and a selectable marker (e.g., ampicillin or tetracycline resistance), for replication and selection, respectively. An "expression vector" refers to a vector that contains the necessary control sequences or regulatory elements for expression of the antibodies including antibody fragments of the invention, in bacterial or eukaryotic cells.

10

SEQ ID NO	DESCRIPTION	NUCLEOTIDE SEQUENCE
52	VRC26.25 VH (Original Seq)	caggtgcagttggtggagtcctgggggagggcgtgggtccagcctgggacgtcc ctgagactctcctgtgcagcctctcaattcaggtttgatgggtatggcatg cactgggtccggccaggccccaggcaagggtgagtggtggcatctata tcacatgatggaattaaaaagtatcacgcagaaaaagtgtggggccgcttc accatctccagagacaattccaagaacacactgtatctacaaatgaacagc ctgcgacctgaggacacggctctctactactgtgcgaaagatttgcgagaa gacgaatgtgaagagtgggtggcggatgattttgggaaacaactcccttgc gcaaagtcacggcgccgcttgggttgaattgctgataactggggccaaggg acaatgggtcacctgtctctca
53	VRC26.25 VL (Original Seq)	cagtctgtgctgacgcagcgcgcctcagtgctgctgggccccaggacagaag gtcaccatctcctgtctctggaaacacctccaacattggcaataattttgtg tcctggtaaccaacagcgcgcgcgcgcagagcccccaactcctcatttatgaa actgacaagcgaccctcagggattcctgaccgattctctgcttccaagtct ggtacgtcaggcacccctggccatcacccgggtgcagactggggacgaggcc gattattactgcccacatgggctgccagcctgagttccgcgcgtgtcttc ggaactgggaccaaggtcatcgtccta
54	35O22 VH (Original Seq)	caggggtcaactagtcagtcctggagctgaattgaaaaagcctggcgccctcg gtgaagatttctctgtaagacttcgggttatagggttaatttctatcatatt aattggattcgacaaaactgcaggacgtggacctgagtggtggatggatgac agcccttacagtggtgacaaaaacctcgacactgcctttcaagacagagtc attatgacgacagacacagaagtcctctgtgacctcattcacgtccacgggc gcagcctacatggaaataaggaacctgaaatttgacgacacaggcacctat ttctgtgcaaaaggcctcctgctgacgggtcgtcgacgtggcttcccttat ttgtggggccagggtaccctactcacctgtctcgtca
55	35O22 VL (Original Seq)	cagtctgtgctgacgcagtcctgcctccgtgtctctgggtctcttggacagtcg gtcaccatctcctgcactggaccaatagtggttggcagtcacaaatct atctcctgggtatcagtggtggccccaggcagagccccacactcatcatttat gaggacaatgaaagggccccgggaatctctcctcgcttctctgggtacaag tcgtattgggtcgccctacttgacaatctctgatctccggcctgaagacgag accacttactactgttgtcctacactcacaatagcggctgtgtcttcggg actggggaccaaggtctccgtcttg
56	10E8v4 VH (Original Seq)	gaggttagactgggtggagtcaggaggggggcttgtgaagcccggtgggtct ctccgcctgagctgttctgcctccggcttggatttcgataacgcctggatg acctgggtcaggcagcctccaggttaagggaactggagtggtgggaagaatc acaggtccaggcgagggtggtccgtggactacgcggaatctgttaaaggg cggtttacaatctcaagggacaataccaagaataccttgatattggagatg aacaacgtgagaactgaagacaccggatattacttctgtgccagaacaggc aaatactacgacttctgggtccggctatccccctggcgaggaatattttcaa gactggggtcagggaacccttgggtatcgtgtcctcc

57	10E8v4 VL (Original Seq)	tccgaactgactcaggaccctgccgtctctgtggcactgaagcagactgtg actattacttggcaggcgactcactcggagccactacgttctctggat cagaagaaacccggccaggcacctgtgctgttctacggaaagaacat aggccatctggcatccccgaccgcttttctggcagtgcacagggaaccga gccagtctgaccattaccggcgccaggctgaggacgaagccgattactat tgacgtctccgggataagagcggctccagactgagcgtgttcggaggagga actaaactgaccgtcctc
58	VRC26.25 VH (Optimized Seq 1)	cagggtgcagctgggtggagagcggcgccggcgtgggtgcagccaggcacctcc ctgaggctgtcttctgtgcagcaagccagttcagatttgatggctacggcatg cactgggtgcccaggcaccaggcaaggcctggagtgggtagcctctatc agccacgacggcatcaagaagtaccacgcccagagaaagtgtggggcagggtc accatctcccgcgataactctaaaaacacactgtatctgcagatgaactcc ctgaggcccaggacacccgacctgtactattgcgccaaggacctgcgcgag gatgagtgtgaggagtgggtgggtccgacgattttggcaagcagctgccttgc gcaaagagcaggggaggcctgggtgggaatcgccgataattggggccagggc accatgggtgacagtgtctagc
59	VRC26.25 VH (Optimized Seq 2)	cagggtgcagctgggtggagtccggaggaggagtgggtgcagccaggcacctct ctgaggctgagctgtgcagcatcccagttcagatttgatggctacggatg cactgggtgaggcaggcaccaggcaaggcctggagtgggtggccagcatc tcccacgacggcatcaagaagtaccacgcccagagaaagtgtggggcagggtc accatctctcgcgataaacgacaagaatacactgtatctgcagatgaacagc ctgaggcccaggacacccgacctgtactattgcgccaaggacctgcgcgag gatgagtgtgaggagtgggtgggtccgacgattttggcaagcagctgccttgc gcaaagagcaggggaggactgggtgggaatcgccgacaattggggccagggc accatgggtgacagtgtctagc
60	VRC26.25 VL (Optimized Seq 1)	cagagcgtgctgacccagccaccttccgtgtctgcgcgaccaggacagaag gtgaccatcagctgttccggcaacacatccaatatcggaacaaatttcgtg tcttggtaccagcagaggccagggaaggccaccacagctgctgatctatgag acagacaagcggccttccggcatcccagatagattttctgccagcaagtcc ggcaccagcggcacactggcaatcaccggcctgcagacaggcgacgaagct gattactattgcgcaacctgggcagcctccctgagctccgccagggtgttc ggaaccggacaacaaagtgtatcgtgctggtg
61	VRC26.25 VL (Optimized Seq 2)	cagagcgtgctgacccagccaccttccgtgtctgcgcgaccaggacagaag gtgaccatcagctgttccggcaacacatccaatatcggaacaaatttcgtg tcttggtaccagcagaggcctggaagagcaccacagctgctgatctatgag acagacaagaggccctccggcatccctgatcgcttttctgccagcaagtcc ggcaccagcggcacactggcaatcaccggactgcagacaggcgacgaggca gattactattgcgcaacctgggcagcctccctgtctagcgccagggtgttc ggcaccggcacaaaagtgtatcgtgctggtg
62	PGDM1400 VH (Optimized Seq 1)	caggcacagctgggtgcagagcggaccagaggtgaggaagccaggcacctct gtgaaggtgagctgtgaagccctggcaacaccctgaagacatacgcctg cactgggtgcggtctgtgcccaggacaggcctgcagtggatgggatggatc tctcacgagggcgacaagaaagtgtatcgtggagcgggtcaaggccaaggtg acaatcgattgggacagatccaccaacacagcctacctgcagctgtctggc ctgaccagcggcgatacagccgtgtactactgtgccaaagggtcttaagcac cggctgagagactacgcccctggacgatgacggcgccctgaactgggcccgtg gatgtggactatctgtccaatctggagtcttggggacagggaaaccgcagtg acagtgtctagc
63	PGDM1400 VH (Optimized Seq 2)	caggcacagctgggtgcagagcggaccagaggtgaggaagccaggcacctct gtgaaggtgagctgtgaagccctggcaacaccctgaagacatacgcctg cactgggtgcggtctgtgcccaggacaggcctgcagtggatgggatggatc agccacgagggcgacaagaaagtgtatcgtggagcgggttaaggccaaggtg acaatcgattgggacagaagcaccatacagcctatctgcagctgtccggc ctgacctctggcgatacagccgtgtactattgcgccaagggtctccaagcac cggctgagagactacgcccctggacgatgacggcgccctgaattgggcagtg gacgtggactatctgagtaatctggagtcttgggggacagggcaccgcagtg acagtgtctagc
64	PGDM1400 VL (Optimized Seq 1)	gattttgtgctgacccagctctccacacagcctgtccgtgacacccggcgag tctgccagcatctctgcaagtctagccacagcctgatccacggcgacagg aacaattacctggcctgggtacgtgcagaagccaggccgacccctcagctg ctgatctatctgtgcatcctctagggcctccggagtccagatcgcttctct ggcagcggctccgataaaggactttaccctgaagatcagccgggtggagaca gaggacgtgggcacatactattgtatgcaggggccgagaatcaccttgga tttgggcagggaaactaaagtgcacatcaaa

65	PGDM1400 VL (Optimized Seq 2)	gatttcgtgctgaccagctctccacatagctctgagcgtgacacccggcgaa agcgcatcaattttcttgtaaatcatctcatagctctgatccacggcgatagg aacaattacctggcctggtagctgcagaagccaggcccgagccctcagctg ctgatctacctggcaagctccaggggcatccggagtgccagatcgcttctct ggcagcggctccgataaggactttaccctgaagatctccgggtggagaca gaggacgtgggcacatactattgcatgcagggcagagagctctccttgacc ttcggccagggcacaaagggtggacatcaag
66	35O22 VH (Optimized Seq 1)	cagggccagctgggtgcagagcggagcagagctgaagaagcctggagccagc gtgaagatctcctgttaagacatctggctaccgggttcaacttttatcacatc aattggatcaggcagaccgcaggaaggggaccagagtggatgggctggatc tccccctactctggcgataagaacctggccccagccttcaggacagagtg atcatgaccacagataccgaggtgcccagtgaccagcttcacctccaccgga gccgcctacatggagatcaggaatctgaagttcgacgatacaggcacctat ttttgcgcaaagggcctgctgagggacggctcctctacctggctgccttac ctgtggggacagggcacccctgctgacagtgcctcc
67	35O22 VH (Optimized Seq 2)	cagggccagctgggtgcagagcggagcagagctgaagaagccaggagcctct gtgaagatcagctgttaagacatccggctaccgggttcaacttttatcacatc aattggatcaggcagaccgcaggaaggggaccagagtggatgggctggatc tccccctactctggcgataagaacctggccccagccttcaggacagagtg atcatgaccacagataccgaggtgcccagtgaccagcttcacctccaccgga gccgcctacatggagatccggaatctgaagttcgacgatacaggcacctat ttttgcgcaaagggcctgctgagagacggctctagcacatggctgccatac ctgtggggacagggcacccctgctgacagtgcctct
68	35O22 VL (Optimized Seq 1)	cagtcctgtgctgaccagctctgccagcgtgtccggctctctgggacagagc gtgaccatctcctgtacaggccccaacagcgtgtgctgtagccacaagagc atctcctggtagcagtggtccaccaggaagggcacctaccctgatcatctat gaggacaatgagcgggccccaggcatctccccagattctctggtacaag tcttattggagcgcctacctgacaatcagcgcctgcgccccgaggatgag acaacatactattgctgttctctatacccaactctggctgcgtgtttggc acaggcaccaaggtgtccgtgctg
69	35O22 VL (Optimized Seq 2)	caagcgtgctgaccagtcgcctctctgtgagcggctccctgggcccagctct gtgaccatcagctgtacaggccccaactcctgtgtgctgttctcacaagtct atcagctgtgtagcagtggtccaccaggaagggcacctaccctgatcatctat gaggacaatgagagggcaccaggaatcagccctcgttctccggctacaag tcttattggagcgcctacctgaccatttccgacctgcgccccgaggatgag accacatactattgctgtagctatacccaactcctggctgcgtgtttggc acaggcaccaaggtgagcgtgctg
70	10E8v4_V5R_S1 00cF VH (Optimized Seq)	gagggtcggtgcgggagagcggcgcggtggtgaagccaggcggctct ctgagactgtcctgttctgccagcggcttcgactttgataatgcctggatg acatgggtgcggcagcctcctggcaaggggctggagtgggtgggaagaatc accggaccaggagagggatggtctgtggactacgcccagagcgtgaagggc cgggttcaccatcagcagagataaactaaaaatacactgtatctggagatg aacaatgtgcggaccgaggacacaggctactatttctgcgcagaaacggc aagtactatgatttctggtttggctacccccctggcgaggagtattttcag gactggggccagggcacccctggtcatcgtgagcagc
71	10E8v4 VL	agcagctgacccaggaccccgccgtgtccgtggccctgaagcagacagtg accatcacatgcagggcgactccctgcgctctcactacgccagctggtat cagaagaagccaggacaggcaccctgctgctgttctacggcaagaacaat cggccttcggcatcccagatagatttccggctctgccagcggaaacagg gccagcctgaccatcacaggagcacaggcagaggatgaagcagattactat tgttctctcgggacaagtcggctctagactgagcgtgttcggcggcgga accaagctgacagtgcctg

Table 4. Nucleotide Sequences of variable domain anti-HIV antibodies.

Nucleotide sequences of the Penta-Nabs shown in Table 3 are provided below.

1. PentaNAb_{1.0} 10E8v4-5X-35O22-5X-N6-5X-PGT121-5X-VRC26.25-His

SEQ ID NO:81

GAATTCGCCGCCACCATGGGATGGAGCTGTATTATTCTGTTTCTGGTCGCTAC
CGCTACCGGAGTGCATTCTTCTGAACTGACCCAGGACCCCGCCGTGAGCGTG
GCCCTGAAGCAGACCGTGACAATCACCTGCAGGGGCGACAGCCTGCGCTCCC
ACTACGCCAGCTGGTATCAGAAGAAGCCTGGCCAGGCCCCAGTGCTGCTGTT
5 CTACGGCAAGAACAATAGGCCCTCCGGCATCCCTGATCGCTTTTCCGGCTCTG
CCAGCGGAAACAGGGCCAGCCTGACAATCACCGGAGCACAGGCAGAGGACG
AGGCAGATTACTATTGCAGCTCCCGGGACAAGTCCGGCTCTAGACTGAGCGT
GTTTCGGCGGCGGCACCAAGCTGACAGTGCTGGGAGGAGGAGGCAGCGGCGG
AGGAGGCTCCGGAGGCGGCGGCTCTGAGGTGCGGCTGGTGGAGTCTGGAGG
10 AGGCCTGGTGAAGCCAGGAGGCAGCCTGAGACTGAGCTGTTCCGCCTCTGGC
TTCGACTTTGATAATGCCTGGATGACATGGGTGCGGCAGCCACCTGGCAAGG
GCCTGGAGTGGGTGGGAAGAATCACCGGACCAGGAGAGGGATGGTCTGTGG
ACTACGCCGAGAGCGTGAAGGGCCGGTTCACCATCTCCAGAGATAACACCAA
GAATACACTGTATCTGGAGATGAACAATGTGCGGACCGAGGACACAGGCTAC
15 TATTTCTGCGCCAGAACCGGCAAGTACTATGATTTTTGGAGCGGCTACCCACC
CGGCGAGGAGTATTTTCAGGACTGGGGCCAGGGCACCCCTGGTCATCGTGAGC
AGCGGCGGCGGCGGCAGCGGCGGCGGCTCCGGAGGAGGCGGCTCTGGA
GGAGGAGGCAGCGGGGGAGGGGGCAGCCAGTCCGTGCTGACCCAGTCTGCC
AGCGTGTCCGGCTCTCTGGGACAGAGCGTGACCATCTCCTGTACAGGCCCCA
20 ACAGCGTGTGCTGTAGCCACAAGAGCATCTCCTGGTACCAGTGGCCTCCAGG
AAGGGCACCTACCCTGATCATCTATGAGGACAATGAGCGGGCCCCAGGCATC
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CGACCTGCGCCCCGAGGATGAGACAACATACTATTGCTGTTCTATACCCACA
ACTCTGGCTGCGTGTTTGGCACAGGCACCAAGGTGTCCGTGCTGGGCGGCGG
25 CGGCAGCGGGGGCGGGGGCTCCGGAGGGGGCGGCTCTCAGGGCCAGCTGGT
GCAGAGCGGAGCAGAGCTGAAGAAGCCTGGAGCCAGCGTGAAGATCTCCTG
TAAGACATCTGGCTACCGGTTCAACTTTTATCACATCAATTGGATCAGGCAGA
CCGCAGGAAGGGGACCAGAGTGGATGGGCTGGATCTCCCCCTACTCTGGCGA
TAAGAACCTGGCCCCAGCCTTCCAGGACAGAGTGATCATGACCACAGATACC
30 GAGGTGCCAGTGACCAGCTTCACCTCCACCGGAGCCGCCTACATGGAGATCA
GGAATCTGAAGTTCGACGATACAGGCACCTATTTTTGCGCAAAGGGCCTGCT
GAGGGACGGCTCCTCTACCTGGCTGCCTTACCTGTGGGGACAGGGCACCCCTG

CTGACAGTGAGCTCCGGCGGCGGGGGCAGCGGCGGCGGGGGCTCCGGAGGA
GGAGGCTCTGGAGGAGGGGGCAGCGGAGGAGGCGGCTCCTACATCCACGTG
ACCCAGTCCCCATCTAGCCTGTCTGTGAGCATCGGCGATCGGGTGACCATCAA
CTGTCAGACATCTCAGGGCGTGGGCAGCGACCTGCACTGGTATCAGCACAAG
5 CCTGGCAGGGCCCCAAAGCTGCTGATCCACCACACATCCTCTGTGGAGGATG
GAGTGCCAAGCCGCTTCTCCGGCTCTGGATTCCACACCTCCTTTAATCTGACA
ATCTCTGACCTGCAGGCCGACGATATCGCCACCTACTATTGCCAGGTGCTGCA
GTTCTTTGGCCGGGGCTCCAGACTGCACATCAAGGGAGGAGGAGGCTCCGGG
GGCGGAGGCTCTGGCGGCGGCGGCAGCCGGGCCCACCTGGTGCAGAGCGGC
10 ACCGCCATGAAGAAGCCTGGCGCCAGCGTGAGAGTGTCTGTCAGACATCTG
GCTACACCTTCACCGCCCACATCCTGTTCTGGTTTAGGCAGGCACCAGGAAGA
GGCCTGGAGTGGGTGGGCTGGATCAAGCCCCAGTATGGAGCAGTGAACCTCG
GAGGAGGCTTTCGGGACAGAGTGACACTGACCCGGGACGTGTACAGAGAGA
TCGCCTATATGGATATCAGGGGCCTGAAGCCAGACGATACCGCCGTGTACTA
15 TTGCGCCAGGGACCGCTCCTACGGCGATAGCTCCTGGGCACTGGACGCATGG
GGACAGGGCACCACAGTGGTGGTGAGCGCCGGCGGCGGAGGCTCCGGCGGC
GGGGGCTCTGGAGGAGGCGGCAGCGGAGGGGGAGGCTCCGGAGGGGGAGGC
TCTAGCGACATCTCCGTGGCCCCCTGGCGAGACAGCCAGAATCTCTTGTGGCG
AGAAGTCTCTGGGCAGCAGGGCCGTGCAGTGGTACCAGCACAGGGCAGGAC
20 AGGCACCATCTCTGATCATCTATAACAATCAGGATAGGCCAAGCGGCATCCC
TGAGCGGTTTCAGCGGCTCCCCCGACAGCCCTTTTGGCACCACAGCCACACTG
ACCATCACATCCGTGGAGGCAGGCGACGAAGCCGATTACTATTGCCACATCT
GGGATTCCAGAGTGCCAACCAAGTGGGTGTTTCGGAGGAGGAACCACACTGAC
AGTGCTGGGAGGGGGGGGCTCTGGCGGCGGGGGCAGCGGGGGAGGAGGCTC
25 CCAGATGCAGCTGCAGGAGAGCGGACCAGGCCTGGTGAAGCCTAGCGAGAC
ACTGAGCCTGACATGTTCTGTGAGCGGCGCCTCCATCTCTGACAGCTACTGGT
CTTGATCAGACGGAGCCCCGGCAAGGGCCTGGAATGGATCGGCTACGTGCA
CAAGTCCGGCGATACAACTATTCCCCATCTCTGAAGTCTCGGGTGAACCTGT
CTCTGGACACCAGCAAGAATCAGGTGAGCCTGTCCCTGGTGGCAGCAACCGC
30 AGCAGATAGCGGCAAGTACTATTGCGCCAGAACACTGCACGGCAGGCGCATC
TACGGCATCGTGGCCTTTAACGAGTGGTTCACCTACTTTTATATGGACGTGTG
GGGCAATGGCACCCAGGTGACAGTGTCTCTGGCGGGGGCGGCTCCGGAGGC

GGAGGCTCTGGCGGGGGCGGCAGCGGCGGGGGCGGCTCCGGGGGAGGCGGC
TCTCAGAGCGTGCTGACCCAGCCACCTTCCGTGTCTGCCGCACCAGGACAGA
AGGTGACCATCAGCTGTTCCGGCAACACATCCAATATCGGCAACAATTCGT
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5 ACAGACAAGCGGCCTTCCGGCATCCCAGATAGATTTTCTGCCAGCAAGTCCG
GCACCAGCGGCACACTGGCAATCACCGGCCTGCAGACAGGCGACGAAGCTG
ATTACTATTGCGCAACCTGGGCAGCCTCCCTGAGCTCCGCCAGGGTGTTCGGA
ACCGGAACAAAAGTGATCGTGCTGGTGGGCGGCGGAGGCTCTGGCGGAGGC
GGCAGCGGCGGGGGGGGCTCCCAGGTGCAGCTGGTGGAGAGCGGCGGCGGC
10 GTGGTGCAGCCAGGCACCTCCCTGAGGCTGTCTTGTGCAGCAAGCCAGTTCA
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ATCTGCAGATGAACTCCCTGAGGCCCCGAGGACACCGCCCTGTACTATTGCGC
15 CAAGGACCTGCGCGAGGATGAGTGTGAGGAGTGGTGGTCCGACTACTATGAT
TTTGGCAAGCAGCTGCCTTGCGCAAAGAGCAGGGGAGGCCTGGTGGGAATCG
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CCACCATCACCATCATTAGTGAAAGCTT

2. PentaNAb_{2.0} 10E8v4__S100cF-5X-35O22-5X-N6-5X-PGT121-5X-VRC26.25-His
20 SEQ ID NO:82

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CGCTACCGGAGTGCATTCTTCTGAACTGACCCAGGACCCCGCCGTGAGCGTG
GCCCTGAAGCAGACCGTGACAATCACCTGCAGGGGCGACAGCCTGCGCTCCC
ACTACGCCAGCTGGTATCAGAAGAAGCCTGGCCAGGCCCCAGTGCTGCTGTT
25 CTACGGCAAGAACAATAGGCCCTCCGGCATCCCTGATCGCTTTTCCGGCTCTG
CCAGCGGAAACAGGGCCAGCCTGACAATCACCGGAGCACAGGCAGAGGACG
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AGGAGGCTCCGGAGGCGGCGGCTCTGAGGTGCGGCTGGTGGAGTCTGGAGG
30 AGGCCTGGTGAAGCCAGGAGGCAGCCTGAGACTGAGCTGTTCCGCCTCTGGC
TTCGACTTTGATAATGCCTGGATGACATGGGTGCGGCAGCCACCTGGCAAGG
GCCTGGAGTGGGTGGGAAGAATCACCGGACCAGGAGAGGGATGGTCTGTGG

ACTACGCCGAGAGCGTGAAGGGCCGGTTCACCATCTCCAGAGATAACACCAA
GAATACACTGTATCTGGAGATGAACAATGTGCGGACCGAGGACACAGGCTAC
TATTTCTGCGCCAGAACCGGCAAGTACTATGATTTTTGGTTTGGCTACCCACC
CGGCGAGGAGTATTTTCAGGACTGGGGCCAGGGCACCCCTGGTCATCGTGAGC
5 AGCGGCGGCGGCGGCAGCGGCGGCGGCGGCTCCGGAGGAGGCGGCTCTGGA
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20 GAGGGACGGCTCCTCTACCTGGCTGCCTTACCTGTGGGGACAGGGCACCCCTG
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GAGTGCCAAGCCGCTTCTCCGGCTCTGGATTCCACACCTCCTTTAATCTGACA
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30 ACCGCCATGAAGAAGCCTGGCGCCAGCGTGAGAGTGTCTGTGAGACATCTG
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10 ACCATCACATCCGTGGAGGCAGGCGACGAAGCCGATTACTATTGCCACATCT
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15 CTTGGATCAGACGGAGCCCCGGCAAGGGCCTGGAATGGATCGGCTACGTGCA
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20 GGGCAATGGCACCCAGGTGACAGTGTCTCTGGCGGGGGCGGCTCCGGAGGC
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3. PentaNAb_{2.0} Reverse VRC26.25-5X-PGT121-5X-N6-5X-35O22-5X-
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25 SEQ ID NO:83
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 20 AGTGAAGCTT

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 SEQ ID NO:84

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5 5. PentaNAb_{3.0} Reverse PGDM1400-5X-PGT121-5X-N6-5X-35O22-5X-
10E8v4S100cF-His
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SEQ ID NO:88

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 5 cacacaagctccgtggaggatggagtccaagccgcttcagcggctccggattccacac
 agctttaatctgacaatctccgacctgcaggccgacgatatcgccacctactattgccag
 gtgctgcagttcttggcaggggctccgcctgcacatcaaggcgggcgggcggtctggg
 gggcgggggcagcggcgggggggggctccgggggaggaggctctggcgaggggggcagccag
 ggccagctggtagagcggagcagagctgaagaagccaggagcctctgtgaagatcagc
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 ggaaggggaccagagtggatgggtggatctcccctactctggcgataagaacctggcc
 ccagccttcaggacagagtgatcatgaccacagataccgaggtgccagtgaccagcttc
 acctccaccggagccgctacatggagatccggaatctgaagttcgacgatacaggcacc
 tatttttgcgccaagggcctgctgagagacggctctagcacatggctgccatacctgtgg
 15 ggacagggcacctgctgacagtgtcctctggaggaggaggctccggggcgggcggtct
 ggaggaggaggctctcaaagcgtgctgaccagtcgcctctgtgagcggtccctgggc
 cagtctgtaccatcagctgtacaggcccaactccgtgtgctgttctcacaagtctatc
 agctggtaccagtggccaccaggaagggcacctaccctgatcatctatgaggacaatgag
 agggcaccaggaatcagccctcgcttctccggctacaagtctattggagcgctacctg
 20 accatttccgacctgcgccccgaggatgagaccacatactattgctgtagtatacccac
 aactccggctgctgtttggcacaggcaccaaggtgagcgtgctgggaggagggggctct
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 tctgttctgccagcggttcgactttgataatgcctggatgacatgggtgcggcagcct
 25 cctggcaaggggctggagtgggtgggaagaatcaccggaccaggagaggatggtctgtg
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 ctgtatctggagatgaacaatgtgcggaccgaggacacaggctactatttctgcgccaga
 accggcaagtactatgatttctggttggctacccccctggcgaggagtatttccaggac
 tggggccagggcaccctggatcatcgtgagcagcgggggggaggctccggcgggggggggc
 30 tctggaggagggggctctagcgagctgaccaggaccccccggtgtccgtggccctgaag
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 cagaagaagccaggacaggcacccgtgctgctgttctacggcaagaacaatcggccttc

ggcatcccagatagattttccggctctgccagcggaaacagggccagcctgaccatcaca
 ggagcacaggcagaggatgaagcagattactattgttcctctcgggacaagtccggctct
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 AACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGGACC
 5 GTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCC
 CGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGA
 CCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA
 TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTG
 TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGG
 10 AGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGA
 AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTAC
 ACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCT
 GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG
 GGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCG
 15 TGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGA
 CAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGCTGCA
 TGAGGCTCTGCACAGCCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
 GGGTAAATGA

Methods for producing antibodies, such as those disclosed herein, are known in the
 20 art. For example, DNA molecules encoding light chain variable regions and/or heavy
 chain variable regions can be chemically synthesized using the sequence information
 provided herein. Synthetic DNA molecules can be ligated to other appropriate nucleotide
 sequences, including, e.g., expression control sequences, to produce conventional gene
 expression constructs encoding the desired antibodies. Production of defined gene
 25 constructs is within routine skill in the art. Alternatively, the sequences provided herein
 can be cloned out of hybridomas by conventional hybridization techniques or polymerase
 chain reaction (PCR) techniques, using synthetic nucleic acid probes whose sequences are
 based on sequence information provided herein, or prior art sequence information
 regarding genes encoding the heavy and light chains.

