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(54) Title: MULTISPECIFIC ANTIBODIES TARGETING HUMAN IMMUNODEFICIENCY VIRUS AND METHODS OF USING THE SAME

(57) Abstract: The present disclosure relates to multispecific antibodies targeting the human immunodeficiency virus-1 (HIV-1) envelope, methods for their production, pharmaceutical compositions containing said antibodies and uses thereof in treatment and prevention of HIV infection.

MULTISPECIFIC ANTIBODIES TARGETING HUMAN IMMUNODEFICIENCY VIRUS AND METHODS OF USING THE SAME

RELATED APPLICATIONS

The present application claims priority to and the benefit of U.S. Provisional Application Ser. No. 62/409,097, filed on October 17, 2016, the contents of which is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

The present disclosure relates to multispecific antibodies targeting the human immunodeficiency virus-1 (HIV-1) envelope, methods for their production, pharmaceutical compositions containing said antibodies and uses thereof in treatment and prevention of HIV infection.

BACKGROUND

The human immunodeficiency viruses type 1 (HIV-1) infects and causes the destruction of human CD4+ lymphocytes, resulting in the development of acquired immunodeficiency sy ndrome (AIDS). The entry of HIV-1 into host cells is mediated by the viral envelope glycoproteins (Env), which are displayed as trimeric spokes on the surface of the HIV virion. The trimeric Env complex, consisting of three of each of exterior envelope glycoprotein, gp120, and the gp41 transmembrane envelope glycoprotein, are associated in the viral membrane. Besides mediating virus entry, HIV-1 Env complex is also the sole target of neutralizing antibody responses.

Recent advances in the discovery of broadly neutralizing antibodies (bNAbs) targeting the HIV-1 envelope glycoproteins (Env) have awakened great interest in their use as pre-exposure prophylaxis for prevention and as therapeutic agents, particularly in combination with antiretroviral treatment (ART) for HIV remission and eradication. bNAb isolation and characterization has been accelerated via the integration of emerging functional and structural information and new technologies of single B cell sorting and cloning. bNAbs are therapeutically beneficial as they possess high capacity for viral neutralization. Additionally, bNAbs can facilitate fragment crystallizable (Fc)-mediated effector functions that promote cell lysis and/or clearance of infected cells that express HIV-1 Env on the cell surface via antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity.

The characterization of HIV-1 bNAbs and their cognate epitopes on the Env spikes has identified five conserved Env sites of vulnerability including the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), and the gp120-gp41 interface. Passive immunization with bNAbs is being explored as a means for prevention in healthy individuals and as treatment for HIV infected patients. Passive immunization in humans has proven highly effective in treating many infections such as hepatitis A, hepatitis B, rabies, and respiratory syncytial viruses. However, these viruses have relatively low genetic diversity compared to the extreme antigenic diversity observed for circulating HIV-1 isolates which leads to greatly confounded outcome. 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. However, the HIV virus rapidly develops resistance mutations under a single bNAb pressure, which suggests that passive treatment with a single bNAb is unlikely to result in long-term viremia suppression. Some of the Env mutations associated with bNAb resistance can significantly reduce viral fitness. Therefore, simultaneously targeting different Env epitopes may completely compromise viral replication, as mutations that confer resistance to each bNAb often accumulate to severely reduce viral fitness. Additionally, treatment of simian/human immunodeficiency virus infection in non-human primate models demonstrated that passive immunotherapy with bNAb cocktails prevent mother to child transmission, suppress viremia and, in contrast to combinatorial antiviral therapy (cART) treatments, as well as facilitate CD8+ T-cell immunity for durable suppression of virus replication. Compared to both cART and single bNAb treatments, preliminary data on bNAb cocktails suggest significant advantages for the treatment and management of HIV-1 infection.

While antibody cocktails demonstrated improved efficacy in preclinical studies, multispecific “single agents” are desirable for manufacturing purposes as well as for improved avidity that may result in enhanced neutralization breadth and potency. Bi-NAbs with two Env-epitope binding sites have been generated using CrossMab formats with up to 97% virus coverage. However, their neutralization breadth could be further improved and truly bivalent binding has yet to be experimentally demonstrated. Most recently, one study showed that swapping the IgG1 hinge for a more flexibly IgG3 hinge lacking disulfide bonds (denoted IgG3C-) greatly improved the potency of anti-HIV CrossMabs. While both the CrossMab and IgG3C- designs have significantly improved the potency and breadth of antibodies against HIV, they only target two epitopes, one corresponding to each antigen-

binding Fragment (Fab) arm. This limits the potential increase of avidity that would result from simultaneous engagement of multiple functional moieties. Furthermore, the traditional CrossMab format imposes steric constraints that may impede true bivalent engagement of the Fab arms due to the rigidity of the dimeric IgG Fc fragment where the Fabs are placed.

One study has demonstrated the use of DNA-linkers as "molecular rulers" to connect Fab moieties of two bNAbs resulting in molecules capable of intra-spike crosslinking to enhance the avidity and potency of bNAbs. However, the chemical conjugation process required for connecting Fabs with DNA-linker in this method limits its feasibility and application scale.

SUMMARY OF THE DISCLOSURE

In one aspect, the disclosure features a multispecific antibody, or an antigen-binding fragment thereof, comprising a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof, wherein the first antibody and the second antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and wherein the VH from the first light chain and the VL from the second light chain are connected by one or more linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by one or more linkers. In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by a two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers. In one embodiment, the linker is not a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly), the peptide Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 292), a single Ser, a single Va, the dipeptide Arg-

Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser, Thr-Val-Ala-Ala-Pro, Gln-Pro-Lys-Ala-Ala, Gln-Arg-Ile-Glu-Gly, Ala-Ser-Thr-Lys-Gly-Pro-Ser, Arg-Thr-Val-Ala-Ala-Pro-Ser, Gly-Gln-Pro-Lys-Ala-Ala-Pro, and His-Ile-Asp-Ser-Pro-Asn-Lys. In one embodiment, the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the envelope protein. In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers. In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers. In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers. In one embodiment, the multispecific antibody of any of the above aspects or embodiments further comprises a third antibody which specifically binds to a third epitope. In one embodiment, the multispecific antibody of any of the above aspects or embodiments further comprises a fourth antibody which specifically binds to a third epitope. In one embodiment, the third epitope is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV. In one embodiment, the third epitope is different from the first epitope and the second epitope. In one embodiment, the first antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1. In one embodiment, the first antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1. In one embodiment, the antibody that binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 is selected from the group consisting of: VRC03, VRC06, VRC07, 3BNC117, IOMA, and N6. In one embodiment, the antibody that binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 is selected from the group consisting of: PGT121, PGT122, PGT128, PGT135, 10-1074, and BG18. In one embodiment, the first antibody binds to an

epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1. In one embodiment, the first antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1. In one embodiment, the antibody that binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 is selected from the group consisting of: 35022, N123-VRC34.01, 3BC315, and PGT151. In one embodiment, the antibody that binds to an epitope in the MPER of the of the envelope protein of HIV-1 is selected from the group consisting of: 10E8, 10E8v4, 10E8v4 S100cF, Dh511.2_k3, Z13, 4E10, and 2F5. In one embodiment, the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 3, 4, 5 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 7, 8, 9. In one embodiment, the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 11, 12, 13 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 15, 16, 17. In one embodiment, the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 32, 33, 