30 Standard techniques of molecular biology may be used to prepare DNA sequences
 coding for the antibodies or fragments of the antibodies of the present invention. Desired
 DNA sequences may be synthesized completely or in part using oligonucleotide synthesis

techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecules of the present invention or fragments thereof.

5 Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and F(ab')₂ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, e.g. mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. Suitable mammalian
10 host cells include CHO, HEK293T, PER.C6, myeloma or hybridoma cells.

In some embodiments, antibodies according to the invention may be produced by i) expressing a nucleic acid sequence according to the invention in a cell, and ii) isolating the expressed antibody product. Additionally, the method may include iii) purifying the antibody.

15 For the antibodies of the present invention to be expressed, the protein coding sequence should be "operably linked" to regulatory or nucleic acid control sequences that direct transcription and translation of the protein. As used herein, a coding sequence and a nucleic acid control sequence or promoter are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or
20 translation of the coding sequence under the influence or control of the nucleic acid control sequence. The "nucleic acid control sequence" can be any nucleic acid element, such as, but not limited to promoters, enhancers, IRES, introns, and other elements described herein that direct the expression of a nucleic acid sequence or coding sequence that is operably linked thereto. The term "promoter" will be used herein to refer to a
25 group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II and that when operationally linked to the protein coding sequences of the invention lead to the expression of the encoded protein. The expression of the antibodies of the present invention can be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when exposed to some
30 particular external stimulus, such as, without limitation, antibiotics such as tetracycline, hormones such as ecdysone, or heavy metals. The promoter can also be specific to a

particular cell-type, tissue or organ. Many suitable promoters and enhancers are known in the art, and any such suitable promoter or enhancer may be used for expression of the antibodies of the invention. For example, suitable promoters and/or enhancers can be selected from the Eukaryotic Promoter Database (EPDB).

5 Nucleic acids encoding desired antibodies can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK 293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and myeloma cells that do not otherwise produce IgG
10 protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions. Specific expression and purification conditions will vary depending upon the expression system employed.

15 Following expression, the antibodies and/or antigens of the invention can be isolated and/or purified or concentrated using any suitable technique known in the art. For example, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, immuno-affinity chromatography, hydroxyapatite chromatography, lectin chromatography, molecular
20 sieve chromatography, isoelectric focusing, gel electrophoresis, or any other suitable method or combination of methods can be used.

In some embodiments, the antibodies can be made using recombinant DNA methods as described in U.S. Pat. No. 4,816,567. The polynucleotides encoding a monoclonal antibody can be isolated from mature B-cells or hybridoma cell, such as by
25 RT-PCR using oligonucleotide primers that specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using conventional procedures. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors, which when transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or
30 myeloma cells that do not otherwise produce immunoglobulin protein, monoclonal antibodies are generated by the host cells.

The anti-HIV antibodies can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues. It should be understood that the antibodies of the invention may differ from the exact sequences illustrated and described herein. Thus, the invention contemplates deletions, additions and substitutions to the sequences shown, so long as the sequences function in accordance with the methods of the invention. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, leucine can be replaced with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid can be made.

The polynucleotide(s) encoding a monoclonal antibody can further be modified in a number of different manners using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light and heavy chains of, for example, a mouse monoclonal antibody can be substituted 1) for those regions of, for example, a human antibody to generate a chimeric antibody or 2) for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate the desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region can be used to optimize specificity, affinity, etc. of a monoclonal antibody.

For the purposes of the present invention, it should be appreciated that modified antibodies can comprise any type of variable region that provides for the association of the antibody with the polypeptides of HIV such as the envelope protein.

Besides their configuration, it is known in the art that the constant region mediates several effector functions. For example, binding of the C1 component of complement to antibodies activates the complement system. Activation of complement is important in the

opsonisation and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can also be involved in autoimmune hypersensitivity. Further, antibodies bind to cells via the Fc region, with a Fc receptor site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which
5 are specific for different classes of antibody, including IgG (gamma receptors), IgE (eta receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent
10 cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

In certain embodiments, the anti-HIV antibodies provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, the deletion or inactivation (through point mutations or other means) of a
15 constant region domain can reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it may be that constant region modifications, consistent with this invention, moderate complement binding and thus reduce the serum half-life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region can be used to eliminate disulfide linkages or
20 oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. Similarly, modifications to the constant region in accordance with this invention can easily be made using well known biochemical or molecular engineering techniques well within the purview of the skilled artisan.

Methods

25 The multispecific anti-HIV antibodies of the invention are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as the treatment, cure, functional cure, or prevention of HIV infection. The methods of use may be in vitro, ex vivo, or in vivo methods.

In some embodiments, the multispecific antibodies disclosed herein may be used
30 as neutralizing antibodies, passively administered or given via gene therapies.

In one aspect, the anti-HIV antibodies are useful for detecting the presence of HIV in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue.

5 Certain other methods can be used to detect binding of anti-HIV antibodies to antigens such as envelope protein. Such methods include, but are not limited to, antigen-binding assays that are well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and
10 immunohistochemistry (IHC).

 In certain embodiments, the antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or
15 molecular interaction.

 In certain embodiments, the antibodies are immobilized on an insoluble matrix. Immobilization entails separating the antibody from any antigen that remains free in solution. This conventionally is accomplished by either insolubilizing the antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et
20 al., U.S. Pat. No. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the antibody after formation of a complex between the antibody and antigen, e.g., by immunoprecipitation.

 The present invention provides for methods of treating or preventing HIV infection comprising administering a therapeutically effective amount of a multispecific
25 antibody as described herein to a subject (e.g., a subject in need of treatment). In some embodiments, the subject is a human.

 Subjects at risk for HIV-related diseases or disorders include patients who have come into contact with an infected person or who have been exposed to HIV-1 in some other way. Administration of a prophylactic agent can occur prior to the manifestation of

symptoms characteristic of HIV-1-related disease or disorder, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

Methods for preventing an increase in HIV-1 virus titer, virus replication, virus proliferation or an amount of an HIV-1 viral protein in a subject are further provided. In one embodiment, a method includes administering to the subject an amount of an anti-HIV antibody effective to prevent an increase in HIV-1 titer, virus replication or an amount of an HIV-1 protein of one or more HIV strains or isolates in the subject.

For in vivo treatment of human patients, the patient is usually administered or provided a pharmaceutical formulation including a multispecific anti-HIV antibody of the invention. When used for in vivo therapy, the antibodies of the invention are administered to the patient in therapeutically effective amounts (i.e., amounts that eliminate or reduce the patient's viral burden). The antibodies can be administered to a human patient, in accordance with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The antibodies may be administered parenterally, when possible, at the target cell site, or intravenously. Intravenous or subcutaneous administration of the antibody is preferred in certain embodiments. Therapeutic compositions of the invention are administered to a patient or subject systemically, parenterally, or locally.

For parenteral administration, the antibodies can be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable, parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils and ethyl oleate are also used. Liposomes are used as carriers. The vehicle contains minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. In some embodiments, the antibodies are typically formulated in such vehicles at concentrations of about 1 mg/ml to 10 mg/ml.

The dose and dosage regimen depends upon a variety of factors readily determined by a physician, such as the nature of the infection and the characteristics of the particular cytotoxic agent or growth inhibitory agent conjugated to the antibody (when used), e.g., its therapeutic index, the patient, and the patient's history. Generally, a

therapeutically effective amount of an antibody is administered to a patient. In particular embodiments, the amount of antibody administered is in the range of about 0.1 mg/kg to about 20 mg/kg of patient body weight. Depending on the type and severity of the infection, about 0.1 mg/kg to about 20 mg/kg body weight (e.g., about 0.1-15 mg/kg/dose) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. The progress of this therapy is readily monitored by conventional methods and assays and based on criteria known to the physician or other persons of skill in the art.

Antibodies of the invention can be coupled to a drug for delivery to a treatment site or coupled to a detectable label to facilitate imaging of a site comprising cells of interest, such as cells infected with HIV. Methods for coupling antibodies to drugs and detectable labels are well known in the art, as are methods for imaging using detectable labels. Labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an antibody of the invention and an epitope of interest (an HIV epitope) can be facilitated by attaching a detectable substance to the antibody. Suitable detection means include the use of labels such as radionucleotides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S , or ^3H . Such labeled reagents may be used in a variety of well-known assays, such as radioimmunoassays, enzyme immunoassays, e.g., ELISA, fluorescent immunoassays, and the like.

The antibodies can be tagged with such labels by known methods. For instance, coupling agents such as aldehydes, carbodiimides, dimaleimide, imidates, succinimides,

bid-diazotized benzadine and the like are used to tag the antibodies with the above-described fluorescent, chemiluminescent, and enzyme labels. An enzyme is typically combined with an antibody using bridging molecules such as carbodiimides, periodate, diisocyanates, glutaraldehyde and the like. Various labeling techniques are described in Morrison, *Methods in Enzymology* 32b, 103 (1974), Syvanen et al., *J. Biol. Chem.* 284, 3762 (1973) and Bolton and Hunter, *Biochem J.* 133, 529(1973).

In one embodiment, the antibodies can be administered as immunoconjugates, conjugated to a second molecule. For example, the second molecule can be a toxin, a label, a radioisotope, a drug, or a chemical compound.

10 An antibody according to the invention may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive metal ion or radioisotope. Examples of radioisotopes include, but are not limited to, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, Bi-213, Pd-109, Tc-99, In-111, and the like. Such antibody conjugates can be used for modifying a given biological response; the drug
15 moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, TLR agonists (such as TLR7 agonist), or monomethylauristatin E.

20 Other therapeutic regimens can be combined with the administration of the anti-HIV antibody of the present invention. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preferably such
25 combined therapy results in a synergistic therapeutic effect.

For any application, the antibody, antigen binding fragment, or nucleic acid encoding the antibody or antigen binding fragment 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
30 with nucleoside analog reverse-transcriptase inhibitors (such as zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine, entecavir, and apricitabine),

nucleotide reverse transcriptase inhibitors (such as tenofovir and adefovir), non-nucleoside reverse transcriptase inhibitors (such as efavirenz, nevirapine, delavirdine, etravirine, and rilpivirine), protease inhibitors (such as saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, fosamprenavir, atazanavir, tipranavir, and darunavir),
5 entry or fusion inhibitors (such as maraviroc and enfuvirtide), maturation inhibitors, (such as bevirimat and vivecon), or a broad spectrum inhibitors, such as natural antivirals. In some examples, a disclosed antibody or active fragment thereof or nucleic acids encoding such is administered in conjunction with IL-15, or conjugated to IL-15.

Single or multiple administrations of the compositions including the antibody,
10 antigen binding fragment, or nucleic acid encoding the antibody or antigen binding fragment, that are 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 treat the patient. The dosage can be administered once, but may be applied
15 periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy.

One approach to administration of nucleic acids is direct administration with plasmid DNA, such as with a mammalian expression plasmid. The nucleotide sequence encoding the disclosed antibody, or antibody binding fragments thereof, can be placed
20 under the control of a promoter to increase expression. Another approach is to administer the nucleic acids in the form of mRNA.

In some embodiments, the subject is administered cells that are engineered to express the anti-HIV antibody. In some embodiments, the cells are engineered immune cells, such as B cells. In some embodiments, the cells are engineered, autologous cells.

25 In another approach to using nucleic acids, an anti-HIV antibody, or antibody binding fragment thereof can also be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, cytomegalovirus or other viral vectors can be used to express the antibody. For example, vaccinia vectors and methods useful protocols are described in
30 U.S. Pat. No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the disclosed antibodies (see Stover, Nature 351:456-460, 1991).

Compositions

The present invention also encompasses compositions comprising one or more antibodies of the invention. In certain embodiments, the compositions are pharmaceutical compositions. In some embodiments, formulations are prepared for storage and use by combining an antibody with a pharmaceutically acceptable vehicle (e.g. carrier, excipient) (*Remington, The Science and Practice of Pharmacy 20th Edition* Mack Publishing, 2000). Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (e.g. octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight polypeptides (e.g. less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG).

For the treatment or prevention of HIV, the appropriate dosage of an antibody or combination of antibodies of the present invention can depend on a variety of factors, such as the severity and course of the disease, the responsiveness of the disease, whether the antibody or agent is administered for therapeutic or preventative purposes, previous therapy, patient's clinical history, and so on all at the discretion of the treating physician. The antibody or agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. In certain embodiments, dosage is from 0.01 μ g to 100 mg per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain embodiments, the antibody or combination of antibodies is given once every two weeks or once every three weeks. In certain

embodiments, the dosage of the antibody is from about 0.1 mg to about 20 mg per kg of body weight. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Effective dosages and schedules for administering embodiments of the present invention can be determined empirically. In some embodiments, and effective amount of one or more antibodies are administered to neutralize, treat, prevent or eradicate HIV infection. In some embodiments, compositions comprising one or more nucleic acid molecules of the invention are administered to the subject. In some embodiments, genetic constructs capable of inducing production of antibodies of the present invention may be administered to a patient in need thereof.

Controlled-release parenteral formulations 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 protein, such as a cytotoxin or a drug, 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, N.Y., pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, N.Y., pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the antibody 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
5 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 are known (see U.S. Pat. No. 5,055,303; U.S. Pat. No. 5,188,837; U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; U.S. Pat. No. 4,837,028; U.S. Pat. No. 4,957,735; U.S. Pat. No. 5,019,369; U.S. Pat. No. 5,055,303;
10 U.S. Pat. No. 5,514,670; U.S. Pat. No. 5,413,797; U.S. Pat. No. 5,268,164; U.S. Pat. No. 5,004,697; U.S. Pat. No. 4,902,505; U.S. Pat. No. 5,506,206; U.S. Pat. No. 5,271,961; U.S. Pat. No. 5,254,342 and U.S. Pat. No. 5,534,496).

In some embodiments, the compositions of the invention may be injectable suspensions, solutions, sprays, lyophilized powders, syrups, elixirs and the like. Any
15 suitable form of composition may be used. To prepare such a composition, a nucleic acid or vector of the invention, having the desired degree of purity, is mixed with one or more pharmaceutically acceptable carriers and/or excipients. The carriers and excipients must be "acceptable" in the sense of being compatible with the other ingredients of the composition. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at
20 the dosages and concentrations employed, and include, but are not limited to, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, or combinations thereof, buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride;
25 phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides,
30 disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes);

and/or non-ionic surfactants such as TWEEN™ PLURONICS™ or polyethylene glycol (PEG).

The compositions can be designed to introduce the antibodies, nucleic acids or expression vectors to a desired site of action and release it at an appropriate and controllable rate. Methods of preparing controlled-release formulations are known in the art. For example, controlled release preparations can be produced by the use of polymers to complex or absorb the immunogen and/or immunogenic composition. A controlled-release formulations can be prepared using appropriate macromolecules (for example, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine sulfate) known to provide the desired controlled release characteristics or release profile. Another possible method to control the duration of action by a controlled-release preparation is to incorporate the active ingredients into particles of a polymeric material such as, for example, polyesters, polyamino acids, hydrogels, polylactic acid, polyglycolic acid, copolymers of these acids, or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these active ingredients into polymeric particles, it is possible to entrap these materials into microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsule and poly-(methylmethacrylate) microcapsule, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in New Trends and Developments in Vaccines, Voller et al. (eds.), University Park Press, Baltimore, Md., 1978 and Remington's Pharmaceutical Sciences, 16th edition.

The compositions can be administered using any suitable delivery method including, but not limited to, intramuscular, intravenous, intradermal, mucosal, and topical delivery. Such techniques are well known to those of skill in the art. More specific examples of delivery methods are intramuscular injection, intradermal injection, and subcutaneous injection. However, delivery need not be limited to injection methods. Further, delivery of DNA to animal tissue has been achieved by cationic liposomes (Watanabe et al., (1994) Mol. Reprod. Dev. 38:268-274; and WO 96/20013), direct injection of naked DNA into animal muscle tissue (Robinson et al., (1993) Vaccine 11:957-960; Hoffman et al., (1994) Vaccine 12: 1529-1533; Xiang et al., (1994) Virology

199: 132-140; Webster et al., (1994) Vaccine 12: 1495-1498; Davis et al., (1994) Vaccine 12: 1503-1509; and Davis et al., (1993) Hum. Mol. Gen. 2: 1847-1851), or intradermal injection of DNA using "gene gun" technology (Johnston et al., (1994) Meth. Cell Biol. 43:353-365). Alternatively, delivery routes can be oral, intranasal or by any other suitable route. Delivery also be accomplished via a mucosal surface such as the anal, vaginal or oral mucosa.

Dosing schedules (or regimens) can be readily determined for the particular subject and composition. Hence, the composition can be administered one or more times to the subject. Preferably, there is a set time interval between separate administrations of the composition. While this interval varies for every subject, typically it can range from 10 days to several weeks, and is often 2, 4, 6 or 8 weeks. In some embodiments, the interval can be typically from 2 to 6 weeks.

The compositions of the invention can be administered alone, or can be co-administered, or sequentially administered, with other HIV immunogens and/or HIV immunogenic compositions, e.g., with "other" immunological, antigenic or vaccine or therapeutic compositions thereby providing multivalent or "cocktail" or combination compositions of the invention and methods of employing them. Again, the ingredients and manner (sequential or co-administration) of administration, as well as dosages can be determined taking into consideration such factors as the age, sex, weight, species and condition of the particular subject, and the route of administration.

Kits

The present invention also includes kits useful in performing diagnostic and prognostic assays using the antibodies of the present invention. Kits of the invention include a suitable container comprising an HIV-1 antibody of the invention in either labeled or unlabeled form. In addition, when the antibody is supplied in a labeled form suitable for an indirect binding assay, the kit further includes reagents for performing the appropriate indirect assay. For example, the kit includes one or more suitable containers including enzyme substrates or derivatizing agents, depending on the nature of the label. Control samples and/or instructions are also included.

Application of the teachings of the present invention to a specific problem is within the capabilities of one having ordinary skill in the art in light of the teaching contained herein. Examples of the compositions and methods of the invention appear in the following non-limiting Examples.

5

EXAMPLES

Example 1. Multi-specific antibodies for HIV-1 and their utility

Here, 3D structure information of broadly neutralizing antibodies (bNAbs) in complex with HIV-1 envelope glycoproteins (Env) was used to perform rational design of penta-specific antibodies (PentaNAbs) capable of targeting five major Env neutralizing epitopes. These inventions, herein referred to as “PentaNAbs”, consist of five functional single chain fragment variable (ScFv) moieties connected in tandem via (G4S)_n linkers, such that penta-valence engagement of five individual epitopes on the HIV-1 Env by each respective moiety is possible. The bNAb functional moieties incorporated in the design target five major HIV neutralization epitopes including the i) V1/V2 apex glycan, ii) V3-base glycan, iii) CD4 binding site, iv) gp120/gp41 interface, and v) the membrane proximal external region (MPER). The sequence information of the penta-specific antibodies is listed in Table 4. In addition, these PentaScFv entities were fused to the fragment crystallizable region (Fc) region of IgG1 to accommodate effector functions in a Penta-IgG format.

To design the PentaScFvs, the physical distance between the compatible N/C termini of parental bNAb variable heavy (VH) and light chain (VL) domains was estimated to identify the shortest linker combinations allowing optimal VH/VL connection within each bNAb ScFv entity. Furthermore, to generate molecules capable of simultaneously engaging (“draping” the HIV-1 Env) contiguous epitopes on the HIV-1 Env surface, the spatial relationship between each neighboring bNAb moiety in the state of cognate epitope binding was assessed. It was found that epitopes located on adjacent protomers of the HIV-1 trimer are often more proximate to each other than those on the same protomer. Thus, in some designs, two functional bNAb moieties binding to the same protomer (intra-protomer mode) were arranged and in some cases to separate protomers (inter-protomers) of the HIV-1 Env trimer. Finally, PentaNAbs with bNAb moieties connected from N to C terminus in two orientations were designed: from the MPER to

V1V2 apex (forward order) or from the V1V2 apex to MPER (reverse order) followed by the Fc fragment to form PentaNAb IgG1 molecules. The purpose of these designs is to minimize potential steric hindrance imposed by the addition of Fc fragment to the C terminus of the connected ScFv entities. PentaNAb2.0 was initially constructed, which
5 consists of functional moieties of five bNAbs including 10E8v4 S100cF, 35O22, N6, PGT121, and VRC26.25 to test the rationale. In this design, an molecular orientation was used, namely “Forward” orientation with which the MPER bNAb 10E8v4 S100cF moiety is at the N terminus of the PentaNAb and the V1V2 glycan bNAb VRC26.25 moiety on the distal side followed by Fc fragment.

10 Using a 208-virus panel representing the worldwide diverse circulating HIV-1 virus isolates to assess neutralization breadth, PentaNAb2.0, in both ScFv and IgG form, displayed improved virus isolate coverage of 99.6%, as compared to individual bNAbs 10E8, VRC01, PGT121 and 35O22, which had 97.6%, 90.4%, 63.9%, and 45.2% coverage, respectively. Most significantly, the PentaNAb2.0, in both ScFv and IgG form,
15 displayed significantly improved IC₅₀ geomeans of 0.006 and 0.072 µg/mL, respectively, as compared to 10E8, which has an IC₅₀ mean of 0.299 µg/mL with the best neutralization breadth among all known bNAbs. In addition, PentaNAb2.0 ScFv displays neutralization potency and breadth superior to previously engineered tri- and tetra-specific antibodies to HIV-1 Env. Furthermore, PentaNAb2.0, in both ScFv and IgG
20 form effectively neutralizes VRC01-resistant replication-competent viral isolates from patients participated in the clinical trial involving passive transfer of VRC01. Finally, PentaNAbs were engineered with a “Reverse” orientation with which the V1V2 glycan bNAb VRC26.25 or PGDM1400 moiety is at the N terminus of the PentaNAb and the MPER bNAb 10E8v4 S100cF moiety on the distal side followed by Fc fragment. The
25 neutralization capacities of these PentaNAb iterations were assessed with a panel of 26 HIV-1 Env-pseudoviruses in TZM-bl target cells. It was found that all of the PentaNAbs in the ScFv format displayed a greater breadth and potency than their respective IgG1 counterparts with Fc fragment. Additionally, it was observed that the PentaNAbs in the “reverse” order displayed a greater potency than the “forward” order while maintaining
30 the same breadth. Taken together, penta-specific antibodies were engineered that are capable of engaging five major neutralization epitopes of HIV-1 envelope glycoproteins possess superior antiviral activities to conventional HIV-1 bNAbs and previously

engineered tri- and tetra-specific HIV-1 bNAbs, which may serve as effective agents for HIV-1 prevention, treatment and diagnosis.

Results

5 *Spatial relationships between HIV-1 Env-specific bNAb epitopes and the design of connecting linker length*

To constitute PentaNAb molecules, five top lead antibodies displaying the best neutralization potency and breadth among bNAbs within each individual epitope cluster were first selected. For example, in one combination VRC26.25 or PGDM1400, PGT121, N6, 35O22, and 10E8v4 V5R S100cF from the V1/V2, V3-glycan, CD4bs, gp120/gp41
10 interface, and MPER epitope cluster, respectively were picked. In order to develop a PentaNAb capable of simultaneously engaging five separate epitopes, the physical distance between the compatible variable heavy (VH) and light (VL) chain region termini (e.g. VH C-terminus followed by N-terminus of VL) of the selected bNAbs was measured to identify the shortest combinations (Fig 2). The PentaNAb was designed such that the
15 VH and VL of each individual bNAb entity was connected between termini with flexible linkers such that the flexible linker would traverse the shortest unimpeded distance possible. It is believed that joining the individual entities of the PentaNAb between the termini with the shortest unimpeded distance in between would avoid excess linker length, which would i) improve protein folding, ii) improve the expression level/yield of
20 the molecule, and iii) improve the coordination of simultaneous engagement of all five epitopes.

Five G4S linkers were used to connect moieties of bNAbs targeting the V1V2 (e.g. VRC26.25) and V3 (e.g. PGT121) glycans. The distance between V1/V2 and the V3-glycan targeting antibodies VRC26.25 or PGDM1400 and PGT121, respectively, was
25 not determined as crystallography data of gp120 bound VRC26.25 or PGDM1400 is currently unavailable. Using the EMD data for VRC26.25 bound to BG505 SOSIP.664, EMD-5856 (Doria-Rose *et al.*, Nature 509, 55-62 (2014)), the crystal structure of the VRC26.25 Fab (PDB: 5DT1) (Doria-Rose *et al.*, J Virol 90, 76-91 (2015)), as well as the crystal structure of PGT122 bound BG505 SOSIP.664 (PDB: 5FYK) (Stewart-Jones *et al.*,
30 *Cell* 165, 813-826 (2016)), a model of VRC26.25 & PGT121 bound Env trimer were generated by manual superposition of BG505 trimer elements. This model was

subsequently used to roughly estimate the spatial relationship between VRC26.25 and PGT121 with results similar to other modeling attempts using a combination of molecular dynamics (MD) and molecular dynamics flexible-fitting (MDFF) (Gorman *et al.*, Nat Struct Mol Biol 23, 81-90 (2016)). Similarly this EM model, EMD-5856 (Doria-Rose *et al.*, Nature 509, 55-62 (2014)), was used to manually fit the crystal structure of the PGDM1400 Fab (PDB: 4RQQ) (Sok *et al.*, Proc Natl Acad Sci U S A 111, 17624-17629 (2014)), with the crystal structure of PGT122 bound BG505 SOSIP.664 (PDB: 5FYK) (Stewart-Jones *et al.*, Cell 165, 813-826 (2016)), to briefly estimate the spatial relationship between PGDM1400 and PGT121, since PGDM1400 approaches the gp120 apex with an angle similar to VRC26.25. According to the EM data, both VRC26.25 and PGDM1400 are quaternary-dependent antibodies targeting the V1/V2 epitope at the trimer apex (Doria-Rose *et al.*, J Virol 90, 76-91 (2015); Sok *et al.*, Proc Natl Acad Sci U S A 111, 17624-17629 (2014)) (Fig 1), with VH/VL termini close to one another. Based on the above stated analysis, a linker of sufficient length (e.g. a linker with five G4S units) is utilized to connect VRC26.25 or PGDM1400 with PGT121.

Five G4S linkers were used to connect the V3-glycan targeting PGT121 and the CD4bs targeting N6 ScFv entities. Previous studies determined that the shortest distance between PGT122 (a surrogate for PGT121) and VRC01 (a surrogate for N6) is between adjacent protomers within an Env trimer (inter-protomer). Using the PDB model 5FYK (Stewart-Jones *et al.*, Cell 165, 813-826 (2016)), determined the distances between the VRC01 and PGT121 functional variable domains as the following: 37 Å between the C-terminus of VRC01 VH domain and the N-terminus of PGT121 VL domain with VRC01(VL-VH)-PGT121(VL-VH) topology, and 53 Å between the C-terminus of PGT121 VL and the N-terminus of VRC01 VH with PGT121(VH-VL)-VRC01(VH-VL) topology (Figure 8A). Furthermore, negative stain electron microscopy data demonstrated that one of the Bi-ScFvs previously generated, dVRC01(VL-VH)-5X-PGT121(VL-VH), was able to simultaneously engage both the PGT121 and VRC01 epitopes in an inter-protomer manner as we had predicted (Steinhardt *et al.*, Nat Commun 9, 877 (2018)), which supports rationale for this linker design.

The shortest distance between the CD4bs targeting bNAb moiety, N6 (with VRC01 as surrogate), and the gp120/gp41 interface targeting 35O22 entity was determined to be an intra-protomer distance. Using the model in PDB 5FYK (Stewart-

Jones *et al.*, Cell 165, 813-826 (2016)), the distance between the 35O22 and N6 functional variable domains were determined to be 57 Å between the C-terminus of 35O22 VH domain and the N-terminus of N6 VL domain with the topology of 35O22(VL-VH)-N6(VL-VH), and 47 Å between the C-terminus of N6 VL and the N-terminus of 35O22 VH with the topology of N6(VH-VL)-35O22(VH-VL) (Figure 8B), respectively. Thus, five G4S linkers were used to connect N6 and 35O22.

Finally, the shortest distances between the gp120/gp41 interface targeting 35O22 entity and the MPER targeting 10E8v4 entity was determined to be inter-protomer manner. Using PDB 5IQ7 superimposed with PDB 5FYK (Stewart-Jones *et al.*, Cell 165, 813-826 (2016); Kwon *et al.*, J Virol 90, 5899-5914 (2016)), a model for Env engaged with both 35O22 and 10E8 was generated. In this model, the distance between the 10E8v4 and 35O22 functional variable domains were determined to be 48 Å between the C-terminus of 10E8v4 VH domain and the N-terminus of 35O22 VL domain with the topology of 10E8v4(VL-VH)-35O22(VL-VH), and 25 Å between the C-terminus of 35O22 VL and the N-terminus of 10E8v4 VH with the topology of 35O22(VH-VL)-10E8v4(VH-VL) (Figure 8C), respectively. Therefore, five G4S linkers were used to connect 35O22 and 10E8v4.

PentaNAbs display exceptional neutralization potency and breadth

Based on the analysis of spatial relationship between the five major bNAB epitopes on HIV-1 Env surface, and the design of connecting linker length stated above, PentaNAb2.0 (Fig. 2, Table 5) was composed consisting of bNAB moieties of VRC26.25, PGT121, N6, 35O22, and 10E8v4_S100cF from the V1/V2, V3-glycan, CD4bs, gp120/gp41 interface, and MPER epitope cluster, respectively.

Name	Order	Orientation
PentaNAb _{1.0}	10E8v4, 35O22, N6, PGT121, VRC26.25	10E8v4-5X-35O22-5X-N6-5X-PGT121-5X-VRC26.25
PentaNAb _{2.0}	10E8v4_S100cF, 35O22, N6, PGT121, VRC26.25	10E8v4_S100cF-5X-35O22-5X-N6-5X-PGT121-5X-VRC26.25
PentaNAb _{2.0} Reverse	VRC26.25, PGT121, N6, 35O22, 10E8v4_S100cF	VRC26.25-5X-PGT121-5X-N6-35O22-10E8v4_S100cF
PentaNAb _{3.0}	10E8v4_S100cF, 35O22, N6, PGT121, PGDM1400	10E8v4_S100cF-5X-35O22-5X-N6-5X-PGT121-5X-PGDM1400
PentaNAb _{3.0} Reverse	PGDM1400, PGT121, N6, 35O22, 10E8v4_S100cF	PGDM1400-5X-PGT121-5X-N6-5X-35O22-5X-10E8v4_S100cF
PentaNAb _{4.0}	10E8v4_V5R_S100cF, 35O22, N6, PGT121, PGDM1400	10E8v4_V5R_S100cF-5X-35O22-5X-N6-5X-PGT121-5X-PGDM1400
PentaNAb _{4.0} Reverse	PGDM1400, PGT121, N6, 35O22, 10E8v4_V5R_S100cF	PGDM1400-5X-PGT121-5X-N6-5X-35O22-5X-10E8v4_V5R_S100cF

Table 5. Summary of PentaNAb names, order of individual antibody entities and orientation of variable light and variable heavy chains.

The PentaNAb2.0 -ScFvs and full length IgGs were expressed in mammalian 293FreeStyle cells and purified by Ni⁺ and protein A columns, respectively. All molecules were characterized by SDS-PAGE gels, size exclusion chromatography (SEC), and dynamic light scattering (DLS) (data not shown). Furthermore, the binding specificities of these constructs were validated by ELISA binding assay (not shown), and Bio-Layer Interferometry (BLI), which demonstrated binding to numerous Env ligands including the CD4bs specific RSC3 probe, an MPER peptide, and full length BG505 SOSIP.664 and JR-FL SOSIP.664 Env trimers (data not shown).

A comprehensive virus panel consisting of 208 pseudotype virus strains representing the worldwide diversified circulating HIV-1 virus isolates was used to analyze and compare the PentaNAb2.0 ScFv and IgG with TetraNAb, TriNAb and BiNAb designs in a previous invention disclosure (PCT/US2017/057053), as well as prototypic bNAbs including 10E8, VRC01 and PGT121. Both PentaNAb2.0 ScFv and IgG displayed substantially greater breadth and higher potency than the prototypical bNAbs and BiNAbs, while PentaNAb2.0 ScFv displays potency higher than TetraNAb and TriNAb (Figs. 3A, 3B & 4) by approximately 10-fold. The PentaNAb2.0, in both ScFv and IgG form, displayed improved virus isolate coverage of 99.6%, as compared to bNAbs 10E8, VRC01 and PGT121, which had 97.6%, 90.4% and 63.9%, respectively. The PentaNAb2.0, in both ScFv and IgG form, displayed significantly improved IC50 geomeans of 0.006 and 0.072 µg/mL, respectively (Figs 3 & 4). Most significantly, with an IC50 mean of 0.006 µg/mL, the PentaNAb2.0 ScFv is the most potent HIV-1 multiNAb tested to date by the comprehensive 208-virus panel.

Finally, when tested against VRC01-resistant replication-competent viral isolates from patients participating in the clinical trial involving passive transfer of VRC01, the PentaNAb2.0 ScFv and IgG displayed neutralization capacity superior to previous multiNAb designs as well as the prototypical bNAbs (Fig 5A-5C). Taken together, the data suggest that the PentaNAb could serve as a multifunctional therapeutic agent template for the prevention and treatment of HIV-1 infection, outperforming the conventional bNAbs and multi-specific bNAbs engineered previously.

Optimized bNAb orientations enhance the potency of PentaNAbs

PentaNAb2.0 was initially designed and purified in the “Forward” orientation (Fig 2). While PentaNAb2.0 ScFv shows superior potency to Tri- and Tetra-NAb designs, PentaNAb2.0 IgG with Fc fragment fused to the V1V2 glycan antibody moieties at the C terminus of the PentaNAb2.0 ScFv (Fig 2) shows potency virtually identical to Tri- and Tetra-NAb designs. We hypothesized that the steric hindrance imposed by the Fc fragment to the potent V1V2 glycan antibody moieties such as VRC26.25 may result in deleterious effect on neutralization potency.

To test this hypothesis, a series of Penta-ScFvs and full length IgGs (Fig 2, Fig 6) were generated, with combinations and permutations of VH-VL orientations and linker length for optimization (Table 4). Particularly, these designs include either i) the “Forward” orientation: 10E8v4(VL-VH)-35O22(VL-VH)-VRC01(VL-VH)-5X-PGT121(VL-VH)-VRC26.25/PGDM1400(VL-VH) –Fc whereby the Fc fragment connects with VRC26.25 or PGDM1400 (V1V2 glycan bNAb) and protrudes from the apex of the trimer (Fig 2), or ii) the “Reverse” orientation: VRC26.25/PGDM1400(VH-VL)-PGT121(VH-VL)-VRC01(VH-VL)-35O22(VH-VL)-10E8v4(VH-VL)-Fc whereby the Fc fragment connects with 10E8v4 (MPER bNAb) and protrudes from the trimer MPER region (Fig 6A). Additionally, “knob into hole” technology (Ridgway *et al.*, Protein Eng 9, 617-621 (1996)) was utilized to generate a heterodimer of the “Forward” and “Reverse” PentaNAb arms for subsequent evaluation, with LS mutations (M428L/N434S) in the Fc fragment for elongated antibody in vivo half-lives as well as improved biodistribution into the mucosal compartment (Ko *et al.*, Nature 514, 642-645 (2014)) (Fig 6B).

Three selected Penta-ScFvs and full length IgGs were expressed in mammalian 293FreeStyle cells and purified by Ni⁺ and protein A columns (Fig 7), respectively. All the proteins have expected molecular weight, as shown in representative SDS-PAGE gels (Fig 7A), size exclusion chromatography (SEC), and dynamic light scattering (DLS) analysis (data not shown). Furthermore, the binding specificities of these antibodies were validated by ELISA binding assay (not shown), and Bio-Layer Interferometry (BLI) (data not shown).

To compare the neutralization potency and breadth of the Penta-ScFvs and IgGs in the “Forward”, “Reverse” and “Forward/Reverse” orientations, a virus panel of high stringency consisting of 26 tier-2 virus strains (Fig 7B & 7C) was used. Of the multiple iterations tested, it was found that the Penta-ScFv and Penta-IgG in the “Reverse” orientation displayed improved potency while maintaining the same breadth (virus coverage) as those in the “Forward” orientation (Fig 7B & 7C). Somewhat expectedly, the “Forward/Reverse” IgG orientation displayed a potency in between that of the “Forward” and “Reverse” IgG (Fig 7B & 7C). Thus, the “Reverse” orientation of Penta-ScFv and Penta-IgG could be applied to generate antiviral agent in the future.

10 Example 2. Design of Pentaspecific HIV antibodies

Advances in the treatment of human immunodeficiency virus (HIV) infection have led to dramatic improvements in health outcomes of infected individuals receiving antiretroviral therapy (ART) over the course of the last several decades. By suppressing plasma viremia with ART, life expectancy has approached that of uninfected individuals, turning what was once an almost uniformly fatal illness into a manageable chronic disease. Nonetheless, the vast majority of HIV-infected individuals must remain on continuous therapy given that virus inevitably rebounds following discontinuation of ART. Furthermore, current ART requires life-long adherence to daily medication regimens and is associated with significant costs, cumulative toxicities, and the potential for emergence of drug-resistant virus. Consequently, intense efforts have been made in recent years to develop therapeutic strategies aimed at achieving ART-free virologic remission in infected individuals. Among those, recent discoveries of a number of highly potent broadly neutralizing HIV-specific antibodies (bNAbs) isolated from B cells of viremic HIV-infected individuals have materialized as a basis for realistic and feasible approaches for HIV prevention and immunotherapy.

Recent clinical trials conducted to assess the safety, tolerability, and virologic efficacy of multiple infusions of several bNAbs have shown to be safe, well-tolerated, and exhibit antiretroviral activities against HIV. However, these human trials have concomitantly shown that therapies involving single bNAbs are unlikely to suppress plasma viremia for prolonged periods of time due to both pre-existing and emergent HIV mutations that are resistant to the antibody-mediated neutralization. Therefore, it is of

great interest to develop a combination approach to increase the specificity and potency of bNAbs (or bNAb-like) molecules against a broad spectrum of infectious HIV in patients. In order to achieve this goal, we have developed synergistically combined functional epitope-binding moieties from five bNAbs that target the CD4 binding site, V2 and V3 conserved glycans, gp120/gp41 interface, as well as the membrane exterior proximal region into a 'single' penta-specific antibody (penta-NAb).

Thus far, our penta-Nabs exhibited superior degrees of HIV inhibition (neutralization of 99.5% of a 208 pseudo-typed HIV panel), exceptional neutralization capacity (IC₅₀ at 0.006 µg/ml) compared to individual bNAbs, and the capacity to neutralize replication-competent HIV isolates from infected individuals whose virus became resistant to various single bNAbs inferring our penta-Nabs the potential to overcome immune-evasion and eliminate persistent HIV reservoirs.

In this example, it was proposed to:

- 1) generate multiple penta-Nabs by incorporating five candidate bNAbs exhibiting the highest levels of neutralization against >300 individual infectious viral isolates obtained from the persistent viral reservoir of infected individuals receiving ART.
- 2) engineer/modify the Fc region to extend the in vivo half-life of each penta-NAb.
- 3) generate several penta-bNAb/anti-CD3 antibody-based molecules that are capable of binding cell-surface Env and inducing CD8⁺ T cell-mediated killing of HIV reservoirs in vitro and in vivo.
- 4) measure the efficacy of penta-NAbs in NSG mice following administration of peripheral blood mononuclear cells (PBMCs) from ART-naïve, HIV-infected individuals whose viral reservoirs carry sensitive and/or resistant virus to individual bNAbs.
- 5) evaluate the capacity of penta-bNAbs and DART molecules to prevent plasma viral rebound in NSG mice injected with from HIV-infected individuals following discontinuation of ART

The overall outcome of this study will provide the first proof of concept that a "5-in-1" antibody can achieve long-lasting broad-spectrum HIV inhibition in vivo, which will substantially advance our basic understanding of protective immunity against persistent

virus infection and contribute to the development of safe and effective strategies toward ART-free virologic remission.

Results

A clinical trial was previously conducted to investigate whether a bNAb VRC01
5 could prevent plasma viral rebound in infected individuals upon discontinuation of ART. While multiple infusions of VRC01 were safe and well-tolerated, the majority of patients experienced plasma viral rebound due to pre-existing and emergent antibody resistant HIV, suggesting therapeutic strategies involving passive transfer of bNAbs may require a combination(s) of bNAbs that targets more than one region on HIV Env protein in order
10 to achieve sustained virologic control in HIV-infected individuals upon withdrawal of ART. Thus, building upon the outcome of the data, it was proposed to test HIV Env-specific antibodies possessing multi-functional moieties with improved antigen binding and/or virus neutralization capacities, which may translate into superior protective efficacy in subsequent studies. The preliminary data, derived from five of the constructs,
15 demonstrated the feasibility of the methodology and linkage to the studies proposed here. Taking the first step toward generating multi-epitope targeting bNAbs, it was sought to test if one could engineer bi-specific single-chain variable fragment (bi-ScFv) consisting of bNAbs VRC01 and PGT121. As shown in Fig. 9A, a series of VRC01-PGT121 bi-ScFvs and full length IgGs were generated with combinations and permutations of VH-
20 VL orientations and linker length. It was shown using single molecule electron microscopy (EM) analysis that the VRC01-PGT121 bi-ScFv binds Env trimer in a bi-specific manner (Fig. 9B). The virus neutralization capacity of the bispecific molecules (Bi-BNAb) against a virus panel consisting of 208 primary virus isolates was evaluated and demonstrated ~95% virus neutralization breadth, which is substantially expanded
25 compared to that of the parent mAbs (VRC01 90% and PGT121 64%).

Using the knob-into-hole technology, the VRC01-PGT121 Bi-NAb was combined with 1) the moiety of MPER mAb 10E8 to form Tri-NAb; and 2) another Bi-NAb consisting of gp120/gp41 interface mAb and 10E8 in two different N-term to C-term orientations to form Tet-NAb1 and 2. Finally, a scFV was created connecting variable
30 regions of five bNAbs, namely penta-NAb, which engages all of the five conserved epitopes of HIV Env trimer (Fig. 2).

Interestingly, Tri-NAb, Tet-NAbs and Penta-NAb demonstrate remarkably improved neutralization breadth and potency compared to the parental mAbs (Fig. 3A). The superior neutralization breadth and potency possessed by the multi-epitope targeting NAbs are likely caused by cooperative and sometimes synergistic effect. Thus, the engineered multi-epitope targeting NAbs, which target three to five out of the five major conserved HIV Env neutralizing epitopes, could achieve nearly perfect virus coverage with IC₅₀ geometric mean below 0.1 µg/ml. Furthermore, the neutralization capacity of the Penta-scFv and Penta-Nab was tested, which display neutralization breadth and potency outperforming the parental monoclonal antibodies and combinations (Fig. 5C). These multi-NAbs possessing remarkably improved breadth and potency represent excellent functional modules for next generation candidate therapeutic molecules. In order to generate in vivo efficacy data, NSG mice will be utilized that are injected with PBMCs from HIV-infected viremic and aviremic individuals. As shown in Fig. 10, all 8 mice exhibited robust HIV replication following transfer of 8x10⁶ PBMCs from an viremic individual who was ART-naïve. These data demonstrate that the above animal system could be used to measure the efficacy of penta-NAbs and DART molecules against HIV derived from viral reservoirs from infected individuals who may carry sensitive and resistant HIV to certain bNAb.

Example 3

The neutralization profiles of PentaNAb2.0 Reverse ScFv, PentaNAb4.0 Forward ScFv (depicted in FIG. 11A), and PentaNAb4.0 Reverse ScFv (depicted in FIG. 11B) were tested with 208-virus panel (FIG. 14A & B). It was confirmed that PentaNAb2.0 Reverse ScFv has incremental improvement in potency (IC₅₀ geometric mean) compared to the initial design, PentaNAb2.0 ScFv (FIG. 14A & B).

A new multi-specific antibody was constructed, namely HexaNAb 1.0 ScFv (FIG. 12), which contains PentaNAb4.0 Reverse ScFv and variable domains of dB4C7/UB-421, an anti-CD4 antibody (N Engl J Med. 2019 Apr 18; 380(16):1535-1545. PMID: 30995373; patent US7501494B2) to 1) enhance the antiviral breadth, and 2) potentially inhibit the emergence of resistant virus variants as demonstrated by a recent clinical trial described in this cited reference. The HexaNAb 1.0 ScFv shows 100% virus

neutralization breadth and promising potency (IC₅₀ geometric mean ~0.005 ug/mL) (FIG. 14 A & B).

A new version of Penta-specific antibody in full-length IgG form was constructed (Fig. 13), namely PentaNAb4.0 Stem HC LS v2 IgG, expressed as an IgG assembled from a heavy chain and a light chain. With the M428L/N434S (LS) mutation in the Fc domain to elongate antibody *in vivo* half-life, the heavy chain consists of V2 bNAb PGDM1400, V3 bNAb PGT121, and the VH domain of CD4bs bNAb N6. The light chain contains the VL domain of N6 and 35O22 and 10E8v4 S100cF.

The PentaNAb4.0 Stem HC LS v2 IgG demonstrates 99.5% virus coverage (207/208 viruses tested) and high potency (IC₅₀ geometric mean ~0.02 ug/mL) (FIG. 14A & B), which is comparable to the Sanofi Tri-specific antibodies, 10E8v4xPGDM1400/VRC01 and 10E8v4xPGDM1400/N6-LS (FIG. 14B). The 5-specificity feature of the PentaNAb4.0 Stem HC LS v2 IgG may inhibit the emergence of neutralization resistant virus better than the other antibodies possessing less specificities (e.g. Tri-specific antibodies).

PentaNAb4.0 Reverse ScFv

[PGDM1400 (VH-3X-VL)]-5X-[PGT121 (VH-3X-VL)]-5X-[N6 (VH-3X-VL)]-5X-[35O22 (VH-3X-VL)]-5X-[10E8v4 V5R S100cF (VH-3X-VL)]-His

Amino acid sequence (SEQ ID NO:90)

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SGGGGSIHVTVQSPSSLSVSI GDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSG
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LKKPGASVKISCKTSGYRFNFYHINWIRQTAGRPEWMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFT
STGAAYMEIRNLKFDDTGTYFCAGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGGGSGQSVLTQSA
SVSGSLGQSVTISCTGPNVCCSHKSI SWYQWPPGRAPTLLIYEDNERAPGISPRFSGYKSYWSAYLTISDL
RPEDETTYCCSYTHNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSEVRLRESGGGLVKPGGS
LRLSCSASGFDNNAWMTWVRQPPGKGLEWVGRTGPGEGWSVDYAESVKGRFTISRDNKNTLYLEMNNVR
TEDTGYYFCARTGKYDFWFGYPPGEEYFQDWGQGTLLVIVSSGGGGSGGGGSGGGGSGSELTQDPAVSVALKQ
TVTITCRGDSLRSYASWYQKKPGQAPVLLFYGKNNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCS
SRDKSGSRLSVFGGGTKLTVLGS GHHHHHH

DNA sequence (SEQ ID NO.91)

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 TCTCGGGACAAGTCCGGCTCTAGACTGAGCGTGTTTCGGCGGCGGAACCAAGCTGACAGTGCTGGGATCCGGT
 CACCACCATCACCACCACTAG

HexaNAb1.0 Reverse ScFv

[dB4C7-UB-421 (VL-3X-VH)]-5X-[PGDM1400 (VH-3X-VL)]-5X-[PGT121 (VH-3X-VL)]-
 10 5X-[N6 (VH-3X-VL)]-5X-[35O22 (VH-3X-VL)]-5X-[10E8v4 V5R S100cF (VH-3X-VL)]-
 His

Amino acid sequence (SEQ ID NO: 92)

MGWSCIILFLVATATGVHSDIVLTQSPASLAVSLGQRATITCKAGQSVDDYDGD SYMNWYQQKPGQPPKLLIY
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 15 SQVQLVQSGPELKKPGASVKVSKASGYTFTDYVIHWVKQATGQGLEWIGEIYPGSGSAYSNAKFKDRVMT
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 VFGTGTQSVLGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
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 35 FGYPPEEYFQDWGQGLVIVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
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 VLGS GHHHHHH

DNA sequence (SEQ ID NO: 93)

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PentaNAb4.0 Stem HC LS_v2

HC: PGDM1400-5X-PGT121-5X-N6 HC

5 [PGDM1400 (VH-3X-VL)]-5X-[PGT121 (VH-3X-VL)]-5X-[N6 (VH)]-CH1-CH2-CH3

Amino acid sequence (SEQ ID NO: 94)

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15 RPSGIPERFSGSPDspFGTTATLTITsVEAGDEADYyCHIWDsRVPTKWVFGGGTTLTVLGGGSGGGGSGG
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FGGGFRDRVTLTTRDVYREIAYMDIRGLKPDdTAVYYCARDrsYGDSSWALDAWGQGTtVvVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYfPEPVTvSWNSGALTSGVHTfPAVLQSSGLYSLSSVtVTPSSSLGTQTY
ICNVNHKPSNTKVDKKVEPKSCDKthTcPPCPAPEllGGPSVfLFPPKPKDtlMISRTPEVtCVvVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNstYrVvSVLTvLHQDWLNGKEYKCKVSNKALPAPIEKtISKAKGQ
20 PREPQVYtLPPSREEMTKNQVSLtCLVKGFYPSDIAVEWESNGQPENNYKtTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVfSCSVLHEALHSHYtQKSLSLSPGK

DNA sequence (SEQ ID NO: 95)

25 ATGGGCTGGAGCTGCATCATCTCTGTTCTCTGGTGGCAACCGCAACCGGTGTGCACAGCCAGGCACAGCTGGTG
CAGAGCGGACCCGAAAGTGAGAAAACCTGGGACTAGCGTCAAAGTGTGATGTAAAGCCCCCTGGAAAATACCCTG
AAGACCTACGATCTGCACTGGGTGCGGTCCGTGCCTGGACAGGGCCTGCAGTGGATGGGATGGATCTCTCAC
GAGGGCGACAAGAAAAGTGATCGTGGAGCGGTTCAAGGCCAAGGTGACAATCGATTGGGACAGATCCACCAAC
ACAGCCTACCTGCAGCTGTCTGGCCTGACCAGCGGCGATACAGCCGTGTACTACTGTGCCAAGGGCTCTAA
CACC GGCTGAGAGACTACGCCCTGTATGACGATGACGGCGCCCTGAACTGGGCCGTGGATGTGGACTATCTG
30 TCCAATCTGGAGTTCTGGGGACAGGGAACCGCAGTGACAGTGAGCTCCGGAGGAGGAGGCTCCGGCGGCGGA
GGCTCTGGGGGAGGCGGCAGCGATTTTGTGCTGACCCAGTCTCCACACAGCCTGTCCGTGACACCCGGCGAG
TCTGCCAGCATCTCTGCAAGTCTAGCCACAGCCTGATCCACGGCGACAGGAACAATTACCTGGCCTGGTAC
GTGCAGAAGCCAGGCCGACGCCCTCAGCTGCTGATCTATCTGGCATCCTCTAGGGCCTCCGGAGTGCCAGAT
CGCTTCTCTGGCAGCGGCTCCGATAAGGACTTTACCCCTGAAGATCAGCCGGGTGGAGACAGAGGACGTGGGC
35 ACATACTATTGTATGCAGGGCCGAGAATCACCTTGGACATTTGGGCAGGGAACATAAGTTGACATCAAAGGG
GGGGGGGGCTCCGGCGGCGGGGGCTCTGGCGGCGGCGGCAGCGGAGGAGCGGCTCCGGAGGAGGCGGCTCT
CAGATGCAGCTGCAGGAGAGCGGACCAGGACTGGTGAAGCCTTCCGAGACCCTGTCTCTGACATGTTCTGTG
AGCGGCGCCTCCATCTCTGATAGCTACTGGAGCTGGATCAGACGGAGCCCTGGCAAGGGCCTGGAGTGGATC
GGCTACGTGCACAAGTCTGGCGATACAACTATTCCCCATCTCTGAAGAGCCGGGTGAACCTGAGCCTGGAC
40 ACCTCCAAGAAATCAGGTGAGCCTGTCCCTGGTGGCAGCAACCGCAGCAGACAGCGGCAAGTACTATTGCGCC
AGAACACTGCACGGCAGGCGCATCTACGGCATCGTGGCCTTTAACGAGTGGTTACCTACTTTTATATGGAC
GTGTGGGGCAATGGCACCCAGGTGACAGTGTCCTCTGGCGGCGGCGGCTCTGGCGGAGGAGGCAGCGGAGGA
GGAGGCAGCTCCGACATCTCTGTGGCACCTGGAGAGACCGCAAGGATCAGCTGTGGAGAGAAAGTCTCTGGGC
AGCAGGGCCGTGCAGTGGTACCAGCACAGGGCAGGACAGGCACCATCCCTGATCATCTATAACAATCAGGAC
45 CGGCCATCTGGCATCCCCGAGAGATTCTCTGGCAGCCCCGATAGCCCTTTTGGCACCACAGCCACCCTGACA
ATCACCTCCGTGGAGGCCGCGCAGGAAGCAGATTACTATTGCCACATCTGGGACTCCAGAGTGCCAAACCAAG
TGGGTGTTTCGGAGGAGGAACCACTGACAGTGCTGGGCGGCGGAGGCTCCGGCGGGGGCGGCTCTGGAGGC
GGCGGCAGCGGAGGGGGCGGCTCCGGCGGCGGCGGCTCTAGGGCACACCTGGTGCAGAGCGGAACCGCAATG
AAGAAGCCTGGCGCCTCTGTGCGCGTGAGCTGTCAGACATCCGGCTACACCTTCACCGCCACATCCTGTTTC

TGGTTTAGGCAGGCACCCAGGAAGAGGACTGGAGTGGGTGGGCTGGATCAAGCCCCAGTATGGAGCAGTGAAC
 TTCGGAGGAGGCTTTTCGGGACAGAGTGACACTGACCCGGGACGTGTACAGAGAGATCGCCTATATGGATATC
 AGGGGCCTGAAGCCCCGACGATACCGCCGTGTACTATTGCGCCAGGGACCGCTCCTACGGCGATTCTAGCTGG
 GCACTGGACGCATGGGGACAGGGAACACAGTGGTGGTGAGCGCCGCGTCGACCAAGGGCCCATCGGTCTTC
 5 CCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTC
 CCCGAACCTGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTA
 CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTAC
 ATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAA
 ACTCACACATGCCCCACCGTGGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAA
 10 CCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGAC
 CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
 CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCCTGCTGCACCAGGACTGGCTGAATGGCAAGGAG
 TACAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAG
 CCCCAGAAACACAGGTGTACACCCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACC
 15 TGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
 TACAAGACCACGCCTCCCGTGTGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAG
 AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGCTGCATGAGGCTCTGCACAGCCACTACACGCAG
 AAGAGCCTCTCCCTGTCTCCGGGTAAATGA

20 **LC: N6 KC-CK-35022-5X-10E8v4 V5R S100cF**

[N6 (VL)] -CL-5X- [35022 (VH-3X-VL)] -5X- [10E8v4 S100cF (VH-3X-VL)]

Amino acid sequence (**SEQ ID NO: 96**)

MGWSCIILFLVATATGVHSYIHVTQSPSSLSVSIQDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLIHHTSS
 VEDGVPSRFSGSGFHTSFNLITISDLQADDIATYYCQVLQFFGRGSRHLHKRTVAAPSVFI FPPSDEQLKSGT
 25 ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL
 SSPVTKSFNRGECGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 NWIRQTAGRGPEWMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTFFCAK
 GLLRDGSSTWLPYLVWQGTLLTVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 30 KSISWYQWPPGRAPTLIIYEDNERAPGISPRFSGYKSYWSAYLITISDLRPEDETTYCCSYTHNSGCVFGTG
 TKVSVLGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVRLVESGGGLVKPGGSLRLSCSASGFDNDAWMTWVRQPP
 GKGLEWVGRITGPGEWSDVYAESVKGRFTISRDNKNTLYLEMMNVRTEDTGYFCAARTGKYDFWFGYPP
 GEEYFQDWGQGTLLVIVSSGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 QAPVLLFYGKNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSRLSVFGGGTCLTVL

35 **DNA sequence (SEQ ID NO: 97)**

ATGGGCTGGAGCTGCATCATCCTGTTCTTCTGGTGGCAACCGCAACAGGAGTGCACAGCTACATCCAC
 GTGACCCAGTCCCCTTCTCTGTCCGTGTCTATCGGCGATCGCGTGACCATCAACTGTCAGACAAGCCAG
 GGAGTGGGCTCCGACCTGCACTGGTATCAGCACAAAGCCTGGCAGGGCCCCAAAGCTGCTGATCCACCACACA
 AGCTCCGTGGAGGATGGAGTGCCAAGCCGCTTCAGCGGCTCCGGATTCCACACCAGCTTTAATCTGACAATC
 40 TCCGACCTGCAGGCCGACGATATCGCCACCTACTATTGCCAGGTGCTGCAGTTCTTTGGCAGGGGCTCCCGC
 CTGCACATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCT
 GGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGAT
 AACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
 AGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
 45 GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTGGAGGAGGGGGCTCTGGCGGCGGGGGC

AGCGGCGGAGGCGGCTCCGGAGGGGGCGGCTCTGGCGGAGGCGGCAGCCAGGGCCAGCTGGTGCAGAGCGGA
 GCAGAGCTGAAGAAGCCAGGAGCCTCTGTGAAGATCAGCTGTAAGACATCCGGCTACCGGTTCAACTTTTAT
 CACATCAATTGGATCAGGCAGACCGCAGGAAGGGGACCAGAGTGGATGGGCTGGATCTCCCCCTACTCTGGC
 GATAAGAACCTGGCCCCAGCCTTCCAGGACAGAGTGATCATGACCACAGATACCGAGGTGCCAGTGACCAGC
 5 TTACCTCCACCGGAGCCGCCTACATGGAGATCCGGAATCTGAAGTTCGACGATACAGGCACCTATTTTTGC
 GCCAAGGGCCTGCTGAGAGACGGCTCTAGCACATGGCTGCCATACCTGTGGGGACAGGGCACCCTGCTGACA
 GTGTCCTCTGGAGGAGGAGGCTCCGGGGGCGGCGGCTCTGGAGGAGGAGGCTCTCAAAGCGTGCTGACCCAG
 TCCGCCTCTGTGAGCGGCTCCCTGGGCCAGTCTGTGACCATCAGCTGTACAGGCCCAACTCCGTGTGCTGT
 TCTCACAAGTCTATCAGCTGGTACCAGTGGCCACCAGGAAGGGCACCTACCCTGATCATCTATGAGGACAAT
 10 GAGAGGGCACCAGGAATCAGCCCTCGCTTCTCCGGCTACAACTCTTATTGGAGCGCCTACCTGACCATTTC
 GACCTGCGCCCCGAGGATGAGACCACATACTATTGCTGTAGCTATACCCACAACCTCCGGCTGCGTGTGTTGGC
 ACAGGCACCAAGGTGAGCGTGCTGGGAGGAGGGGGCTCTGGCGGCGGGGGCAGCGGCGGAGGCGGCTCCGGA
 GGGGGCGGCTCTGGCGGAGGCGGCAGCGAGGTGCGGCTGGTGGAGAGCGGCGGCGGCTGGTGAAGCCAGGC
 GGCTCTCTGAGACTGTCCTGTTCTGCCAGCGGCTTCGACTTTGATAATGCCTGGATGACATGGGTGCGGCAG
 15 CCTCCTGGCAAGGGGCTGGAGTGGGTGGGAAGAATCACCGGACCAGGAGAGGGATGGTCTGTGGACTACGCC
 GAGAGCGTGAAGGGCCGGTTTACCATCAGCAGAGATAACACTAAAAATACACTGTATCTGGAGATGAACAAT
 GTGCGGACCGAGGACACAGGCTACTATTTCTGCGCCAGAACCGGCAAGTACTATGATTTCTGGTTTGGCTAC
 CCCCCTGGCGAGGAGTATTTTTCAGGACTGGGGCCAGGGCACCTGGTCATCGTGAGCAGCGGCGGGGGAGGC
 TCCGGCGGGGGGGGCTCTGGAGGAGGGGGCTCTAGCGAGCTGACCCAGGACCCCGCCGTGTCCGTGGCCCTG
 20 AAGCAGACAGTGACCATCACATGCAGGGGCGACTCCCTGCGCTCTCACTACGCCAGCTGGTATCAGAAGAAG
 CCAGGACAGGCACCCGTGCTGCTGTTCTACGGCAAGAACAATCGGCCTTCCGGCATCCAGATAGATTTTCC
 GGCTCTGCCAGCGGAAACAGGGCCAGCCTGACCATCACAGGAGCACAGGCAGAGGATGAAGCAGATTACTAT
 TGTTCCTCTCGGGACAAGTCCGGCTCTAGACTGAGC

mAb dB4C7/UB-421's perfect heavy chain Fv region (SEQ ID NO 98)

25 QVQLVQSGPELKPGASVKVSCKASGYTFTDDYVIHWVKQATGQGLEWIGEIYPGSGSA
YSNAKFKDRVTMTADKSSNTAYMELSSLTSDDTAVYFCARRRGNGTGFAYWGQGLVT
 VSS

mAb dB4C7/UB-421's perfect light chain Fv region (SEQ ID NO. 99)

30 DIVLTQSPASLAVSLGQRATITCKKAGQSVDYDGDSYMNWYQQKPGQPPKLLIYVASNLE
SGIPARFSGSGSGTDFTLNIHPVEENDAATYYCQQSYKDPLTFGQGTKLEIK

CDR1 of Heavy Chain of murine antibody B4 SEQ ID NO. 100 DYVIH

35 CDR2 of Heavy Chain of murine antibody B4 SEQ ID NO. 101 EIYPGSGSAYSNAKFKD

CDR3 of Heavy Chain of murine SEQ ID NO. 102 RGNGTGFAY

antibody B4

CDR1 of Light Chain of murine SEQ ID NO. 103 KAGQSVDDYDGD SYMN

antibody B4

CDR2 of Light Chain of murine SEQ ID NO. 104 VASNLES

5 antibody B4

CDR3 of Light Chain of murine SEQ ID NO. 105 QQSYKDPLT

antibody B4

SEQ ID NO:	Name	Orientation	Amino Acid Sequence
92	HexANAbl.0 Reverse ScFv	(dB4C7/UB-421)-5X-PGDM1400-5X-PGT121-5X-N6-5X-35022-5X-10E8v4 V5R S100cF-His	MGWSCIILFLVATATGVHSDIVLTQSPASLAV SLGQRATITCKAGQSVDDYDGD SYMNWYQQKPG QPPKLLIYVASNLES GIPARFSGSGSGTDFTL NIHPVEENDAATYYCQQSYKDPLTFGQGTKLE IKGGGSGGGGSGGGGSGVQLVQSGPELKKPG ASVKVSCKASGYTFTDYVIHWVKQATGQGLEW IGEIYPGSGSAYSNAKFKDRVMTADKSSNTA YMESSSLTSDDTAVYFCARRNGTGFAYWGQG TLVTVSSGGGSGGGGSGGGGSGGGGSGGGG QAQLVQSGPEVRKPGTSVKVSCKAPGNTLTKTY DLHWVRSVPGQGLQWMGWISHEGDKKVIVERF KAKVTIDWDRSTNTAYLQLSGLTSGDTAVYYC AKGSKHRLRDYALYDDD GALNWAVD VDYLSNL EFWGQGTAVTVSSGGGSGGGGSGGGGSGDFVL TQSPHSLSVTPGESASISCKSSSLIHGDRNN YLAWYVQKPGRSPQLLIYLASSRASGV PDRFS GSGSDKDFTLKISRVEDTVGTYYCMQGRES P WTFGQGTKVDIKGGGSGGGGSGGGGSGGGG GGGSGMQQLQESGPGLVKPSSETLSLTCSVSGA SISDSYWSWIRRS PGKGLEWIGYVHKSGDTNY SPSLKSRVNLSLDTSKNQVSLSLVAATAADSG KYYCARTLHGRIYGI VAFNEWFTYFYMDVWG NGTQVTVSSGGGSGGGGSGGGGSSDISVAPG ETARISCGEKS LGSRAVQWYQHRAGQAPSLII YNNQDRPSGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWD SRVPTKWVFGGGTTLTV LGGGSGGGGSGGGGSGGGGSGGGGSRHLVQ SGTAMKKPGASVRVSCQTSGYTFTAHLFWFR QAPGRGLEWVGWIKPQYGAVNFGGGFRDRVTL TRDVYREIAYMDIRGLKPDDTAVYYCARDRSY GDSSWALDAWGQTTVVVSAGGGSGGGGSGG GGSYIHVTQSPSSLVSIGDRV TINCQTSQGV GSDLHWYQHKPGRAPKLLIHHTSSVEDGVPSR FSGSGFHTSFNLTISDLQADDIATYYCQVLQF FGRGSRHLIKGGGSGGGGSGGGGSGGGGSGG GSGQQLVQSGAELKKPGASVKISCKTSGYRF NFIHINWIRQTAGRGP EWMGWISPYSGDKNLA PAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRN LKFDDTGT YFCAKGLLRDGSSTWLPYLGWQGT LLTVSSGGGSGGGGSGGGGSGSVLTQSASVS GSLGQSVTISCTGPNSVCCSHKSI SWYQWPPG

			RAPTLIIYEDNERAPGISPRFSGYKSYWSAYL TISDLRPEDETTYCCSYTHNSGCVFGTGTKV SVLGGGSGGGSGGGSGGGSGGGSGGGSEVRL RESGGGLVKPGGSLRLSCSASGFDFDANWMTW VRQPPGKGLEWVGRIITGPGEGWSDYAESVKG RFTISRDNKTNTLYLEMNNVRTEDTGYYFCAR TGKYYDFWFGYPPGEEYFQDWGQGTLVIVSSG GGGSGGGSGGGSGGGSGGGSGGGSGGGQTVT ITCRGDSLRSHYASWYQKKPGQAPVLLFYGKN NRPSGIPDRFSGSASGNRASLTITGAQAEDEA DYCSSRDKSGSRLSVFGGGTCLTVLGS GHHH HHH
94	PentaNAb4.0 Stem HC LS_v2 (heavy chain)	PGDM1400-5X- PGT121-5X-N6 HC	MGWSCIILFLVATATGVHSQAQLVQSGPEVRK PGTSVKVSCKAPGNTLKTLDLHWVRSVPGQGL QWMGWISHEGDKKVIVERFKAKVTIDWDRSTN TAYLQLSGLTSGDTAVYYCAKGSKHRLRDYAL YDDD GALNWAVD VDYLSNLEFWGQGTAVTVSS GGGSGGGSGGGSGGGSGGGSGGGSGGGSGG SASISCKSSHSLIHGDRNNYLAWYVQKPGRSP QLLIYLASSRASGVPDRFSGSGSDKDFTLKIS RVETEDVGTYYCMQGRES PWTFGQGTKV DIKG GGGSGGGSGGGSGGGSGGGSGGGSGGQMQLQESG PGLVKPSETLSLTCSVSGASISDSYWSWIRRS PGKGLEWIGYVHKSGDTNYSPLKSRVNLSD TSKNQVSLSLVAATAADSGKYCARTLHGRI YGIVAFNEWFTYFYMDVWNGTQVTVSSGGGG SGGGSGGGSGGGSDISVAPGETARISCGEKS LG SRAVQWYQHRAGQAPSLIIYNNQDRPSGIPER FSGSPDSPFGTTATLTITISVEAGDEADYYCHI WDSRVPTKWVFGGGTTLTVLGGGSGGGSGGGSGG GGSGGGSGGGSGGGSGGGSGGGSGGGSGG VSCQTSGYTFTAHLFWFRQAPGRGLEWVGWI KPQYGAVNFGGGFRDRVTLTRDVYREIAYMDI RGLKPDDTAVYYCARDRSYGDSSWALDAWGQG TTVVVSAASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVTPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMI SRTPEVTCVVDVSHED PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPV L DSDGSGFFLYSKLTVDKSRWQQGNV FSCSVLHE ALHSHYTQKSLSLSPGK
96	PentaNAb4.0 Stem HC LS_v2 (light chain)	N6 KC-CK- 35O22-5X- 10E8v4 S100cF	MGWSCIILFLVATATGVHSYIHVTQSPSSLSV SIGDRVINCQTSQGVGSDLHWYQHKPGRAPK LLIHHTSSVEDGVPSRFSGSGFHTSFNLTISD LQADDIATYYCQVLQFFGRGSRHLIKRTVAAP SVFI FPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSF NRGECGGGSGGGSGGGSGGGSGGGSGGGSGG QLVQSGAELKKPGASVKISCKTSGYRNFYHI NWIRQTAGRGPEWMGWISPYSGDKNLAPAFQD RVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDD TGTYFCAKGLLRDGSSTWLPYLGWQGTLLTVS SGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG SVTISCTGPNSVCCSHKSI SWYQWPPGRAPTL

			IIYEDNERAPGISPRFSGYKSYWSAYLTIISDL RPEDETTYCCSYTHNSGCVFGTGTKVSVLGG GSGGGGSGGGGSGGGGSGGGGSEVRLVESGG GLVKPGGSLRLSCSASGFDNNAWMTWVRQPP GKGLEWVGRITGPGEWSVDYAESVKGRFTIS RDNTKNTLYLEMNNVRTEDTGYF CARTGKYY DFWFGYPPGEEYFQDWGQGLVIVSSGGGGSG GGGSGGGGSSSELTQDPAVSVALKQTVTITCRG DSLRSHYASWYQKKPGQAPVLLFYGKNNRPSG IPDRFSGSASGNRASLTITGAQAEDEADYYCS SRDKSGSRLSVFGGGTKLTVL
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**PentaNAb2.0:10E8v4_s100cF-5X-35022-5X-N6-5X-PGT121-5X-VRC26.25-IgG3C
hinge-IgG1 LS (SEQ ID NO.106)**

5 ATGGGATGGAGCTGTATTATTCTGTTTCTGGTCGCTACCGCTACCGGAGTGCATTCTTCTGAACTGACCCAG
 GACCCCGCCGTGAGCGTGGCCCTGAAGCAGACCGTGACAATCACCTGCAGGGGCGACAGCCTGCGCTCCCAC
 TACGCCAGCTGGTATCAGAAGAAGCCTGGCCAGGCCCCAGTGCTGCTGTTCTACGGCAAGAACAATAGGCCC
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 GCAGAGGACGAGGCAGATTACTATTGCAGCTCCCGGGACAAGTCCGGCTCTAGACTGAGCGTGTTCCGGCGGC
 10 GGCACCAAGCTGACAGTGCTGGGAGGAGGAGGCAGCGCGGAGGAGGCTCCGGAGGCGGCGGCTCTGAGGTG
 CGGCTGGTGGAGTCTGGAGGAGGCCTGGTGAAGCCAGGAGGCAGCCTGAGACTGAGCTGTTCCGCCTCTGGC
 TTCGACTTTGATAATGCCTGGATGACATGGGTGCGGCAGCCACCTGGCAAGGGCCTGGAGTGGGTGGGAAGA
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 GATAACACCAAGAATACACTGTATCTGGAGATGAACAATGTGCGGACCGAGGACACAGGCTACTATTTCTGC
 15 GCCAGAACC GGCAAGTACTATGATTTTTGGTTTGGCTACCCACCCGGCGAGGAGTATTTTCAGGACTGGGGC
 CAGGGCACCCCTGGTCATCGTGAGCAGCGGCGGCGGCGGCGGCGGCTCCGGAGGAGGCGGCTCT
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 20 AGATTCTCTGGCTACAAGTCTTATTGGAGCGCTACCTGACAATCAGCGACCTGCGCCCCGAGGATGAGACA
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 GAGCTGAAGAAGCCTGGAGCCAGCGTGAAGATCTCCTGTAAGACATCTGGCTACCGGTTCAACTTTTATCAC
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 25 AAGAACCTGGCCCCAGCCTTCCAGGACAGAGTGATCATGACCACAGATACCGAGGTGCCAGTGACCAGCTTC
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 30 AACTGTGAGACATCTCAGGGCGTGGGCAGCGACCTGCACTGGTATCAGCACAAAGCCTGGCAGGGCCCCAAAG
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 35 ACATCTGGCTACACCTTCACCGCCACATCCTGTTCTGGTTTAGGCAGGCACAGGAAGAGGCCTGGAGTGG
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 40 GGGGGAGGCTCTAGCGACATCTCCGTGGCCCCCTGGCGAGACAGCCAGAATCTCTGTGGCGAGAAGTCTCTG
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 5 TACTATTGCGCCAGAACACTGCACGGCAGGCGCATCTACGGCATCGTGGCCTTTAACGAGTGGTTTACCTAC
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 GGCTCTGGCGGGGGCGGCAGCGGCGGGGGCGGCTCCGGGGGAGGCGGCTCTCAGAGCGTGTGACCCAGCCA
 CCTTCCGTGTCTGCCGACCCAGGACAGAAGGTGACCATCAGCTGTTCCGGCAACACATCCAATATCGGCAAC
 AATTTTCGTGTCTTGGTACCAGCAGAGGCCAGGAAGGGCACCACAGCTGCTGATCTATGAGACAGACAAGCGG
 10 CCTTCCGGCATCCCAGATAGATTTTCTGCCAGCAAGTCCGGCACCAGCGGCACACTGGCAATCACCGGCCTG
 CAGACAGGCGACGAAGCTGATTACTATTGCGCAACCTGGGCAGCCTCCCTGAGCTCCGCCAGGGTGTTCGGA
 ACCGGAACAAAAGTGATCGTGCTGGTGGGCGGCGGAGGCTCTGGCGGAGGCGGCAGCGCGGGGGGGGCTCC
 CAGGTGCAGCTGGTGGAGAGCGGCGGCGGCGTGGTGCAGCCAGGCACCTCCCTGAGGCTGTCTTGTGCAGCA
 AGCCAGTTTCAATTTGATGGCTACGGCATGCACTGGGTGCGCCAGGCACCAGGCAAGGGCCTGGAGTGGGT
 15 GCCTCTATCAGCCACGACGGCATCAAGAAGTACCACGCCGAGAAAGTGTGGGGCAGGTTTACCATCTCCCGC
 GATAACTCTAAAAACACACTGTATCTGCAGATGAACTCCCTGAGGCCCCGAGGACACCGCCCTGTACTATTGC
 GCCAAGGACCTGCGCGAGGATGAGTGTGAGGAGTGGTGGTCCGACTACTATGATTTTGGCAAGCAGCTGCCT
 TGCGCAAAGAGCAGGGGAGGCCTGGTGGGAATCGCCGATAATTGGGGCCAGGGCACCATGGTGACAGTGTCT
 AGCGGATCCGGAGAGCTGAAGACCCCTCTGGGCGATACCACACACACATCCCCACGGAGCCCCGAGCCAAAG
 20 TCCTCTGACACCCACCCCCCTAGCCCTAGATCCCCCTGAGCCAAAGAGCTCCGATACACCACCCCTTCTCCA
 AGGAGCCCCGAGCCTAAGTCTAGCGACACCCACCCCTTGCCCCGCTGTCCAGCACCAGAGCTGCTGGGA
 GGACCAAGCGTGTTCCTGTTTCCACCAAGCCTAAGGATACACTGATGATCTCTCGCACCCCGAGGTGACA
 TGCGTGGTGGTGGACGTGAGCCACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
 CACAATGCCAAGACCAAGCCCAGGGAGGAGCAGTACAACAGCACCTATCGCGTGGTGTCCGTGCTGACAGTG
 25 CTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGTCCAATAAGGCCCTGCCTGCCCAATC
 GAGAAGACAATCAGCAAGGCAAAGGGACAGCCAAGGGAGCCACAGGTGTACACCTGCCCTCAAGCCGCGAG
 GAGATGACCAAGAACCAGGTGTCCCTGACATGTCTGGTGAAGGGCTTCTATCCTAGCGATATCGCCGTGGAG
 TGGGAGTCCAATGGCCAGCCAGAGAACAATTACAAGACCACACCCCTGTGCTGGACTCCGATGGCTCTTTC
 TTTCTGTATTCTAAGCTGACCGTGGACAAGAGCCGCTGGCAGCAGGGCAACGTGTTACAGCTGCTCTGTGCTG
 30 CACGAAGCCCTGCACAGCCATTACACCCAGAAGAGCCTGAGCCTGAGCCCCGAAAA

Amino acid: (SEQ ID NO.107)

MGWSCIILFLVATATGVHSELTDPAVSVALKQTVTITCRGDSLRSYASWYQKKPGQAPVLLFYGKNNRP
 SGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSRLSVFGGGTKLTVLGGGSGGGSGGGGSEV
 35 RLVESGGGLVKPGGSLRLSCSASGFDNDNAWMTWVRQPPGKLEWVGRITGPGEWSVDYAESVKGRFTISR
 DNTKNTLYLEMNVRTEDTGYFFCARTGKYDFWFGYPPGEEYFQDWGQGLVIVSSGGGSGGGSGGGGSGGGG
 GGGSGGGGSQSVLTQSASVSGSLGQSVTISCTGPNVCCSHKSIISWYQWPPGRAPTLIIYEDNERAPGISP
 RFSGYKSYWSAYLTISDLRPEDETTYCCSYTHNSGCVFGTGTVSVLGGGSGGGSGGGSGGGSGGGSGGGSGG
 40 ELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWGMWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSF
 TSTGAAYMEIRNLKFDDTGTFFCAKGLLRDGSSTWLPYLWGQGLTLTVSSGGGSGGGSGGGSGGGSGGGSGG
 GGSYIHVTQSPSSLSVSIQDRVTINCQTSQVGSGLHWYQHKPGRAPKLLIHHTSSVEDGVPSRFSGSGFHT
 SFNLTISDLQADDIATYYCQVLQFFGRGSRHLIKGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
 TSGYTFTHAILFWFRQAPGRGLEWVGWIKPQYGAIVNFGGGRDRVTLTRDVYREIAYMDIRGLKPDATVYY
 CARDRSYGDSSWALDAWQGTTVVVSAGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
 45 GSRVQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSDRVPT
 KWVFGGGTTLTVLGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
 LEWIGYVHKSQDNTYSPSLKSRVNLSDTSKNQVSLSLVAATAADSGKYCARTLHGRRYIGIVAFNEWFTY
 FYMDVWNGTQVTVSSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
 50 NFVSWYQQRPGRAPQLLIYETDKRPSGIPDRFSASKSGTSLTGLTGLQTDGDEADYYCATWAASLSARVFG
 TGTKVIVLVGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGG
 ASISHDGIKKYHAEKVGWGRFTISRDNKNTLYLQMNSLRPEDTALYYCAKDLREDECEEWWSDDYDFGKQLP
 CAKSRGGLVGIADNWQGTMTVTVSSSGGELKTPLDGTTHTSPRSPEPKSSDTPPPSPRSPEPKSSDTPPPSP
 RSPPEPKSSDTPPPCPRCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSVL
HEALHSHYTQKSLSLSPGK

5 The studies presented above, using prototypic HIV bNAbs to create multispecific Env binding functional moieties with enhanced breadth and/or potency through structure-based rational design and iterative optimization, as well as combinations of the optimized bispecific Abs to form multi-specific Abs will serve as a methodological foundation for the eventual expansion of this line of investigation in regard to the more imaginative anti-viral agents described in this disclosure.

10 Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

15 While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

What is claimed is:

1. A multispecific anti-HIV antibody that binds to multiple epitopes on HIV envelope protein, wherein the antibody comprises:
 - 5 i. an amino acid sequence that binds to a V1/V2 apex glycan epitope;
 - ii. an amino acid sequence that binds to a V3-base glycan region epitope;
 - iii. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope;
 - 10 iv. an amino acid sequence that binds to a gp120/gp41 interface epitope; and
 - v. an amino acid sequence that binds to a membrane proximal external region (MPER) epitope.
- 15 2. The multispecific anti-HIV antibody of claim 1, wherein the antibody simultaneously binds the multiple epitopes.
3. The multispecific anti-HIV antibody of any of claims 1-2, wherein the amino acid sequences of i-v) are present on a single polypeptide chain.
4. The multispecific anti-HIV antibody of any of claims 1-3, wherein the antibody is
 - 20 capable of neutralizing at least 99% of the HIV viruses listed in Table 1 with an IC50 value of less than 50 µg/mL.
 5. The multispecific anti-HIV antibody of claim 4, wherein the antibody has an IC50 geomean of less than or equal to 0.10 µg/ml.
 6. The multispecific anti-HIV antibody of claim 4, wherein the antibody has an IC50
 - 25 geomean of less than or equal to 0.072 µg/ml.
 7. The multispecific anti-HIV antibody of claim 4, wherein the antibody has an IC50 geomean of less than or equal to 0.006 µg/ml.
 8. The multispecific anti-HIV antibody of claim 4, wherein the antibody is capable of neutralizing at least 99.5% of the HIV pseudoviruses listed in Table 1 with an
 - 30 IC50 value of less than 50 µg/mL.
 9. The multispecific anti-HIV antibody of any of claims 1-8, wherein the amino acid sequences of parts i-v) comprise amino acid sequences of single chain fragment

variable (ScFv) moieties, wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody.

- 5 10. The multispecific anti-HIV antibody of claim 9, wherein one or more of the ScFv moieties is organized such that the VL is at the amino terminal end of the ScFv moiety and the VH is at the carboxy terminal end of the ScFv moiety.
11. The multispecific anti-HIV antibody of claim 9, wherein one or more of the ScFv moieties is organized such that the VH is at the amino terminal end of the ScFv moiety and the VL is at the carboxy terminal end of the ScFv moiety.
- 10 12. The multispecific anti-HIV antibody of claim 9, wherein each ScFv moiety is organized such that the VL is at the amino terminal end of the ScFv moiety and the VH is at the carboxy terminal end of the ScFv moiety.
13. The multispecific anti-HIV antibody of any of claims 9-12, wherein the VL and VH sequences are separated by one or more linking amino acids.
- 15 14. The multispecific anti-HIV antibody of claim 13, wherein the linking amino acids comprise one or more tetra-glycine serine (G₄S) linkers.
15. The multispecific anti-HIV antibody of claim 14, wherein the VL and VH sequences are separated by 1-10 tetra-glycine serine (G₄S) linkers.
16. The multispecific anti-HIV antibody of claim 15, wherein the VL and VH
20 sequences are separated by 3 tetra-glycine serine (G₄S) linkers.
17. The multispecific anti-HIV antibody of any of claims 1-16, wherein the antibody further comprises an Fc region of an immunoglobulin or a variant thereof.
18. The multispecific anti-HIV antibody of any of claims 1-17, wherein the antibody comprises a first and second polypeptide chain, wherein the first and second
25 polypeptide chains each comprise
 - a. five ScFv moieties, wherein each ScFv moiety on a single chain recognizes an individual epitope, wherein each ScFv moiety comprises an amino acid sequence from a light chain variable region (VL) and an amino acid sequence from a heavy chain variable region (VH) of an antibody;
30 and
 - b. an Fc region of an immunoglobulin or a variant thereof.

19. The multispecific anti-HIV antibody of any of claims 17-18, wherein the immunoglobulin is IgG.
20. The multispecific anti-HIV antibody of any of claims 17-18, wherein the immunoglobulin is IgG1.
- 5 21. The multispecific anti-HIV antibody of any of claims 9-20, wherein the ScFv moieties are separated on the polypeptide chain by one or more linking amino acids.
22. The multispecific anti-HIV antibody of claim 21, wherein the linking amino acids comprise one or more tetra-glycine serine (G₄S) linkers.
- 10 23. The multispecific anti-HIV antibody of claim 22, wherein each ScFv moiety is separated by 5 tetra-glycine serine (G₄S) linkers.
24. The multispecific anti-HIV antibody of any of claims 1-23, wherein the amino acid sequences of parts i-v) of claim 1 are organized on a single polypeptide chain in the following order, from its N-terminus to its C-terminus:
- 15 i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to the V3-base glycan region epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- 20 iv. an amino acid sequence that binds to the gp120/gp41 interface epitope; and
- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope.
- 25 25. The multispecific anti-HIV antibody of any of claims 1-23, wherein the amino acid sequences of parts i-v) of claim 1 are organized on a single polypeptide chain in the following order, from its N-terminus to its C-terminus:
- i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
- 30 ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;

- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the V3-base glycan region epitope; and
- v. an amino acid sequence that binds to the V1/V2 apex glycan epitope.

26. The multispecific anti-HIV antibody of any of claims 1-23 and 25, wherein the antibody comprises a first and second polypeptide chain, wherein the amino acid sequences of parts i-v) of claim 1 are organized in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
- ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- iv. an amino acid sequence that binds to the V3-base glycan region epitope;
- v. an amino acid sequence that binds to the V1/V2 apex glycan epitope; and
- vi. an Fc region of an immunoglobulin or a variant thereof.

27. The multispecific anti-HIV antibody of any of claims 1-24, wherein the antibody comprises a first and second polypeptide chain, wherein the amino acid sequences of parts i-v) of claim 1 are organized in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to the V3-base glycan region epitope;
- iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;

- iv. an amino acid sequence that binds to the gp120/gp41 interface epitope;
- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope; and
- 5 vi. an Fc region of an immunoglobulin or a variant thereof.

28. The multispecific anti-HIV antibody of any of claims 1-25, wherein the antibody comprises

- 1) a first polypeptide chain, wherein the amino acid sequences of parts i-v) of claim 1 are organized in the following order, from its N-terminus to its

- 10 C-terminus:
 - i. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope;
 - ii. an amino acid sequence that binds to the gp120/gp41 interface epitope;
 - 15 iii. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
 - iv. an amino acid sequence that binds to the V3-base glycan region epitope;
 - v. an amino acid sequence that binds to the V1/V2 apex glycan
 - 20 epitope; and
 - vi. an Fc region of an immunoglobulin or a variant thereof; and

- 2) a second polypeptide chain, wherein the amino acid sequences of parts i-v) of claim 1 are organized in the following order, from its N-terminus to its

- 25 C-terminus:
 - i. an amino acid sequence that binds to the V1/V2 apex glycan epitope;
 - ii. an amino acid sequence that binds to the V3-base glycan region epitope;
 - iii. an amino acid sequence that binds to the CD4 binding site
 - 30 (CD4bs) epitope;
 - iv. an amino acid sequence that binds to the gp120/gp41 interface epitope;

- v. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope; and
- vi. an Fc region of an immunoglobulin or a variant thereof.

29. The multispecific anti-HIV antibody of any of claims 1-28, 46-47 and 48-49,

5 wherein:

- i. the amino acid sequence that binds to the epitope of the V1/V2-glycan region comprises an amino acid sequence from an antibody selected from the group consisting of VRC26.25 and PGDM1400;
- 10 ii. the amino acid sequence that binds to the epitope of the V3-glycan region comprises an amino acid sequence from antibody PGT121;
- iii. the amino acid sequence that binds to the epitope of the CD4-binding site (CD4bs) comprises an amino acid sequence from antibody N6;
- iv. the amino acid sequence that binds to the epitope of the gp120/gp41 interface comprises an amino acid sequence from antibody 35O22; and
- 15 v. the amino acid sequence that binds to the epitope of the membrane proximal external region (MPER) comprises an amino acid sequence from an antibody selected from the group consisting of 10E8v4, 10E8v4_S100cF, and 10E8v4_V5R_S100cF.

30. The multispecific anti-HIV antibody of claim 29, wherein

- 20 i. the amino acid sequence from the antibody VRC26.25 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises QFRFDGYG, CDR H2 comprises ISHDGIKK and CDR H3 comprises AKDLREDECEEWSDDFGKQLPCA KSRGGLVGIADN; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises TSNIGNNF, CDR L2 comprises ETD and CDR L3 comprises ATWAASLSSARV;
- 25 ii. the amino acid sequence from the antibody PGDM1400 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GNTLKTYD, CDR H2 comprises ISHEGDKK and CDR H3 comprises AKGSKHRLRDYALDDD GALNWA VDVLYLSNLEF; and an amino
- 30

acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises HSLIHGDRNNY, CDR L2 comprises LAS and CDR L3 comprises MQGRESPTW;

- iii. the amino acid sequence from the antibody PGT121 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GASISDSY, CDR H2 comprises VHKSGDT and CDR H3 comprises ARTLHGRRYIGIVAFNEWFTYFYMDV; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises SLGSRA, CDR L2 comprises NNQ and CDR L3 comprises HIWDSRVPTKWV;
- iv. the amino acid sequence from the antibody N6 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYTFTAHI, CDR H2 comprises IKPQYGAV and CDR H3 comprises AR; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises QGVGSD, CDR L2 comprises HTS and CDR L3 comprises QVLQF;
- v. the amino acid sequence from the antibody 35O22 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GYRFNFIYH, CDR H2 comprises ISPYS GDK and CDR H3 comprises DDTGTYFCAGLLRDGSSTWLPYL; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises NSVCCSHKS, CDR L2 comprises EDN and CDR L3 comprises CSYTHNSGCV;
- vi. the amino acid sequence from the antibody 10E8v4 comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3 comprises TGYFCACTGKYYDFWSGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3,

wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV;

- vii. the amino acid sequence from the antibody 10E8v4_S100cF comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3 comprises

TGYYFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV;

- viii. the amino acid sequence from the antibody 10E8v4_V5R_S100cF comprises an amino acid sequence from the VH region comprising CDR H1, CDR H2 and CDR H3, wherein CDR H1 comprises GFDFDNAW, CDR H2 comprises ITGPGEGWSV and CDR H3 comprises

TGYYFCARTGKYYDFWFGYPPGEEYFQD; and an amino acid sequence from the VL region comprising CDR L1, CDR L2 and CDR L3, wherein CDR L1 comprises RGDSLRSHYAS, CDR L2 comprises GKNNRPS and CDR L3 comprises SSRDKSGSRLSV.

31. The multispecific anti-HIV antibody of claim 29, wherein

- i. the amino acid sequence from the antibody VRC26.25 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:1; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID

NO:5;

- ii. the amino acid sequence from the antibody PGDM1400 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:9; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:13;

- 5 iii. the amino acid sequence from the antibody PGT121 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:17; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:21;
- 10 iv. the amino acid sequence from the antibody N6 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:25; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:29;
- 15 v. the amino acid sequence from the antibody 35O22 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:33; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:37;
- 20 vi. the amino acid sequence from the antibody 10E8v4 comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:41; and VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45;
- 25 vii. the amino acid sequence from the antibody 10E8v4_S100cF comprises the VH region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:49; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45; and
- 30 viii. the amino acid sequence from the antibody 10E8v4_V5R_S100cF comprises the VH region or a variant thereof comprising 1, 2, 3, or 4

conservative amino acid substitutions, wherein the VH region comprises SEQ ID NO:51; and the VL region or a variant thereof comprising 1, 2, 3, or 4 conservative amino acid substitutions, wherein the VL region comprises amino acids SEQ ID NO:45.

- 5 32. The multispecific anti-HIV antibody of any of claims 1-31, wherein the antibody comprises an IgG1 Fc region variant comprising mutations corresponding to M428L and N434S.
- 10 33. The multispecific anti-HIV antibody of any of claims 1-32, wherein the antibody comprises an immunoglobulin Fc region that has been modified to facilitate heterodimerization.
34. The multispecific anti-HIV antibody of any of claims 1-33, wherein the antibody further comprises a sequence tag that facilitates purification of the antibody.
35. The multispecific anti-HIV antibody of claim 34, wherein the sequence tag comprises a polyhistidine tag.
- 15 36. The multispecific anti-HIV antibody of any of claims 1-35, wherein the antibody comprises any of SEQ ID NOS:72-80.
37. One or more vectors comprising a nucleic acid encoding the antibody or polypeptide of any of claims 1-36.
38. A cell comprising the one or more vectors of claim 37.
- 20 39. An engineered cell that expresses the antibody of any of claims 1-36.
40. The cell of claim 39, wherein the cell is an immune cell.
41. The cell of claim 40, wherein the immune cell is a B cell.
42. A pharmaceutical composition comprising an antibody of any of claims 1-36 and/or cells of any of claims 39-41 and a pharmaceutically acceptable carrier.
- 25 43. A method for treating or preventing HIV infection in a subject, comprising administering to the subject an effective amount of the composition of claim 42.
44. The method of claim 43, wherein the composition is administered in combination with another therapy.
45. The method of claim 44, wherein the therapy is an anti-retroviral therapy.
- 30 46. The multispecific anti-HIV antibody of any of claims 1-23, wherein the amino acid sequences of parts i-v) of claim 1 are organized on a single polypeptide chain in the following order, from its N-terminus to its C-terminus:

- i. an amino acid sequence that binds to a CD4 epitope;
- ii. an amino acid sequence that binds to a V1/V2 apex glycan epitope;
- iii. an amino acid sequence that binds to the V3-base glycan region epitope;
- iv. an amino acid sequence that binds to the CD4 binding site (CD4bs) epitope;
- 5 v. an amino acid sequence that binds to the gp120/gp41 interface epitope; and
- vi. an amino acid sequence that binds to the membrane proximal external region (MPER) epitope.

47. The multispecific anti-HIV antibody of claim 46 wherein the amino acid sequence of (i) is derived from the variable domains of the anti-CD4 antibody
10 dB4C7/UB-421.

48. The multispecific anti-HIV antibody of any of claims 1-23, wherein the antibody comprises a first and second polypeptide chain, wherein the first polypeptide chain comprises a heavy chain variable region wherein the heavy chain variable region comprises;

- 15 i. an amino acid sequence that binds to a V1/V2 apex glycan epitope;
- ii. an amino acid sequence that binds to a V3-base glycan region epitope; and
- iii. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope; and

wherein the second polypeptide chain comprises a light chain variable region wherein the light chain variable region comprises:

- 20 i. an amino acid sequence that binds to a CD4 binding site (CD4bs) epitope;
- ii. an amino acid sequence that binds to a gp120/gp41 interface epitope; and
- iii. an amino acid sequence that binds to a membrane proximal external region (MPER) epitope.

49. The multi-specific anti-HIV antibody of claim 48 wherein the Fc domain comprises a
25 M428L/N434S (LS) mutation.

FIG. 1

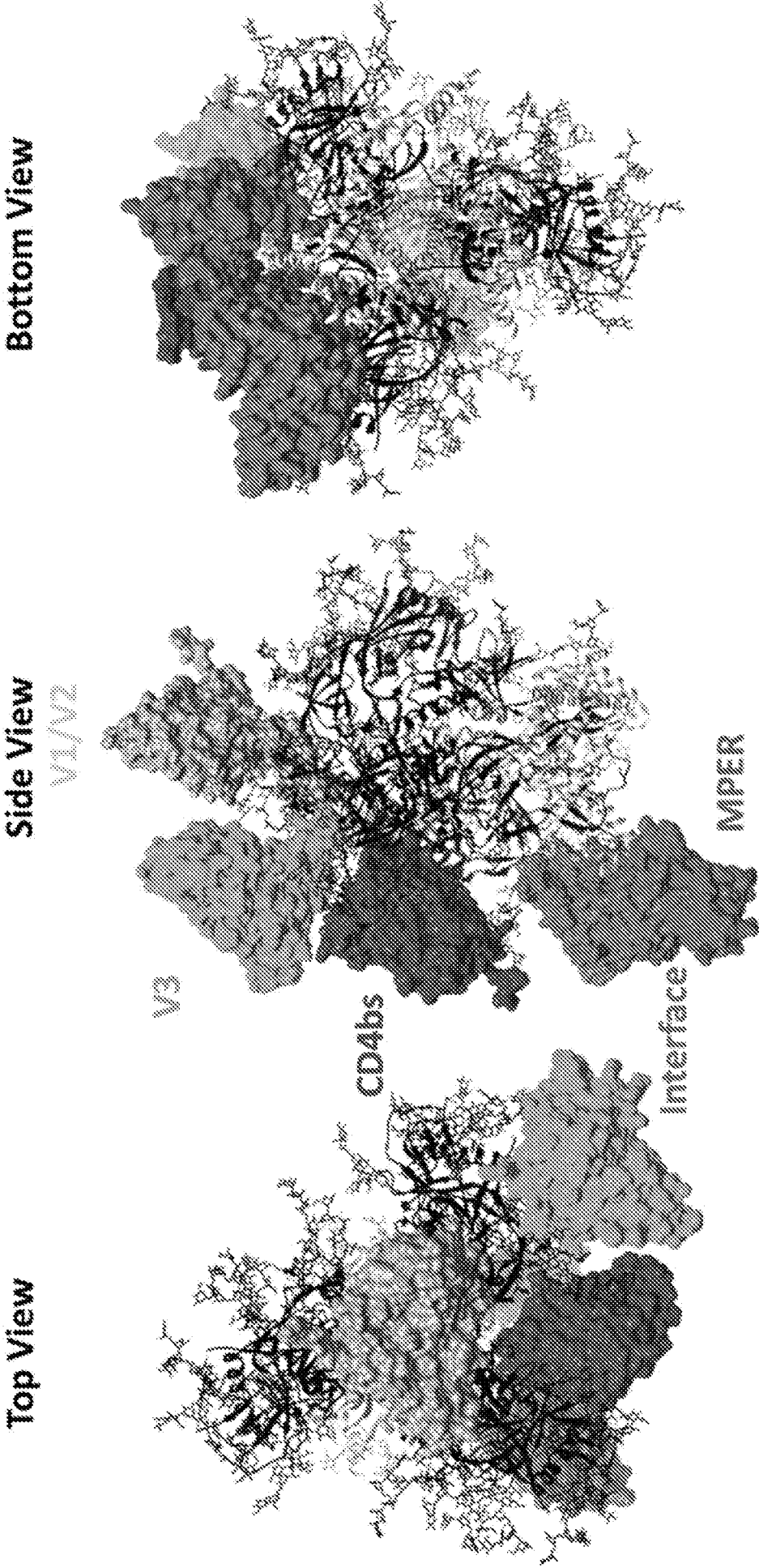


FIG. 2

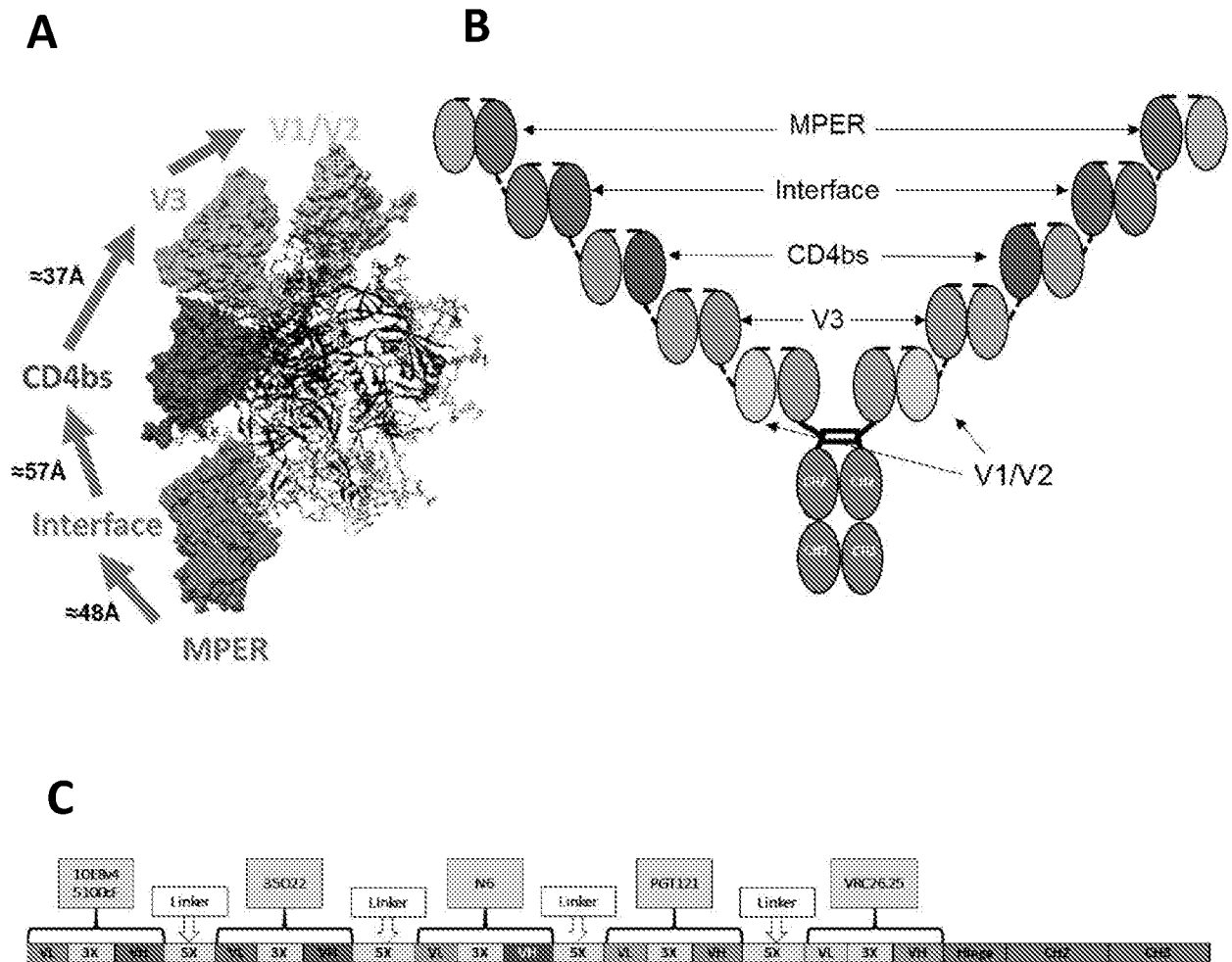


FIG. 3

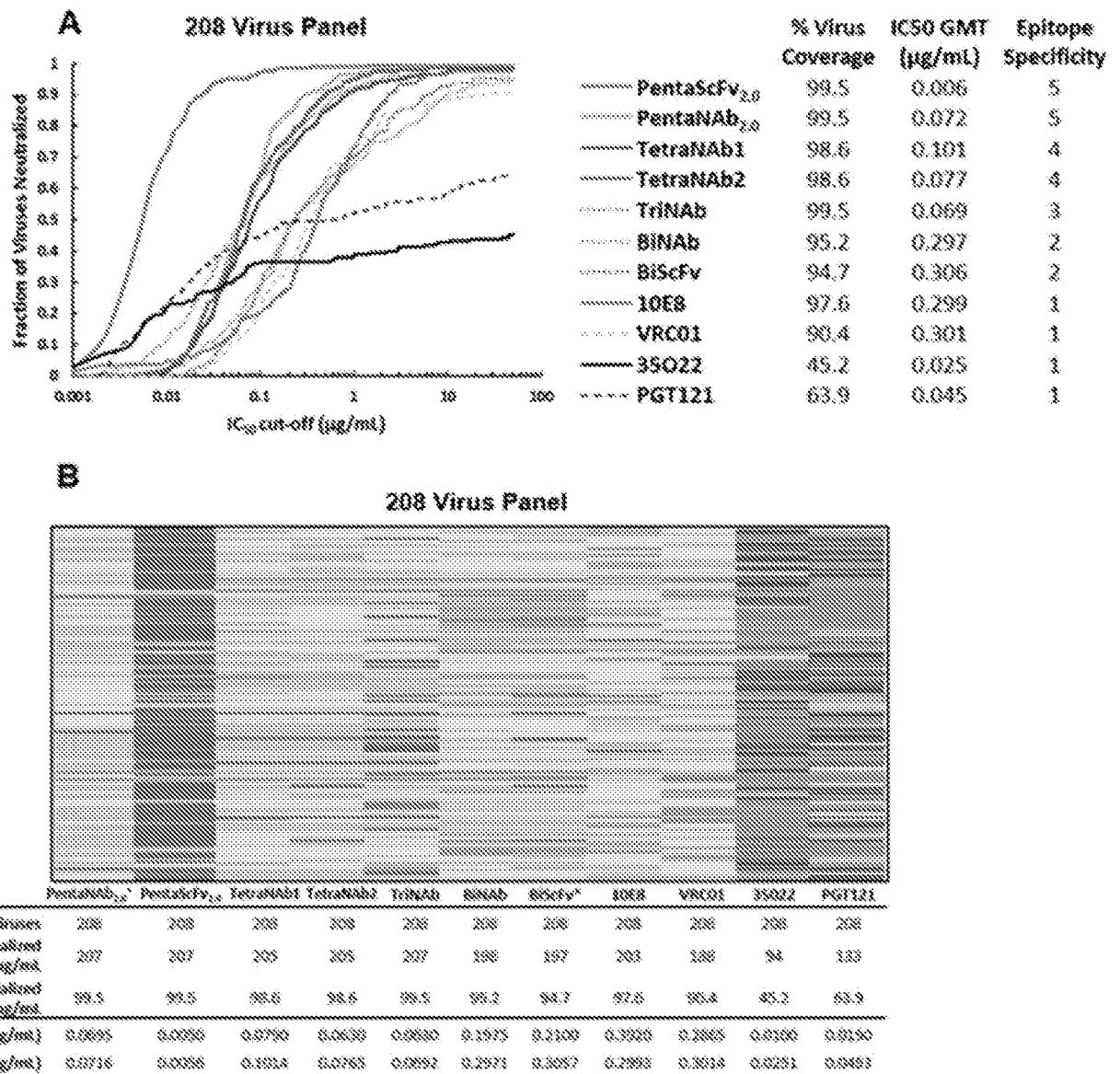


FIG. 4A

Virus ID	Clade	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35022
0260.v5.c36	A	0.099	0.006	0.523	0.039	9.87	0.070
0330.v4.c3	A	0.030	0.002	0.066	0.041	1.12	>50
0439.v5.c1	A	0.665	0.076	0.228	>50	1.23	0.010
3365.v2.c20	A	0.073	0.006	0.060	0.059	1.60	>50
3415.v1.c1	A	0.108	0.010	0.087	>50	4.69	>50
3718.v3.c11	A	0.039	0.004	0.411	1.40	0.838	>50
398-F1 F6 20	A	0.022	0.001	0.102	0.002	0.704	>50
BB201.B42	A	0.019	0.002	0.262	0.003	0.613	>50
BB539.2B13	A	0.057	0.005	0.105	>50	0.591	>50
BG505.W6M.C2	A	0.042	0.005	0.037	0.032	0.689	>50
BI369.9A	A	0.150	0.105	0.047	0.008	0.356	0.007
BS208.B1	A	0.024	0.003	0.027	>50	0.319	>50
KER2008.12	A	0.036	0.002	0.487	2.22	>50	0.0003
KER2018.11	A	0.034	0.004	0.348	>50	1.89	0.005
KNH1209.18	A	0.038	0.002	0.119	0.002	0.406	0.040
MB201.A1	A	0.081	0.006	0.241	0.005	0.411	>50
MB539.2B7	A	0.148	0.016	0.512	>50	>50	1.49
MI369.A5	A	0.079	0.007	0.236	0.022	0.671	0.004
MS208.A1	A	0.056	0.007	0.174	>50	0.187	>50
Q23.17	A	0.012	0.001	0.099	0.004	0.461	0.003
Q259.17	A	0.042	0.003	0.085	>50	4.76	>50
Q769.d22	A	0.110	0.006	0.036	>50	1.91	0.053
Q769.h5	A	0.131	0.005	0.072	>50	2.89	0.635
Q842.d12	A	0.037	0.002	0.034	0.016	2.82	0.006
QH209.14M.A2	A	0.185	0.009	0.026	>50	1.30	>50
RW020.2	A	0.048	0.004	0.217	0.002	0.902	0.009
UG037.8	A	0.119	0.015	0.073	0.065	0.048	0.063
246-F3.C10.2	AC	0.018	0.003	0.254	>50	0.210	0.021
3301.V1.C24	AC	0.069	0.006	0.095	0.009	2.97	0.057
3589.V1.C4	AC	0.059	0.005	0.081	>50	5.77	0.004
6540.v4.c1	AC	0.114	0.006	>50	>50	2.24	>50
6545.V4.C1	AC	0.069	0.005	>50	>50	2.54	>50
0815.V3.C3	ACD	0.055	0.005	0.029	0.020	0.491	0.002
6095.V1.C10	ACD	0.023	0.006	0.631	37.3	0.0005	>50
3468.V1.C12	AD	0.108	0.008	0.058	0.042	0.381	0.005
Q168.a2	AD	0.061	0.009	0.101	>50	0.463	0.051
Q461.e2	AD	0.435	0.043	0.420	>50	2.29	0.001
620345.c1	AE	0.125	0.011	>50	>50	0.989	>50
BJOX009000.02.4	AE	0.109	0.011	1.74	14.7	0.251	>50
BJOX010000.06.2	AE	0.282	0.027	8.40	>50	0.060	3.00
BJOX025000.01.1	AE	0.008	0.001	20.2	>50	0.228	3.00
BJOX028000.10.3	AE	0.014	0.001	0.188	>50	0.167	0.004
C1080.c3	AE	0.020	0.001	2.63	>50	0.108	0.005
C2101.c1	AE	0.128	0.007	0.269	>50	1.20	0.007
C3347.c11	AE	0.078	0.005	0.213	>50	0.019	0.004
C4118.09	AE	0.044	0.003	0.285	>50	0.421	0.0003
CM244.ec1	AE	0.100	0.006	0.116	>50	0.365	0.023

FIG. 4A
(Continued)

Virus ID	Clade	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35022
CNE3	AE	0.125	0.011	1.79	>50	1.37	0.020
CNE5	AE	0.031	0.002	0.398	>50	1.17	>50
CNE55	AE	0.044	0.004	0.358	>50	0.038	>50
CNE56	AE	0.580	0.024	0.525	>50	0.060	>50
CNE59	AE	0.056	0.007	0.368	>50	0.0010	>50
CNE8	AE	0.021	0.0007	0.299	>50	0.140	>50
M02138	AE	0.093	0.007	0.898	>50	0.014	>50
R1166.c1	AE	0.600	0.020	2.09	>50	0.488	>50
R2184.c4	AE	0.070	0.007	0.106	>50	0.576	0.052
R3265.c6	AE	0.486	0.023	0.382	>50	1.58	>50
TH023.6	AE	0.018	0.002	0.546	>50	0.0003	0.086
TH966.8	AE	0.037	0.002	0.390	>50	0.039	>50
TH976.17	AE	0.036	0.003	0.299	>50	0.392	1.00
235-47	AG	0.029	0.004	0.043	0.110	0.244	>50
242-14	AG	0.026	0.002	>50	>50	0.568	>50
263-8	AG	0.049	0.005	0.176	1.23	0.229	>50
269-12	AG	0.096	0.005	0.313	0.164	0.124	>50
271-11	AG	0.020	0.002	0.059	11.7	0.891	0.080
928-28	AG	0.125	0.005	0.394	31.0	0.079	>50
DJ263.8	AG	0.028	0.0010	0.047	0.064	0.009	0.030
T250-4	AG	0.014	0.0010	>50	0.0010	1.07	>50
T251-18	AG	1.59	0.094	4.21	10.8	0.666	>50
T253-11	AG	0.045	0.011	0.397	>50	1.21	2.89
T255-34	AG	0.037	0.003	0.500	>50	0.228	0.005
T257-31	AG	0.039	0.003	1.72	>50	0.336	>50
T266-60	AG	0.100	0.012	1.81	0.160	>50	>50
T278-50	AG	0.241	0.019	>50	>50	0.357	2.27
T280-5	AG	0.078	0.012	0.032	0.002	0.715	>50
T33-7	AG	0.038	0.004	0.018	>50	0.818	1.00
3988.25	B	0.072	0.005	0.494	0.002	0.070	0.609
5768.04	B	0.307	0.028	0.365	0.039	1.63	0.074
6101.10	B	0.076	0.006	0.035	0.002	0.0010	>50
6535.3	B	0.051	0.004	1.93	0.003	0.190	>50
7165.18	B	0.090	0.004	28.2	0.019	0.659	>50
45_01dG5	B	0.126	0.013	0.018	0.002	0.106	>50
89.6.DG	B	0.016	0.0010	0.762	0.016	0.318	>50
AC10.29	B	0.229	0.011	1.81	0.028	0.102	>50
ADA.DG	B	0.052	0.005	0.470	0.002	0.055	>50
BaI.01	B	0.066	0.003	0.095	0.011	0.421	0.002
BaL.26	B	0.137	0.010	0.042	0.010	0.518	0.0010
BG1168.01	B	1.52	0.079	0.869	>50	0.396	>50
BL01.DG	B	0.467	0.039	>50	>50	0.362	0.009
BR07.DG	B	0.128	0.003	1.57	0.064	0.118	0.002
BX08.16	B	0.031	0.002	0.274	0.002	0.213	0.040
CAAN.A2	B	0.256	0.014	1.03	0.005	1.45	0.0010
CNE10	B	0.099	0.005	0.565	0.005	0.014	>50
CNE12	B	0.166	0.010	0.866	0.002	0.301	0.010

FIG. 4A
(Continued)

Virus ID	Clade	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35O22
CNE14	B	0.106	0.068	0.275	0.062	0.151	0.004
CNE4	B	0.249	0.014	0.910	11.5	0.059	0.010
CNE57	B	0.068	0.005	0.563	0.008	0.059	>50
HO86.8	B	0.605	0.024	>50	>50	0.326	>50
HT593.1	B	0.365	0.030	0.476	>50	0.049	>50
HXB2.DG	B	0.105	0.002	0.034	>50	0.003	17.3
JRCSEFJB	B	0.235	0.017	0.362	0.061	0.429	0.070
JRFLJB	B	0.279	0.017	0.028	0.017	0.174	0.020
MN.3	B	0.070	0.008	0.020	>50	0.0003	0.009
PVO.04	B	0.228	0.029	0.511	0.132	1.60	45.0
QH0515.01	B	1.56	0.093	1.01	8.70	2.25	>50
QH0692.42	B	0.530	0.020	1.54	0.940	0.531	0.100
REJO.67	B	0.204	0.011	0.075	8.87	0.302	0.003
RHPA.7	B	0.168	0.010	0.034	0.014	1.01	>50
SC422.8	B	0.115	0.006	0.127	0.098	0.343	>50
SF162.LS	B	0.010	0.0009	0.207	0.004	0.245	>50
SS1196.01	B	0.034	0.0010	0.304	0.002	0.244	0.068
THRO.18	B	0.113	0.008	3.16	>50	0.092	>50
TRJO.58	B	0.675	0.034	0.101	4.31	1.13	0.0010
TRO.11	B	0.109	0.006	0.469	0.006	0.028	>50
WITO.33	B	0.111	0.008	0.102	0.787	0.031	7.24
X2278.C2.B6	B	0.029	0.004	0.151	0.007	0.442	0.0003
YU2.DG	B	0.170	0.013	0.076	0.068	1.17	>50
BJOX002000.03.2	BC	0.042	0.005	>50	0.018	0.384	45.0
CH038.12	BC	0.039	0.002	0.447	0.004	0.271	>50
CH070.1	BC	0.018	0.0010	14.0	0.003	6.65	>50
CH117.4	BC	0.009	0.0008	0.105	>50	0.270	>50
CH119.10	BC	0.081	0.006	0.833	0.029	0.591	38.2
CH181.12	BC	0.014	0.002	0.487	0.007	0.754	10.6
CNE15	BC	0.094	0.006	0.141	19.0	0.844	>50
CNE19	BC	0.020	0.0010	0.247	0.007	0.251	0.0010
CNE20	BC	0.026	0.002	7.39	0.002	0.131	>50
CNE21	BC	0.042	0.004	0.274	0.004	0.979	0.062
CNE40	BC	0.038	0.004	0.433	0.224	0.0010	0.006
CNE7	BC	0.075	0.006	0.187	0.032	0.130	0.003
286.36	C	0.057	0.003	0.223	0.002	1.19	0.009
288.38	C	0.011	0.0010	1.38	0.006	0.435	>50
0013095-2.11	C	0.012	0.0010	0.086	>50	0.009	>50
001428-2.42	C	0.045	0.003	0.014	0.023	1.71	>50
0077_V1.C16	C	0.063	0.008	1.13	>50	1.86	0.005
00836-2.5	C	0.071	0.004	0.122	31.8	0.666	>50
0921.V2.C14	C	0.030	0.003	0.230	>50	0.908	>50
16055-2.3	C	0.042	0.003	0.100	1.02	1.10	>50
16845-2.22	C	0.260	0.016	2.95	9.41	0.020	>50
16936-2.21	C	0.014	0.0007	0.154	0.003	0.264	0.0010
25710-2.43	C	0.033	0.003	0.487	0.014	0.064	>50
25711-2.4	C	0.082	0.006	0.559	0.010	0.516	>50

FIG. 4A
(Continued)

Virus ID	Clade	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35022
25925-2.22	C	0.037	0.002	0.550	0.024	0.402	>50
26191-2.48	C	0.048	0.004	0.183	0.150	1.83	>50
3168.V4.C10	C	0.151	0.011	0.129	0.485	2.83	0.004
3637.V5.C3	C	13.9	1.47	1.97	>50	2.12	>50
3873.V1.C24	C	0.019	0.007	2.81	0.015	5.51	>50
426c	C	0.680	0.030	1.93	>50	0.445	>50
6322.V4.C1	C	0.103	0.015	>50	>50	0.923	>50
6471.V1.C16	C	>50	>50	>50	>50	4.98	>50
6631.V3.C10	C	3.25	0.351	>50	>50	0.934	>50
6644.V2.C33	C	0.080	0.005	0.153	0.018	0.013	0.037
6785.V5.C14	C	0.114	0.008	0.253	0.019	0.701	>50
6838.V1.C35	C	0.046	0.003	0.288	0.119	0.292	>50
96ZM651.02	C	0.075	0.005	0.807	0.009	0.033	>50
BR025.9	C	0.032	0.002	0.528	0.002	0.307	0.005
CAP210.E8	C	0.053	0.003	>50	>50	0.474	0.019
CAP244.D3	C	0.120	0.004	1.34	>50	0.369	>50
CAP256.206.C9	C	0.043	0.003	1.07	0.010	0.713	0.033
CAP45.G3	C	0.030	0.002	6.75	2.08	0.722	0.016
Ce1176.A3	C	0.040	0.003	1.85	0.016	0.252	0.005
CE703010217.B6	C	0.033	0.0010	0.195	0.002	0.096	>50
CNE30	C	0.468	0.020	0.693	0.061	0.456	>50
CNE31	C	0.195	0.017	0.772	0.789	1.32	>50
CNE53	C	0.137	0.008	0.112	0.022	0.213	>50
CNE58	C	0.038	0.005	0.252	>50	0.229	0.070
DU123.06	C	0.015	0.0002	5.70	0.033	0.132	>50
DU151.02	C	0.034	0.002	10.5	0.005	0.461	8.00
DU156.12	C	0.024	0.002	0.077	0.005	0.023	>50
DU172.17	C	0.080	0.003	>50	0.104	0.057	>50
DU422.01	C	0.081	0.004	>50	0.164	0.224	>50
MW965.26	C	0.013	0.003	0.043	0.011	0.0010	5.60
SO18.18	C	0.015	0.0010	0.052	0.002	1.60	>50
TV1.29	C	0.035	0.004	>50	0.118	0.248	>50
TZA125.17	C	0.052	0.004	>50	9.96	0.217	>50
TZBD.02	C	0.251	0.140	0.043	0.005	1.41	>50
ZA012.29	C	0.077	0.010	0.327	0.005	1.47	>50
ZM106.9	C	0.094	0.008	0.264	0.005	>50	>50
ZM109.4	C	0.057	0.005	0.142	13.7	0.161	>50
ZM135.10a	C	0.100	0.004	1.40	1.50	0.033	>50
ZM176.66	C	0.033	0.002	0.045	13.8	0.267	>50
ZM197.7	C	0.113	0.005	0.532	>50	0.055	>50
ZM214.15	C	0.090	0.007	0.957	0.682	2.22	>50
ZM215.8	C	0.027	0.003	0.362	0.014	0.044	0.015
ZM233.6	C	0.019	0.002	1.98	4.14	0.270	0.005
ZM249.1	C	0.046	0.002	0.107	>50	0.830	0.0010
ZM53.12	C	0.028	0.002	0.702	0.002	2.62	>50
ZM55.28a	C	0.080	0.009	0.241	0.070	2.34	>50
3326.V4.C3	CD	0.021	0.002	0.107	>50	1.40	>50

FIG. 4A
(Continued)

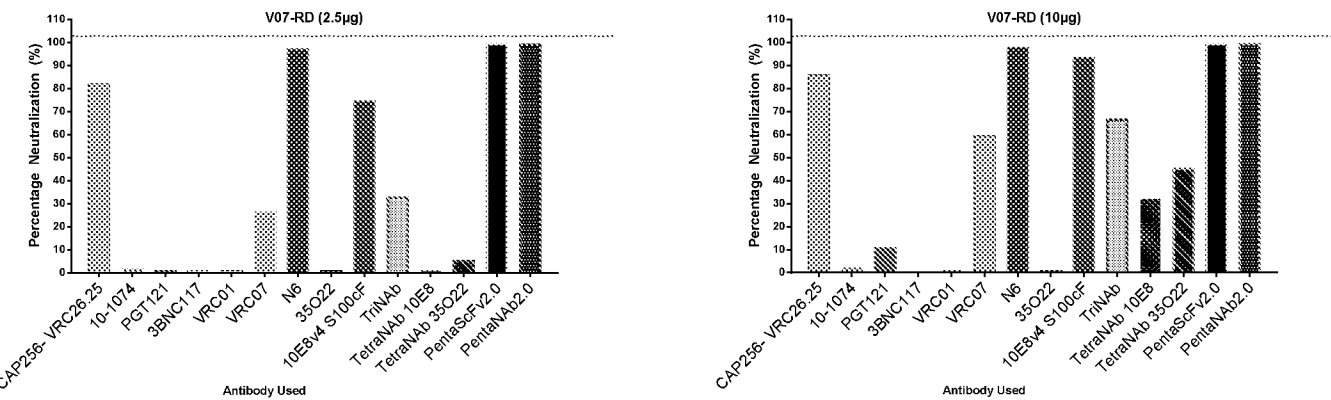
Virus ID	Clade	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35022
3337.V2.C6	CD	0.017	0.0010	0.105	21.1	1.09	0.0009
3817.v2.c59	CD	0.070	0.011	>50	>50	0.229	>50
191821.E6.1	D	0.115	0.014	0.438	>50	1.91	>50
231965.c1	D	0.100	0.015	0.392	>50	11.0	>50
247-23	D	0.049	0.004	1.63	>50	0.344	0.0010
3016.v5.c45	D	0.033	0.004	0.117	>50	0.710	>50
57128.vrc15	D	0.024	0.004	>50	2.16	0.212	0.043
6405.v4.c34	D	0.344	0.015	1.69	0.019	0.461	0.004
A03349M1.vrc4a	D	0.190	0.011	4.42	0.013	0.270	0.0009
A07412M1.vrc12	D	0.070	0.006	0.101	0.012	0.140	>50
NKU3006.ec1	D	1.11	0.157	0.460	>50	0.673	0.004
UG021.16	D	0.234	0.027	0.451	2.41	0.046	>50
UG024.2	D	0.105	0.016	0.219	>50	0.053	>50
P0402.c2.11	G	0.006	0.0010	0.207	0.004	0.057	0.006
P1981.C5.3	G	0.023	0.003	0.336	0.004	0.024	0.0003
X1193.c1	G	0.075	0.009	0.124	0.028	0.341	0.020
X1254.c3	G	0.125	0.016	0.055	0.024	3.67	>50
X1632.S2.B10	G	0.050	0.003	0.131	>50	0.387	0.564
X2088.c9	G	0.129	0.017	>50	0.003	>50	>50
X2131.C1.B5	G	0.033	0.003	0.467	0.010	0.039	>50
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50	>50	>50

FIG. 4B

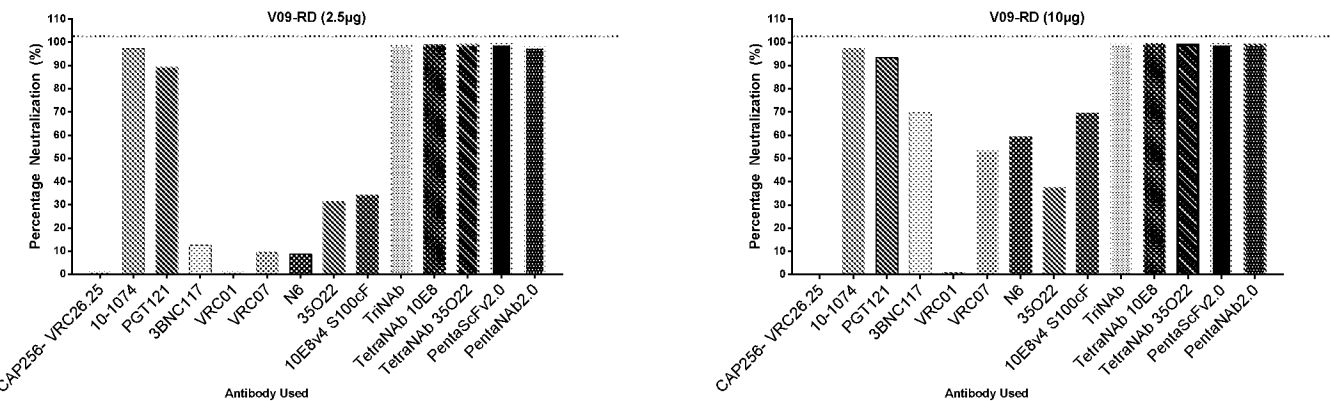
	PentaNAb _{2.0}	PentaScFv _{2.0}	VRC01	PGT121	10E8	35022
# Viruses	208	208	208	208	208	208
Total VS Neutralized						
IC>50 <>50ug/ml	207	207	188	133	203	94
IC>50 <10ug/ml	206	207	184	122	202	89
IC>50 <1.0ug/ml	201	206	152	108	152	79
IC>50 <0.1ug/ml	135	202	40	93	42	75
IC>50 <0.01ug/ml	3	149	0	47	10	45
% VS Neutralized						
IC>50 <>50ug/ml	99.5	99.5	90.4	63.9	97.6	45.2
IC>50 <10ug/ml	99.0	99.5	88.5	58.7	97.1	42.8
IC>50 <1.0ug/ml	96.6	99.0	73.1	51.9	73.1	38.0
IC>50 <0.1ug/ml	64.9	97.1	19.2	44.7	20.2	36.1
IC>50 <0.01ug/ml	1.4	71.6	0.0	22.6	4.8	21.6
Median IC>50	0.070	0.005	0.287	0.019	0.392	0.010
Geometric Mean	0.072	0.006	0.301	0.045	0.299	0.025

FIG. 5

A



B



C

Viruses from VRC01
Clinical Trial

Test Antibody		IC ₅₀ Titer (µg/ml)			
		V07-RD (A)		V09-RD (B)	
		IC ₅₀	IC ₅₀	IC ₅₀	IC ₅₀
Parental mAbs	VRC01	>40	>40	>40	>40
	N6	0.096	0.34	4.88	12.95
	35O22	>40	>40	5.90	>40
	10E8v4S100cF	0.93	2.95	2.20	8.87
	35O22 & 10E8v4S100cF	0.58	3.32	0.36	1.86
Mult-NAbs	Penta-Nab IgG	<0.04	<0.16	0.052	0.13
	Penta-ScFv	<0.04	<0.04	<0.04	<0.04

FIG. 6

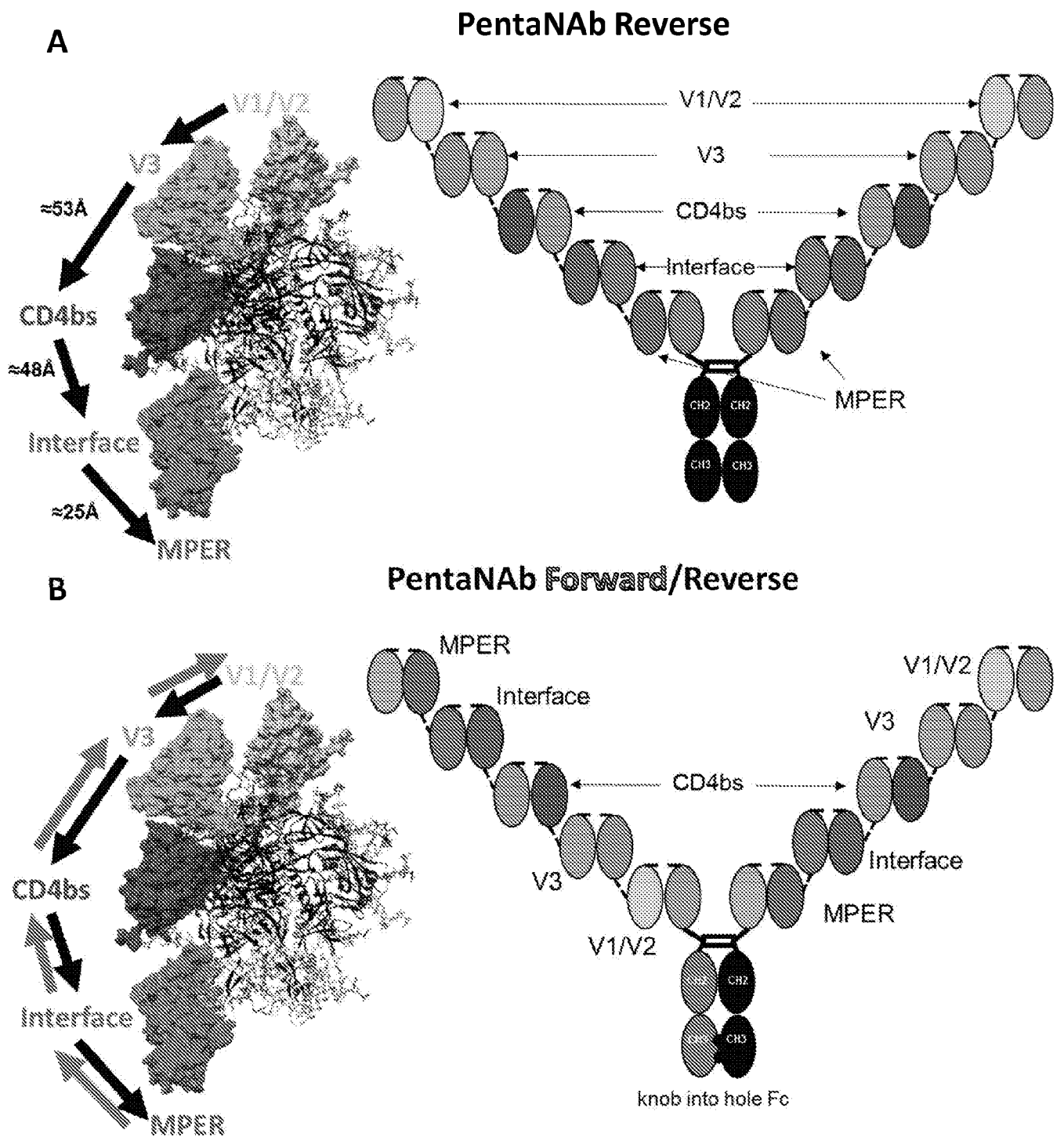


FIG. 7

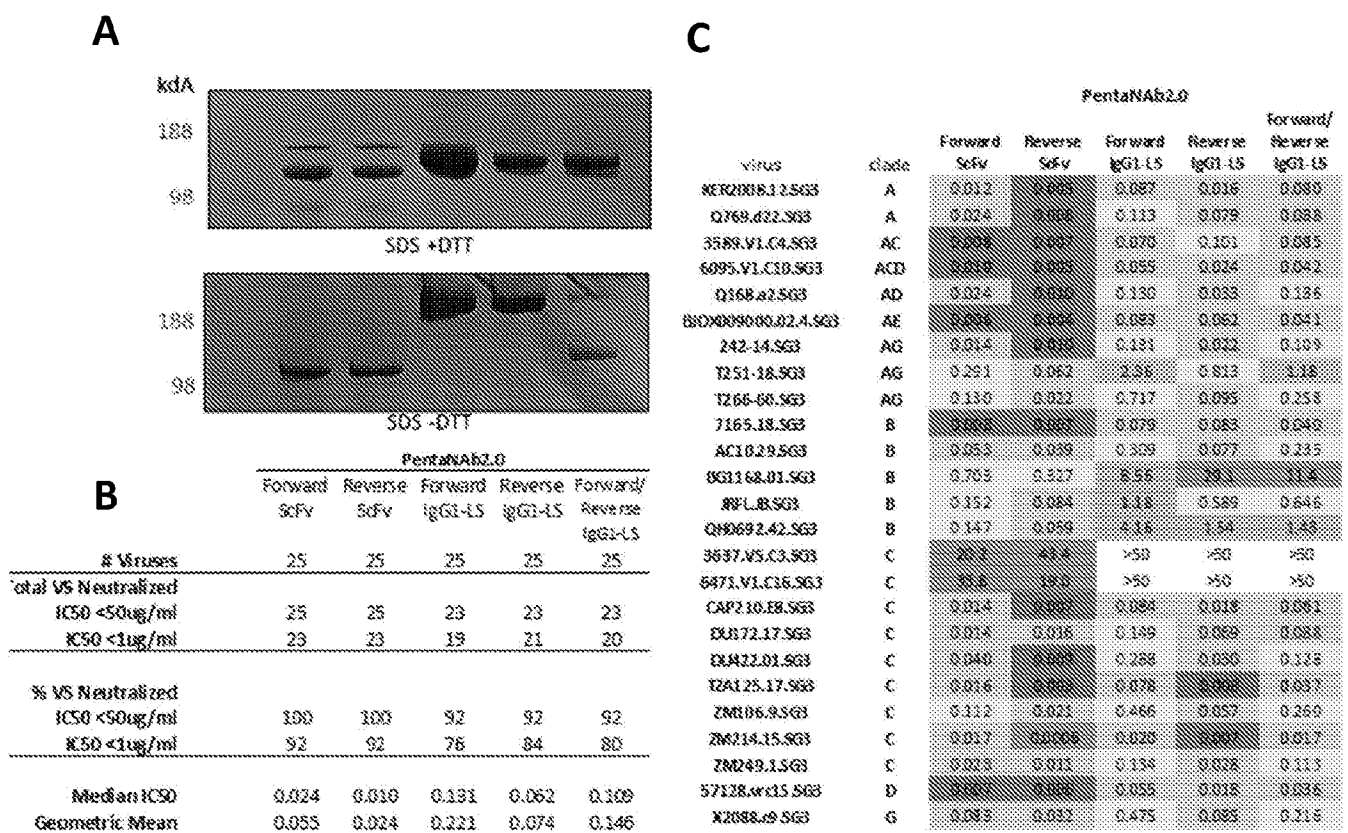


FIG. 8

A				
Distance Between PGT121-VRC01 termini (using PDB: 6FYK)				
Intra-Protomer (1 ↔ 1)				
Orientation	PGT121	VRC01 C-terminus	PGT121 N-terminus Distance (Å)	
VH-VL	VH-VL	VAL 106 V CB	GLN 1 H CB	106.920
VH-VL	VL-VH	VAL 106 V CB	PRO 7 L CB	100.662
VL-VH	VL-VH	VAL 111 U CB	ALA 6 L CB	73.361
VL-VH	VH-VL	VAL 111 U CB	GLN 1 H CB	73.762
Orientation	PGT121	VRC01	PGT121 C-terminus	VRC01 N-terminus Distance (Å)
VH-VL	VH-VL	VAL 106 L CB	GLN 1 U CB	96.325
VH-VL	VL-VH	VAL 106 L CB	VAL 3 V CB	94.519
VL-VH	VL-VH	SER 113 H CB	VAL 3 V CB	93.820
VL-VH	VH-VL	SER 113 H CB	GLN 1 U CB	94.350
Inter-Protomer (1 ↔ 2)				
Orientation	PGT121	VRC01 C-terminus	PGT121 N-terminus Distance (Å)	
VH-VL	VH-VL	VAL 106 V CB	GLN 1 H CB	60.663
VH-VL	VL-VH	VAL 106 V CB	ALA 6 L CB	48.913
VL-VH	VL-VH	VAL 111 U CB	ALA 6 L CB	37.173
VL-VH	VH-VL	VAL 111 U CB	GLN 1 H CB	66.444
Orientation	PGT121	VRC01	PGT121 C-terminus	VRC01 N-terminus Distance (Å)
VH-VL	VH-VL	VAL 106 L CB	GLN 1 U CB	63.470
VH-VL	VL-VH	VAL 106 L CB	VAL 3 V CB	63.459
VL-VH	VL-VH	SER 113 H CB	VAL 3 V CB	66.431
VL-VH	VH-VL	SER 113 H CB	GLN 1 U CB	77.125
B				
Distance Between VRC01-35O22 termini (Using PDB: 6FYK)				
Intra-Protomer (1 ↔ 1)				
Orientation	VRC01	35O22	VRC01 C-terminus	35O22 N-terminus Distance (Å)
VH-VL	VH-VL	VAL 104 V CB	GLN 1 D CB	46.795
VH-VL	VL-VH	VAL 104 V CB	SER 2 E CB	76.268
VL-VH	VL-VH	VAL 111 U CB	SER 2 E CB	82.677
VL-VH	VH-VL	VAL 111 U CB	GLN 1 D CB	83.737
Orientation	35O22	VRC01	35O22 C-terminus	VRC01 N-terminus Distance (Å)
VH-VL	VH-VL	LEU 106 A E CB	GLN 1 U CB	76.856
VH-VL	VL-VH	LEU 106 A E CB	VAL 3 V CB	69.351
VL-VH	VL-VH	SER 113 D CB	VAL 3 V CB	56.967
VL-VH	VH-VL	SER 113 D CB	GLN 1 U CB	81.817
C				
Distance Between 35O22-10E8 termini (Using PDB: 6FYK and 5HQ7)				
Intra-Protomer (1 ↔ 1)				
Orientation	35O22	10E8	35O22 C-terminus	10E8 N-terminus Distance (Å)
VH-VL	VH-VL	LEU 106 E CB	GLU 1 H CB	89.563
VH-VL	VL-VH	LEU 106 E CB	TYR 2 L CB	95.750
VL-VH	VL-VH	SER 113 D CB	TYR 2 L CB	117.288
VL-VH	VH-VL	SER 113 D CB	GLU 1 H CB	123.646
Orientation	10E8	35O22	10E8 C-terminus	35O22 N-terminus Distance (Å)
VH-VL	VH-VL	SER 108 L CB	GLN 1 D CB	115.711
VH-VL	VL-VH	SER 108 L CB	SER 2 E CB	120.145
VL-VH	VL-VH	SER 113 H CB	SER 2 E CB	85.261
VL-VH	VH-VL	SER 113 H CB	GLN 1 D CB	86.240
Inter-Protomer (1 ↔ 2)				
Orientation	35O22	10E8	35O22 C-terminus	10E8 N-terminus Distance (Å)
VH-VL	VH-VL	LEU 106 E CB	GLU 1 H CB	91.172
VH-VL	VL-VH	LEU 106 E CB	TYR 2 L CB	58.521
VL-VH	VL-VH	SER 113 D CB	TYR 2 L CB	82.283
VL-VH	VH-VL	SER 113 D CB	GLU 1 H CB	99.062
Orientation	10E8	35O22	10E8 C-terminus	35O22 N-terminus Distance (Å)
VH-VL	VH-VL	SER 108 L CB	GLN 1 D CB	87.385
VH-VL	VL-VH	SER 108 L CB	SER 2 E CB	86.283
VL-VH	VL-VH	SER 113 H CB	SER 2 E CB	48.278
VL-VH	VH-VL	SER 113 H CB	GLN 1 D CB	86.278
Inter-Protomer (1 ↔ 3)				
Orientation	35O22	10E8	35O22 C-terminus	10E8 N-terminus Distance (Å)
VH-VL	VH-VL	LEU 106 E CB	GLU 1 H CB	24.748
VH-VL	VL-VH	LEU 106 E CB	TYR 2 L CB	45.442
VL-VH	VL-VH	SER 113 D CB	TYR 2 L CB	90.645
VL-VH	VH-VL	SER 113 D CB	GLU 1 H CB	85.162
Orientation	10E8	35O22	10E8 C-terminus	35O22 N-terminus Distance (Å)
VH-VL	VH-VL	SER 108 L CB	GLN 1 D CB	70.113
VH-VL	VL-VH	SER 108 L CB	SER 2 E CB	84.627
VL-VH	VL-VH	SER 113 H CB	SER 2 E CB	71.182
VL-VH	VH-VL	SER 113 H CB	GLN 1 D CB	73.629

FIG. 9

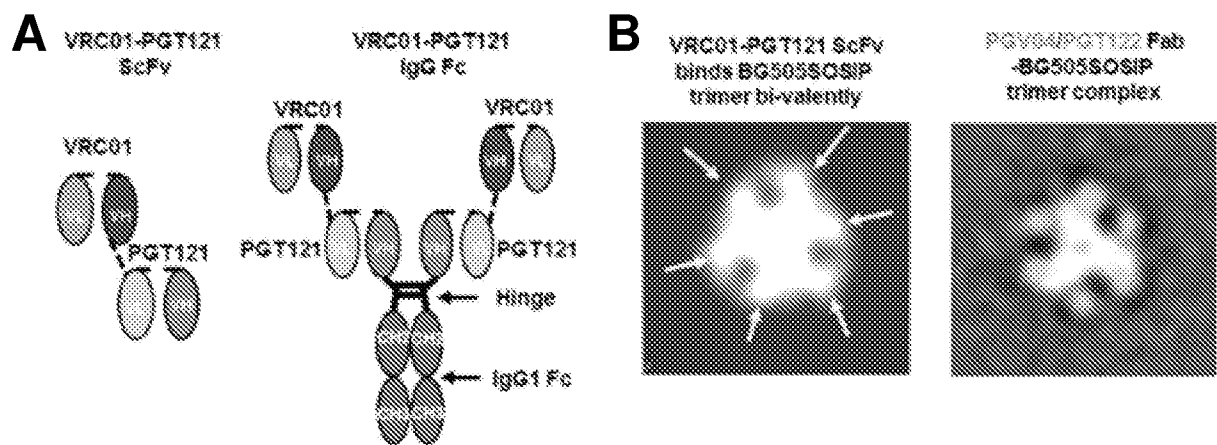


FIG. 10

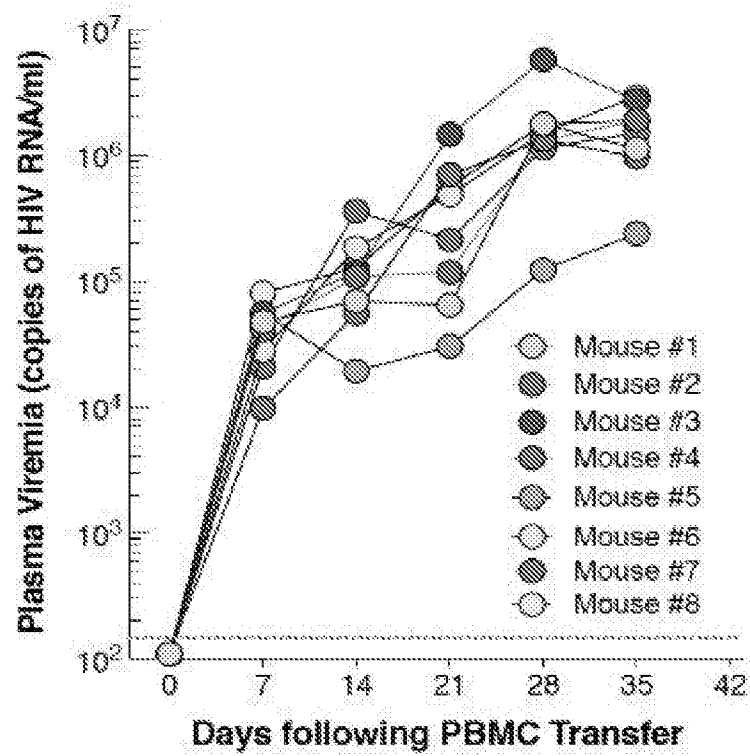
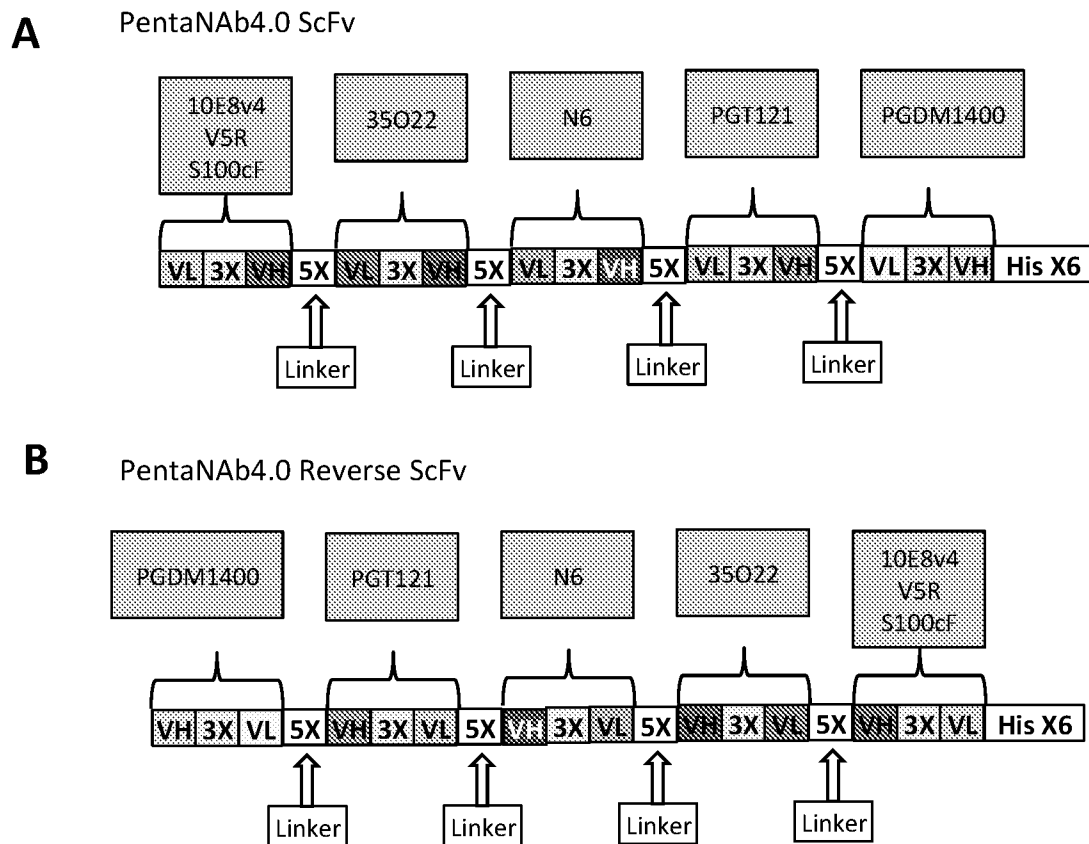


FIG. 11

Linker: (G4S)_n, where n=3 (3x) or 5 (5x)

FIG. 12

HexaNAb1.0 Reverse ScFv

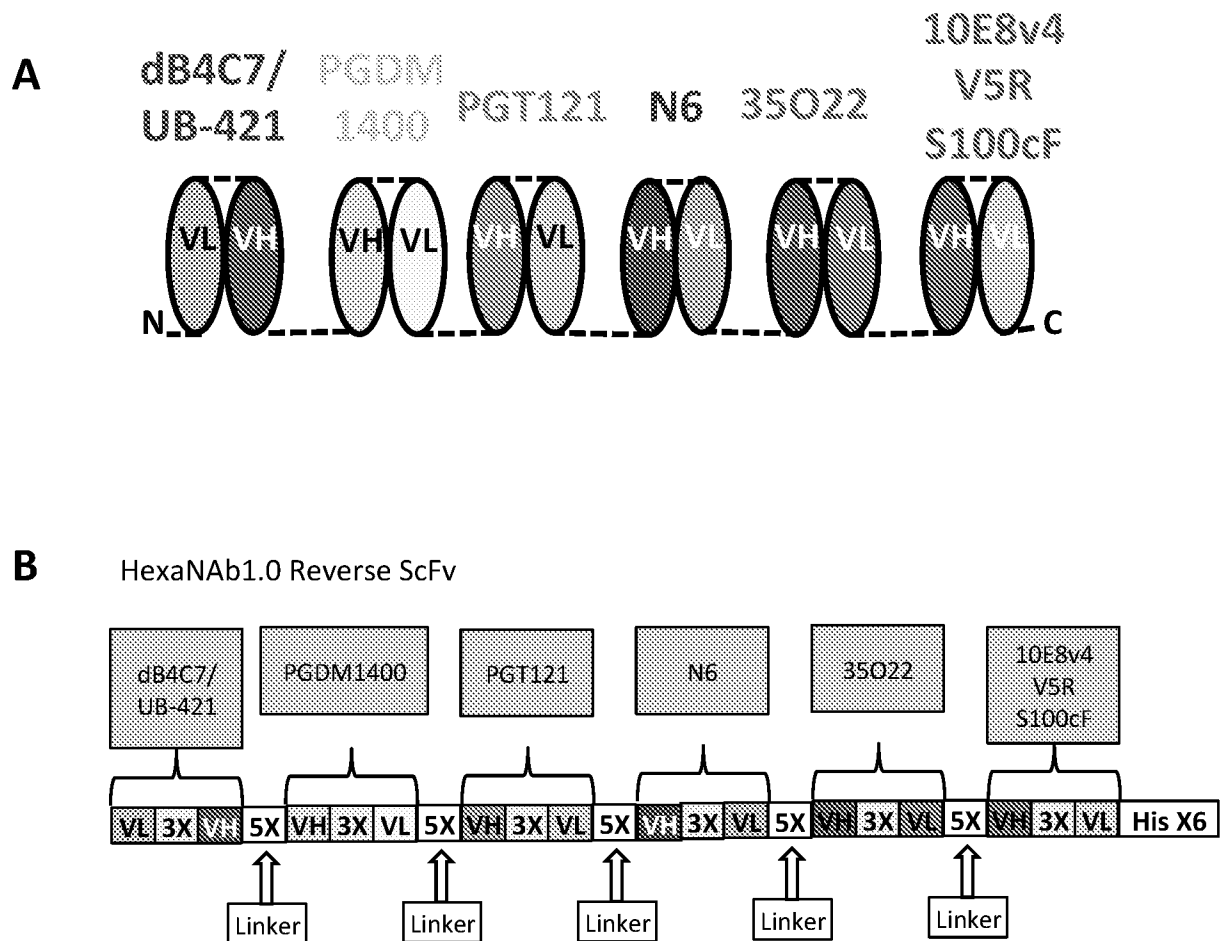
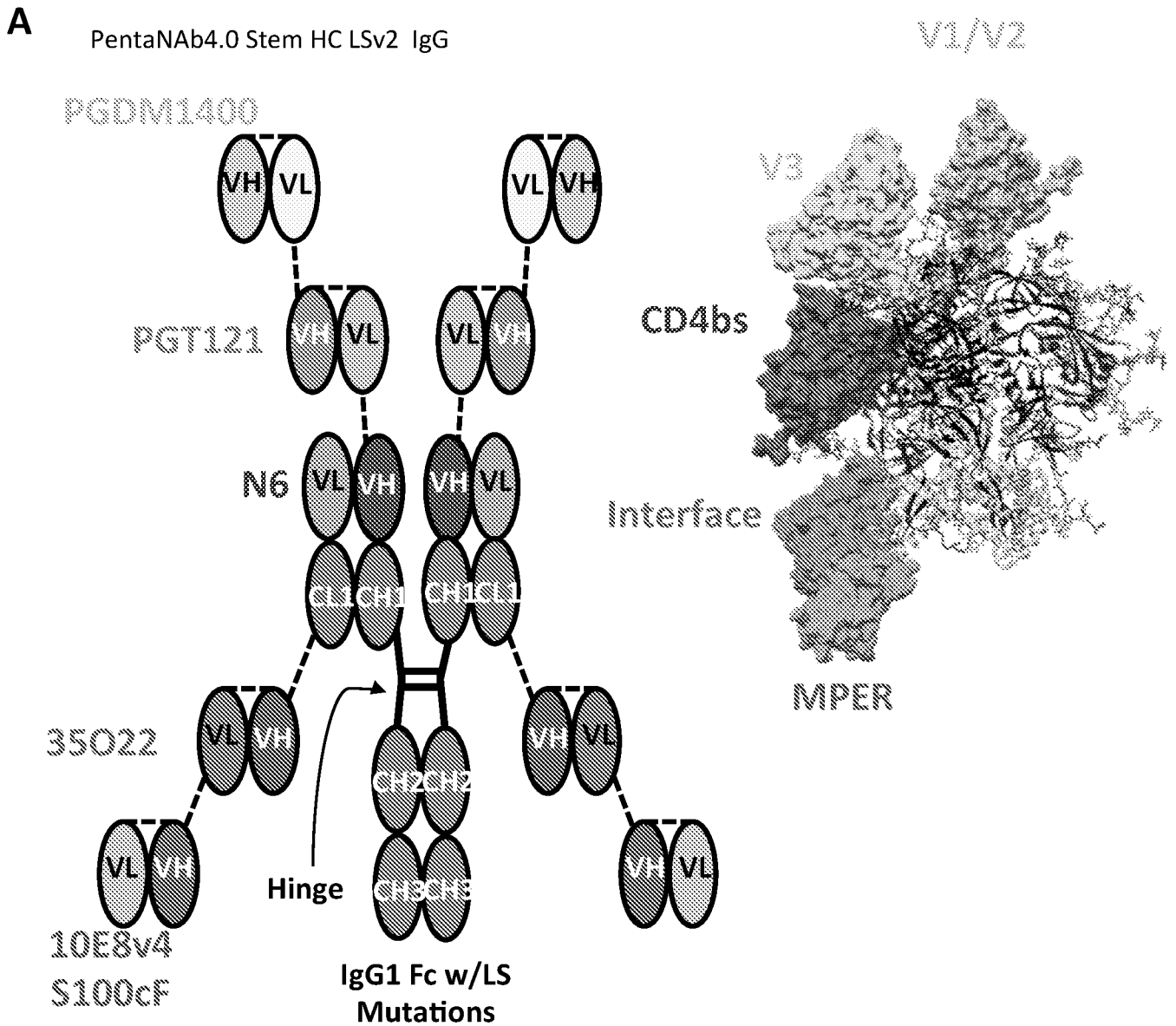
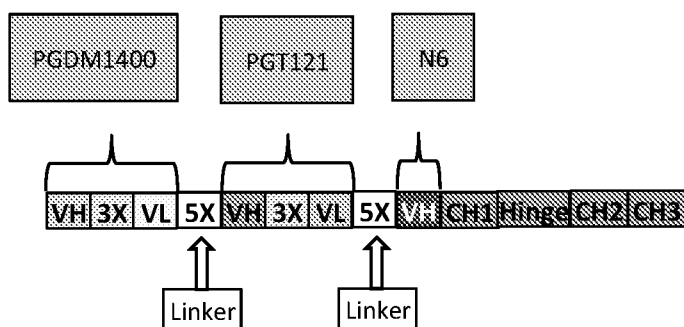


FIG. 13

**B**

PentaNAb4.0 Stem HC LSv2 – Heavy Chain

Linker: (G4S)_n, where n=3 (3x) or 5 (5x)**C**

PentaNAb4.0 Stem HC LSv2 – Light Chain

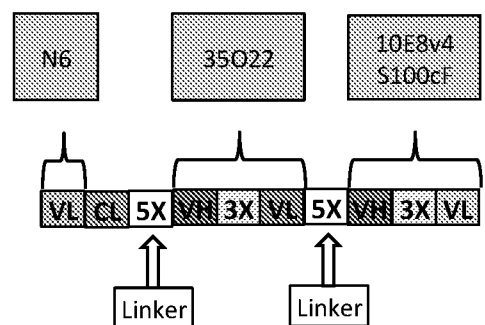


FIG. 14A

Virus ID	Clade	Pentanab		Penta-Nab 2.0		Penta-Nab 4.0		PentaNAB 4.0 Stem		HexaNAB1.0		10E8v4 x		10E8v4 x	
		LS	2.0 ScFv	ScFv	ScFv	Reverse ScFv	HC-LS_v2	Reverse ScFv	VRC01	PGT121	35022	N6	10E8v4	PGDM	
														1400 /	PGDM1400
0260.v5.c36	A	0.197	0.004	0.004	0.019	0.048	0.017	0.002	0.445	0.060	0.070	0.171	7.31	0.025	0.098
0330.v4.c3	A	0.080	0.004	0.002	0.011	0.019	0.003	0.003	0.083	0.066	>50	0.057	1.27	0.012	0.006
0439.v5.c1	A	1.33	0.076	0.079	0.389	2.48	0.168	0.030	0.173	>50	0.010	0.108	1.41	0.417	0.284
3365.v2.c20	A	0.146	0.006	0.004	0.015	0.028	0.008	0.002	0.068	0.121	>50	0.032	0.792	0.017	0.011
3415.v1.c1	A	0.215	0.005	0.008	0.014	0.107	0.025	0.013	0.096	>50	>50	0.053	2.07	0.025	0.020
3718.v3.c11	A	0.078	0.004	0.004	0.003	0.017	0.007	0.002	0.381	0.603	>50	0.033	1.75	0.015	0.006
398-F1_F6_20	A	0.044	0.001	0.002	0.006	0.013	0.012	0.001	0.224	0.023	>50	0.029	1.71	0.021	0.039
BB201.B42	A	0.037	0.002	0.0003	0.003	0.004	0.006	0.001	0.261	0.011	>50	0.187	0.412	0.006	0.0010
BB539.2B13	A	0.113	0.006	0.005	0.030	0.057	0.041	0.016	0.116	>50	>50	0.025	6.77	0.003	0.011
BG505.W6M.C2	A	0.083	0.001	0.001	0.009	0.012	0.022	0.003	0.067	0.049	>50	0.042	0.843	0.018	0.002
B1369.9A	A	0.300	0.105	0.001	0.003	0.023	0.016	0.001	0.236	0.036	0.003	0.176	0.605	0.010	0.011
BS208.B1	A	0.048	0.003	0.001	0.005	0.012	0.007	0.003	0.034	>50	>50	0.019	1.30	0.009	0.005
KER2008.12	A	0.071	0.003	0.002	0.003	0.003	0.003	0.002	0.595	0.578	0.0003	0.468	>50	0.005	0.002
KER2018.11	A	0.068	0.004	0.001	0.003	0.008	0.010	0.003	0.551	>50	0.009	0.279	3.17	0.007	0.003
KNH1209.18	A	0.075	0.002	0.002	0.005	0.013	0.009	0.003	0.128	0.012	0.040	0.092	1.30	0.005	0.016
MB201.A1	A	0.161	0.009	0.006	0.007	0.021	0.015	0.002	0.198	0.002	>50	0.151	0.665	0.154	0.150
MB539.2B7	A	0.296	0.016	0.056	0.023	0.063	0.037	0.021	0.470	>50	1.49	0.231	>50	0.015	0.039
MI369.A5	A	0.158	0.007	0.003	0.013	0.039	0.015	0.003	0.391	0.053	0.003	0.149	0.804	0.002	0.014
MS208.A1	A	0.111	0.007	0.005	0.016	0.054	0.020	0.014	0.220	>50	>50	0.162	1.68	0.0010	0.004
Q23.17	A	0.024	0.001	0.001	0.002	0.006	0.002	0.001	0.085	0.011	0.003	0.035	0.541	0.002	0.0010
Q259.17	A	0.084	0.005	0.008	0.006	0.050	0.013	0.005	0.086	>50	>50	0.289	3.75	0.002	0.015
Q769.d22	A	0.220	0.006	0.023	0.019	0.020	0.009	0.004	0.042	>50	0.053	0.021	0.512	0.0010	0.008
Q769.h5	A	0.262	0.005	0.029	0.006	0.018	0.009	0.006	0.065	>50	0.635	0.038	3.09	0.013	0.006
QH42.d12	A	0.073	0.002	0.002	0.007	0.007	0.007	0.001	0.034	0.023	0.006	0.014	1.98	0.008	0.003
QH209.14M.A2	A	0.369	0.008	0.030	0.031	0.073	0.028	0.014	0.046	>50	>50	0.019	0.750	0.029	0.017
RW020.2	A	0.095	0.004	0.002	0.008	0.015	0.017	0.003	0.276	0.002	0.006	0.069	1.58	0.007	0.023
UG037.8	A	0.238	0.015	0.012	0.047	0.082	0.058	0.022	0.109	0.103	0.063	0.084	0.179	0.036	0.028
246-F3.C10.2	AC	0.035	0.003	0.001	0.005	0.005	0.003	0.003	0.298	>50	0.021	0.035	0.314	0.031	0.011
3301.V1.C24	AC	0.138	0.006	0.003	0.015	0.030	0.014	0.010	0.121	0.021	0.057	0.021	3.58	0.049	0.016
3589.V1.C4	AC	0.117	0.005	0.006	0.009	0.065	0.010	0.005	0.070	>50	0.004	0.045	0.853	0.022	0.001
6540.v4.c1	AC	0.228	0.006	0.004	0.019	0.022	0.012	0.010	>50	>50	>50	0.070	1.57	0.020	0.007
6545.V4.C1	AC	0.137	0.005	0.003	0.006	0.019	0.010	0.004	>50	>50	>50	>50	1.95	0.013	0.002
0815.V3.C3	ACD	0.109	0.002	0.003	0.028	0.094	0.048	0.006	0.034	0.037	0.002	0.031	0.469	0.095	0.084
6095.V1.C10	ACD	0.045	0.002	0.002	0.013	0.019	0.014	0.004	0.783	>50	>50	0.355	3.05	0.006	0.015
3468.V1.C12	AD	0.215	0.009	0.007	0.013	0.024	0.021	0.003	0.059	0.085	0.005	0.029	0.629	0.250	0.061
Q168.a2	AD	0.121	0.008	0.004	0.009	0.018	0.005	0.011	0.112	>50	0.051	0.072	0.464	0.017	0.003
Q461.e2	AD	0.869	0.043	0.011	0.078	0.239	0.075	0.058	0.766	>50	0.001	0.428	3.78	0.116	0.145
620345.c1	AE	0.250	0.011	0.008	0.044	0.635	0.060	0.013	>50	>50	>50	3.88	0.422	2.74	0.061
8J0X009000.02.4	AE	0.218	0.011	0.008	0.020	0.087	0.023	0.005	1.66	4.79	>50	0.388	0.244	0.099	0.096
8J0X010000.06.2	AE	0.564	0.027	0.033	0.003	0.038	0.035	0.006	6.99	>50	3.00	0.689	0.083	0.029	0.015
8J0X025000.01.1	AE	0.015	0.001	0.000	0.001	0.008	0.011	0.001	5.49	>50	3.00	0.072	0.143	0.011	0.010
8J0X028000.10.3	AE	0.028	0.001	0.000	0.000	0.022	0.009	0.000	0.157	>50	0.004	0.000	0.213	0.021	0.014
C1080.c3	AE	0.039	0.004	0.0008	0.003	0.003	0.002	0.0005	1.66	>50	0.005	0.257	0.073	0.005	0.003
C2101.c1	AE	0.255	0.007	0.013	0.005	0.023	0.008	0.005	0.193	>50	0.007	0.085	1.05	0.022	0.009
C3347.c11	AE	0.156	0.006	0.002	0.002	0.014	0.014	0.002	0.101	>50	0.004	0.020	0.027	0.018	0.001
C4118.09	AE	0.087	0.003	0.001	0.003	0.010	0.003	0.003	0.206	>50	0.0003	0.073	0.599	0.009	0.003
CM244.ec1	AE	0.200	0.006	0.004	0.003	0.003	0.002	0.001	0.135	>50	0.023	0.025	0.904	0.0006	0.0009
CNE3	AE	0.250	0.011	0.018	0.013	0.051	0.012	0.015	1.67	>50	0.020	0.059	1.61	0.0010	0.007
CNE5	AE	0.061	0.001	0.002	0.004	0.009	0.003	0.001	0.315	>50	>50	0.100	0.727	0.006	0.002

FIG. 14A (continued)

Virus ID	Clade	Pentanab		Penta-Nab 2.0		Penta-Nab 4.0		PentaNab		HexaNA1.0		10E8v4 x		10E8v4 x		
		2.0 IgG3C	2.0 ScFv	Reverse	ScFv	Reverse	ScFv	4.0 Stem_	Reverse	ScFv	Reverse	ScFv	PGDM	1400 /	PGDM1400	
CNE55	AE	0.088	0.004	0.003	0.007	0.018	0.011	0.002	0.002	0.355	>50	>50	0.126	0.176	0.016	0.008
CNE56	AE	1.16	0.024	0.034	0.040	0.312	0.076	0.012	0.012	0.454	>50	>50	0.133	0.169	0.365	0.209
CNE59	AE	0.111	0.007	0.014	0.020	0.015	0.012	0.002	0.002	0.825	>50	>50	0.220	0.008	0.016	0.008
CNE8	AE	0.041	0.0007	0.0006	0.002	0.004	0.011	0.0007	0.0007	0.490	>50	>50	0.071	0.039	0.009	0.008
M02138	AE	0.186	0.007	0.015	0.022	0.022	0.050	0.019	0.019	1.06	>50	>50	0.483	0.096	0.026	0.038
R1166.c1	AE	1.20	0.020	0.052	0.044	0.161	0.188	0.026	0.026	0.917	>50	>50	0.247	0.235	0.867	0.513
R2184.c4	AE	0.139	0.007	0.008	0.008	0.033	0.021	0.007	0.007	0.106	>50	0.052	0.056	0.347	0.007	0.015
R3265.c6	AE	0.971	0.023	0.167	0.016	0.043	0.015	0.009	0.009	0.564	>50	>50	0.096	1.40	0.025	0.027
TH023.6	AE	0.035	0.002	0.002	0.002	0.004	0.019	0.002	0.002	1.06	>50	0.086	0.027	0.033	0.014	0.009
TH966.8	AE	0.073	0.002	0.001	0.001	0.009	0.030	0.003	0.003	0.489	>50	>50	0.100	0.094	0.019	0.009
TH976.17	AE	0.071	0.002	0.001	0.017	0.386	0.204	0.017	0.017	0.342	>50	1.00	0.105	0.278	0.022	0.135
235-47	AG	0.058	0.004	0.002	0.001	0.012	0.009	0.002	0.002	0.054	0.150	>50	0.020	0.111	0.027	0.008
242-14	AG	0.051	0.002	0.002	0.001	0.015	0.006	0.003	0.003	>50	>50	>50	0.785	0.431	0.019	0.014
263-8	AG	0.098	0.005	0.002	0.001	0.028	0.009	0.003	0.003	0.196	0.447	>50	0.045	0.089	0.010	0.017
269-12	AG	0.192	0.006	0.003	0.016	0.028	0.023	0.009	0.009	0.296	0.142	>50	0.072	0.079	0.386	0.185
271-11	AG	0.039	0.001	0.001	0.001	0.012	0.006	0.003	0.003	0.077	>50	0.080	0.037	0.764	0.016	0.009
928-28	AG	0.250	0.005	0.002	0.016	0.069	0.042	0.012	0.012	0.578	7.34	>50	0.136	0.220	0.031	0.037
DJ263.8	AG	0.055	0.001	0.001	0.001	0.011	0.030	0.003	0.003	0.102	0.042	0.030	0.071	0.021	0.019	0.009
T250-4	AG	0.028	0.001	0.0004	0.001	0.002	0.009	0.0006	0.0006	>50	0.009	>50	0.029	1.30	0.007	0.0010
T251-18	AG	1.18	0.094	0.012	0.001	0.012	0.581	0.003	0.003	3.84	2.96	>50	0.640	0.596	1.33	0.903
T253-11	AG	0.090	0.011	0.001	0.018	0.035	0.071	0.006	0.006	0.340	>50	2.09	0.171	0.700	0.0010	0.009
T255-34	AG	0.073	0.005	0.002	0.001	0.027	0.014	0.003	0.003	0.458	7.62	0.009	0.097	0.360	0.012	0.009
T257-31	AG	0.078	0.002	0.001	0.001	0.018	0.012	0.003	0.003	1.54	>50	>50	0.366	0.443	0.0010	0.006
T266-60	AG	0.200	0.012	0.010	0.100	0.337	0.216	0.125	0.125	2.13	0.144	>50	0.277	>50	0.648	0.223
T278-50	AG	0.481	0.019	0.001	0.001	0.004	0.196	0.001	0.001	>50	>50	2.27	>50	2.96	0.189	1.08
T280-5	AG	0.156	0.012	0.001	0.007	0.028	0.046	0.006	0.006	0.043	0.019	>50	0.024	4.23	0.038	0.007
T33-7	AG	0.076	0.004	0.001	0.012	0.017	0.017	0.003	0.003	0.024	>50	1.00	0.015	0.838	0.008	0.003
3988.25	B	0.144	0.006	0.003	0.016	0.047	0.018	0.011	0.011	0.462	0.014	0.609	0.162	0.177	0.120	0.091
5768.04	B	0.613	0.028	0.021	0.040	0.090	0.043	0.018	0.018	0.424	0.121	0.074	0.121	3.00	0.063	0.069
6101.10	B	0.151	0.006	0.001	0.001	0.023	0.017	0.004	0.004	0.067	0.014	>50	0.016	0.031	0.040	0.007
6535.3	B	0.102	0.004	0.001	0.004	0.010	0.009	0.003	0.003	2.51	0.007	>50	0.225	0.150	0.027	0.006
7165.18	B	0.179	0.004	0.004	0.007	0.026	0.031	0.009	0.009	31.0	0.043	>50	1.71	0.332	2.22	0.210
45_01dG5	B	0.252	0.013	0.003	0.020	0.041	0.025	0.004	0.004	0.037	0.012	>50	0.006	0.390	0.022	0.010
89.6.DG	B	0.032	0.001	0.0009	0.001	0.007	0.021	0.001	0.001	1.31	0.036	>50	0.183	0.389	0.569	0.009
AC10.29	B	0.458	0.011	0.004	0.012	0.065	0.051	0.010	0.010	1.80	0.074	>50	0.450	0.213	0.221	0.132
ADA.DG	B	0.103	0.005	0.001	0.001	0.023	0.025	0.010	0.010	0.584	0.036	>50	0.275	0.051	0.125	0.036
Bal.01	B	0.131	0.001	0.002	0.001	0.007	0.004	0.002	0.002	0.113	0.013	0.012	0.036	0.479	0.012	0.004
Bal.26	B	0.273	0.010	0.005	0.003	0.011	0.006	0.003	0.003	0.047	0.023	0.001	0.023	0.316	0.014	0.004
BG1168.01	B	3.04	0.079	0.106	0.126	0.686	0.652	0.051	0.051	0.549	>50	>50	0.106	0.230	0.793	0.295
BL01.DG	B	0.933	0.039	0.031	0.093	0.216	0.176	0.014	0.014	>50	18.4	0.009	>50	0.226	>50	48.3
BR07.DG	B	0.256	0.003	0.037	0.127	0.113	0.048	0.022	0.022	1.58	0.133	0.009	0.472	0.100	0.638	0.458
BX08.16	B	0.061	0.001	0.0008	0.001	0.005	0.006	0.001	0.001	0.330	0.010	0.040	0.100	0.131	0.011	0.009
CAAN.A2	B	0.511	0.014	0.005	0.018	0.079	0.036	0.008	0.008	1.15	0.021	0.001	0.289	2.78	1.83	0.630
CNE10	B	0.197	0.009	0.001	0.020	0.060	0.011	0.007	0.007	0.756	0.021	>50	0.155	0.302	1.08	0.014
CNE12	B	0.331	0.015	0.004	0.023	0.066	0.031	0.004	0.004	0.703	0.015	0.010	0.190	0.239	1.41	0.617
CNE14	B	0.212	0.006	0.006	0.019	0.043	0.023	0.006	0.006	0.317	0.011	0.004	0.064	0.556	1.02	0.230
CNE4	B	0.497	0.014	0.025	0.044	0.187	0.090	0.022	0.022	0.733	2.16	0.010	0.240	0.106	0.236	0.098
CNE57	B	0.135	0.008	0.002	0.016	0.035	0.016	0.003	0.003	0.532	0.030	>50	0.165	0.217	0.774	0.154
HO86.8	B	1.21	0.024	0.674	0.096	0.211	0.034	0.010	0.010	>50	>50	>50	1.36	0.440	0.220	0.036
HT593.1	B	0.730	0.030	0.030	0.031	0.114	0.126	0.024	0.024	0.531	>50	>50	0.194	0.067	0.198	0.119
HXB2.DG	B	0.209	0.002	0.003	0.001	0.012	0.017	0.002	0.002	0.090	>50	17.3	0.011	0.010	0.043	0.016
JRCSF.J8	B	0.469	0.017	0.014	0.016	0.011	0.008	0.003	0.003	0.350	0.063	0.070	0.162	0.721	0.010	0.006
JRFL.J8	B	0.558	0.017	0.015	0.035	0.091	0.053	0.011	0.011	0.042	0.038	0.030	0.008	0.278	0.119	0.021
MN.3	B	0.140	0.004	0.001	0.015	0.035	0.025	0.001	0.001	0.034	>50	0.009	0.011	0.009	0.009	0.009
PVO.04	B	0.456	0.029	0.027	0.113	0.189	0.171	0.050	0.050	0.508	0.189	43.0	0.244	3.49	0.756	0.373
QH0515.01	B	3.11	0.093	0.163	0.107	0.737	0.427	0.028	0.028	1.15	2.93	>50	0.326	2.02	1.08	0.361

FIG. 14A (continued)

Virus ID	Clade	Pentanab 2.0 IgG3C LS	Pentanab 2.0 ScFv	Penta- Nab 2.0 Reverse ScFv	Penta- Nab 4.0 Reverse ScFv	Penta- Nab 4.0 Reverse ScFv	PentaNab 4.0_Stem_ HC-LS_v2	HexaNab1.0 Reverse ScFv	VRC01	PGT121	35022	N6	10E8v4	10E8v4 x PGDM 1400 / VRC01	10E8v4 x PGDM1400 /N6-LS
QH0692.42	B	1.05	0.020	0.037	0.088	0.142	0.222	0.047	1.58	1.05	0.100	0.579	0.164	0.800	0.721
REJO.67	B	0.407	0.011	0.009	0.006	0.013	0.012	0.003	0.103	2.34	0.003	0.041	0.167	0.012	0.005
RHPA.7	B	0.336	0.010	0.008	0.031	0.056	0.063	0.016	0.064	0.038	>50	0.028	1.12	0.084	0.049
SC422.8	B	0.229	0.006	0.007	0.047	0.105	0.105	0.013	0.142	0.116	>50	0.118	0.310	0.173	0.155
SF162.L5	B	0.020	0.000	0.000	0.204	0.008	0.016	0.002	0.285	0.007	>50	0.072	0.431	0.330	0.109
SS1196.01	B	0.068	0.001	0.002	0.006	0.015	0.022	0.003	0.255	0.014	0.068	0.132	0.155	0.037	0.041
THRO.18	B	0.225	0.006	0.016	0.031	0.124	0.037	0.027	4.39	>50	>50	1.93	0.120	0.226	0.140
TRJO.58	B	1.35	0.034	0.017	0.047	0.089	0.126	0.011	0.128	1.72	0.001	0.147	2.48	0.175	0.268
TRO.11	B	0.218	0.000	0.006	0.020	0.033	0.028	0.007	0.619	0.022	>50	0.152	0.133	0.264	0.153
WITO.33	B	0.221	0.004	0.009	0.210	0.017	0.011	0.004	0.140	0.534	7.24	0.076	0.097	0.001	0.001
X2278.C2.B6	B	0.057	0.004	0.001	0.012	0.032	0.024	0.003	0.121	0.038	0.003	0.033	0.463	0.032	0.038
YU2.DG	B	0.339	0.013	0.004	0.017	0.045	0.082	0.009	0.067	0.041	>50	0.028	0.512	0.214	0.045
BJOX002000.03.2	BC	0.084	0.005	0.002	0.040	0.020	0.013	0.004	>50	0.025	4.0	0.070	0.458	0.006	0.003
CH038.12	BC	0.077	0.007	0.001	0.006	0.020	0.009	0.003	0.333	0.013	>50	0.115	0.408	0.102	0.056
CH070.1	BC	0.035	0.001	0.001	0.001	0.004	0.003	0.003	7.56	0.015	>50	0.143	1.81	0.002	0.002
CH117.4	BC	0.017	0.000	0.000	0.003	0.006	0.003	0.001	0.105	>50	>50	0.043	0.282	0.003	0.003
CH119.10	BC	0.162	0.004	0.003	0.013	0.029	0.013	0.004	0.829	0.032	0.002	0.066	0.869	0.169	0.103
CH181.12	BC	0.027	0.002	0.004	0.018	0.017	0.001	0.013	0.445	0.034	0.006	0.096	0.697	0.002	0.003
CNE15	BC	0.187	0.006	0.005	0.010	0.015	0.006	0.006	0.151	0.30	>50	0.060	1.02	0.008	0.006
CNE19	BC	0.039	0.001	0.0007	0.004	0.009	0.003	0.000	0.215	0.011	0.003	0.038	0.423	0.012	0.003
CNE20	BC	0.052	0.002	0.001	0.005	0.007	0.001	0.001	0.00	0.006	>50	0.028	0.402	0.013	0.003
CNE21	BC	0.083	0.004	0.004	0.010	0.012	0.006	0.003	0.310	0.014	0.062	0.066	0.242	0.002	0.003
CNE40	BC	0.076	0.004	0.003	0.016	0.018	0.029	0.003	0.485	0.279	0.006	0.019	0.006	0.087	0.035
CNE7	BC	0.149	0.006	0.006	0.022	0.057	0.027	0.006	0.397	0.047	0.003	0.069	0.089	0.019	0.022
286.36	C	0.113	0.005	0.002	0.006	0.006	0.003	0.003	0.393	0.013	0.003	0.121	1.67	0.004	0.018
288.38	C	0.022	0.001	0.003	0.004	0.015	0.006	0.006	1.80	0.018	>50	0.366	0.580	0.061	0.024
0013095.2.11	C	0.023	0.001	0.002	0.002	0.006	0.003	0.000	0.127	>50	>50	0.054	0.031	0.004	0.002
001428.2.42	C	0.009	0.003	0.005	0.006	0.007	0.003	0.003	0.022	0.037	>50	0.012	3.56	0.006	0.003
0077_V1.C16	C	0.126	0.009	0.002	0.014	0.033	0.015	0.003	1.18	>50	0.003	0.068	2.08	0.028	0.022
00836.2.5	C	0.141	0.004	0.004	0.007	0.035	0.000	0.004	0.183	>50	>50	0.012	0.607	0.029	0.016
0921.V2.C14	C	0.060	0.003	0.003	0.009	0.026	0.006	0.006	0.277	>50	>50	0.119	1.08	0.027	0.007
16055.2.3	C	0.083	0.001	0.001	0.007	0.013	0.007	0.003	0.087	0.364	>50	0.036	0.910	0.006	0.004
16845.2.22	C	0.520	0.016	0.014	0.014	0.078	0.092	0.008	3.75	2.52	>50	0.608	0.034	0.542	0.198
16936.2.21	C	0.028	0.0007	0.0007	0.002	0.004	0.000	0.000	0.208	0.012	0.001	0.078	0.244	0.011	0.003
25710.2.43	C	0.065	0.003	0.004	0.003	0.010	0.003	0.003	0.487	0.023	>50	0.083	0.056	0.025	0.003
25711.2.4	C	0.163	0.006	0.005	0.014	0.044	0.021	0.006	0.535	0.016	>50	0.087	0.561	0.602	0.215
25925.2.22	C	0.073	0.002	0.001	0.007	0.011	0.004	0.003	0.610	0.050	>50	0.129	0.395	0.015	0.004
26191.2.48	C	0.096	0.004	0.002	0.013	0.032	0.009	0.005	0.196	0.096	>50	0.093	1.28	0.004	0.006
3168.V4.C10	C	0.302	0.011	0.006	0.054	0.112	0.043	0.029	0.175	0.526	0.004	0.153	1.86	0.104	0.114
3637.V5.C3	C	2.7	1.47	>50	3.57	>50	30.6	0.384	7.98	>50	>50	0.331	2.31	5.40	1.12
3873.V1.C24	C	0.037	0.002	0.002	0.006	0.012	0.022	0.002	1.08	0.040	>50	0.117	3.16	0.014	0.006
426c	C	1.36	0.030	0.072	0.096	0.773	0.160	0.027	1.86	>50	>50	0.256	1.09	1.58	0.322
6322.V4.C1	C	0.205	0.015	0.007	0.000	0.003	0.028	0.003	>50	>50	>50	0.069	1.37	0.032	0.004
6471.V1.C16	C	>50	>50	>50	>50	>50	>50	0.426	>50	>50	>50	>50	7.55	>50	>50
6631.V3.C10	C	6.50	0.351	0.507	0.292	3.38	1.13	0.055	>50	>50	>50	0.115	1.55	>50	0.510
6644.V2.C33	C	0.159	0.008	0.004	0.005	0.007	0.007	0.002	0.294	0.034	0.037	0.102	0.136	0.016	0.012
6785.V5.C14	C	0.228	0.008	0.002	0.013	0.024	0.011	0.009	0.462	0.063	>50	0.293	2.11	0.001	0.015
6838.V1.C35	C	0.092	0.005	0.001	0.009	0.010	0.007	0.001	0.358	0.066	>50	0.065	0.620	0.012	0.013
962M651.02	C	0.150	0.004	0.002	0.006	0.019	0.017	0.003	0.824	0.025	>50	0.109	0.081	0.085	0.043
BRQ25.9	C	0.064	0.002	0.001	0.004	0.008	0.007	0.002	0.378	0.010	0.003	0.047	0.634	0.018	0.003
CAP210.E8	C	0.106	0.005	0.001	0.003	0.039	0.013	0.003	>50	5.55	0.019	>50	0.798	0.201	0.014
CAP244.D3	C	0.239	0.004	0.002	0.002	0.021	0.077	0.015	0.999	>50	>50	0.168	0.519	0.338	0.055
CAP256.206.C9	C	0.085	0.005	0.000	0.001	0.005	0.004	0.0007	0.649	0.010	0.033	0.194	1.34	0.020	0.006
CAP45.G3	C	0.059	0.002	0.000	0.002	0.010	0.005	0.001	6.09	1.04	0.016	0.067	0.666	0.008	0.002
Ce1176.A3	C	0.079	0.003	0.002	0.007	0.022	0.009	0.006	3.12	0.027	0.003	0.334	0.431	0.001	0.011
CE703010217.B6	C	0.065	0.001	0.000	0.001	0.004	0.006	0.000	0.248	0.009	>50	0.034	0.135	0.017	0.003

FIG. 14A (continued)

Virus ID	Clade	Pentanab		Penta-Nab 2.0	Penta-Nab 4.0	Penta-Nab 4.0	PentaNab	HexaNab1.0		10E8v4 x						10E8V4 x	
		2.0 IgG3C	2.0 ScFv	Reverse	ScFv	Reverse	4.0_Stem	Reverse	ScFv	PGDM	1400 /	PGDM1400	PGDM	/N6-L5			
		LS	ScFv	ScFv	ScFv	ScFv	HC-L5_v2	Reverse	VRC01	PGT121	35022	N6	10E8v4	VRC01	/N6-L5		
CNE30	C	0.935	0.020	0.029	0.062	0.188	0.044	0.045	0.863	0.126	>50	0.253	0.582	1.17	0.630		
CNE31	C	0.389	0.017	0.024	0.096	0.206	0.151	0.014	1.05	0.676	>50	0.394	1.28	0.382	0.266		
CNE53	C	0.274	0.048	0.003	0.013	0.037	0.027	0.007	0.114	0.028	>50	0.068	0.263	0.031	0.036		
CNE58	C	0.075	0.005	0.002	0.007	0.020	0.008	0.003	0.210	0.13	0.070	0.029	0.304	0.016	0.003		
DU123.06	C	0.029	0.0002	0.001	0.003	0.006	0.004	0.001	0.07	0.048	>50	0.081	0.113	0.021	0.008		
DU151.02	C	0.068	0.002	0.003	0.008	0.012	0.015	0.003	0.13	0.012	0.00	0.068	0.418	0.012	0.014		
DU156.12	C	0.047	0.003	0.001	0.003	0.009	0.006	0.001	0.094	0.016	>50	0.016	0.030	0.019	0.003		
DU172.17	C	0.159	0.002	0.001	0.004	0.021	0.014	0.003	>50	0.003	>50	0.040	0.085	1.07	0.066		
DU422.01	C	0.162	0.004	0.003	0.020	0.067	0.026	0.009	>50	0.063	>50	0.046	0.366	2.96	0.090		
MW965.26	C	0.025	0.003	0.002	0.003	0.006	0.019	0.001	0.045	0.019	0.60	0.013	0.011	0.008	0.003		
SO18.18	C	0.030	0.001	0.004	0.030	0.033	0.005	0.012	0.041	0.005	>50	0.014	0.854	0.004	0.008		
TV1.29	C	0.069	0.004	0.002	0.006	0.009	0.007	0.003	>50	0.176	>50	>50	0.368	0.009	0.003		
TZA125.17	C	0.103	0.004	0.001	0.005	0.027	0.020	0.003	>50	1.75	>50	1.56	0.358	0.081	0.045		
TZBD.02	C	0.502	0.140	0.005	0.012	0.030	0.018	0.013	0.111	0.034	>50	0.061	0.008	0.008	0.003		
ZA012.29	C	0.154	0.008	0.004	0.021	0.032	0.024	0.012	0.297	0.012	>50	0.041	1.40	0.031	0.030		
ZM106.9	C	0.187	0.008	0.003	0.003	0.023	0.017	0.003	0.239	0.012	>50	0.044	>50	0.016	0.023		
ZM109.4	C	0.113	0.005	0.001	0.011	0.032	0.026	0.004	0.152	1.87	>50	0.090	0.208	0.021	0.030		
ZM135.10a	C	0.199	0.004	0.002	0.006	0.026	0.057	0.004	1.29	0.788	>50	0.225	0.151	0.036	0.021		
ZM176.66	C	0.066	0.002	0.0005	0.003	0.030	0.007	0.002	0.050	0.65	>50	0.015	0.917	0.009	0.008		
ZM197.7	C	0.225	0.009	0.004	0.035	0.069	0.050	0.003	0.603	>50	>50	0.178	0.102	0.049	0.055		
ZM214.15	C	0.179	0.007	0.002	0.100	0.574	0.477	0.114	1.20	0.362	>50	0.178	2.22	1.87	0.591		
ZM215.8	C	0.054	0.003	0.001	0.003	0.006	0.005	0.001	0.401	0.034	0.015	0.091	0.063	0.004	0.003		
ZM233.6	C	0.037	0.001	0.002	0.004	0.007	0.004	0.0009	3.37	0.827	0.005	0.125	0.293	0.005	0.006		
ZM249.1	C	0.091	0.002	0.001	0.007	0.034	0.017	0.003	0.110	>50	0.001	0.031	0.936	0.004	0.007		
ZM53.12	C	0.056	0.002	0.0009	0.003	0.006	0.004	0.002	1.20	0.003	>50	0.256	3.82	0.016	0.004		
ZM55.28a	C	0.160	0.005	0.002	0.042	0.075	0.043	0.014	0.293	0.007	>50	0.035	3.23	0.084	0.024		
3326.V4.C3	CD	0.041	0.002	0.001	0.003	0.015	0.007	0.002	0.075	>50	>50	0.002	1.06	0.014	0.003		
3337.V2.C6	CD	0.033	0.001	0.001	0.012	0.051	0.013	0.008	0.098	1.70	0.0009	0.021	0.853	0.009	0.012		
3817.v2.c59	CD	0.140	0.011	0.101	0.399	7.45	0.028	0.086	>50	0.008	>50	0.293	0.845	2.48	0.055		
191821.E6.1	D	0.230	0.014	0.003	0.089	0.144	0.046	0.021	0.622	>50	>50	0.211	2.57	0.069	0.073		
231965.c1	D	0.199	0.015	0.014	0.019	0.060	0.021	0.017	0.489	>50	>50	0.080	3.08	0.029	0.017		
247.23	D	0.098	0.004	0.002	0.004	0.018	0.005	0.002	2.45	>50	0.001	0.181	0.224	0.024	0.003		
3016.v5.c45	D	0.066	0.004	0.002	0.006	0.048	0.020	0.020	0.106	>50	>50	0.035	0.312	0.006	0.014		
57128.vrc15	D	0.047	0.004	0.002	0.007	0.028	0.010	0.005	>50	1.47	0.043	0.720	0.161	0.056	0.030		
6405.v4.c34	D	0.687	0.015	0.009	0.017	0.078	0.067	0.016	1.82	0.038	0.004	0.136	0.853	4.08	0.575		
A03349M1.vrc4a	D	0.380	0.011	0.002	0.014	0.034	0.035	0.003	4.56	0.009	0.0009	1.15	0.318	0.213	0.157		
A07412M1.vrc12	D	0.140	0.006	0.017	0.070	0.097	0.019	0.020	0.145	0.043	>50	0.080	0.516	0.007	0.018		
NKU3006.ec1	D	0.221	0.157	0.134	0.287	1.33	1.41	0.016	0.542	>50	0.003	0.388	0.466	1.10	0.572		
UG021.16	D	0.468	0.027	0.072	0.092	0.092	0.105	0.029	0.511	1.11	>50	0.071	0.096	0.027	0.028		
UG024.2	D	0.209	0.016	0.066	0.200	0.253	0.176	0.021	0.279	>50	>50	0.041	0.070	0.038	0.028		
P0402.c2.11	G	0.011	0.001	0.0009	0.002	0.002	0.006	0.0007	0.165	0.012	0.004	0.058	0.221	0.007	0.003		
P1981.C5.3	G	0.046	0.003	0.001	0.004	0.008	0.005	0.002	0.326	0.003	0.0003	0.232	0.078	0.036	0.028		
X1193.c1	G	0.150	0.008	0.001	0.007	0.017	0.021	0.003	0.167	0.052	0.020	0.110	0.595	0.055	0.040		
X1254.c3	G	0.249	0.016	0.007	0.015	0.045	0.073	0.011	0.054	0.030	>50	0.033	0.880	0.021	0.092		
X1632.S2.B10	G	0.100	0.005	0.001	0.003	0.022	0.013	0.003	0.161	>50	0.564	0.084	0.258	0.0010	0.013		
X2088.c9	G	0.258	0.017	0.007	0.019	0.086	0.044	0.021	>50	0.007	>50	0.063	>50	>50	0.297		
X2131.C1.B5	G	0.086	0.003	0.001	0.005	0.030	0.013	0.003	0.546	0.014	>50	0.140	0.045	0.088	0.071		
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50		
SVA.MLV	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50		

FIG. 14B

	Pentanab 2.0 IgG3C	Pentanab 2.0 ScFv	Penta- Nab 2.0 Reverse ScFv	Penta- Nab 4.0 Reverse ScFv	Penta- Nab 4.0 Reverse ScFv	PentaNAB 4.0_Stem_ HC-LS_v2	HexaNA1.0 Reverse ScFv	VRC01	PGT121	35022	N6	10E8v4	10E8v4 x PGDM 1400 / VRC01	10E8v4 x PGDM1400 /N6-LS
# Viruses	208	208	208	208	208	208	208	208	208	208	208	208	208	208
Total VS Neutralized														
IC50 <50ug/ml	207	207	206	207	206	207	208	188	135	94	202	203	204	207
IC50 <10ug/ml	206	207	206	207	206	206	208	185	132	89	202	203	204	206
IC50 <1.0ug/ml	194	206	206	206	202	204	208	149	113	79	196	147	187	204
IC50 <0.1ug/ml	85	202	199	195	177	185	204	35	91	75	109	32	150	165
IC50 <0.01ug/ml	0	149	165	106	41	65	145	0	10	45	5	2	54	82
% VS Neutralized														
IC50 <50ug/ml	100	100	99	100	99	100	100	90	65	45	97	98	98	100
IC50 <10ug/ml	99	100	99	100	99	99	100	89	63	43	97	98	98	99
IC50 <1.0ug/ml	93	99	99	99	97	98	100	72	54	38	94	71	90	98
IC50 <0.1ug/ml	41	97	96	94	85	89	98	17	44	36	52	15	72	79
IC50 <0.01ug/ml	0	72	79	51	20	31	70	0	5	22	2	1	26	39
Median IC50	0.139	0.005	0.003	0.009	0.023	0.017	0.005	0.328	0.038	0.010	0.086	0.463	0.022	0.015
Geometric Mean	0.143	0.006	0.004	0.011	0.028	0.020	0.005	0.339	0.076	0.025	0.091	0.419	0.034	0.021

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/057089

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/42; C07K 16/10 (2020.01)

CPC - C07K 16/1063; C07K 2317/31; C07K 2317/76 (2020.01)

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/136.1; 424/160.1 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2018/075564 A1 (UNIVERSITY OF MARYLAND, COLLEGE PARK et al) 26 April 2018 (26.04.2018) entire document	1, 3 ----- 2
Y	US 2018/0118816 A1 (IGM BIOSCIENCES, INC.) 03 May 2018 (03.05.2018) entire document	2
A	WO 2017/093985 A1 (CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS et al) 08 June 2017 (08.06.2017) entire document	1-3
A	WO 2017/133640 A1 (NATIONAL CENTER FOR AIDS/STD CONTROL AND PREVENTION, CHINESE CENTER FOR DISEASE CONTROL AND PREVENTION et al) 10 August 2017 (10.08.2017) entire document	1-3
A	US 2015/0218257 A1 (THERACLONE SCIENCES, INC. et al) 06 August 2015 (06.08.2015) entire document	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 January 2020

Date of mailing of the international search report

29 JAN 2020

Name and mailing address of the ISA/US

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/057089

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.: 4-49
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:

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.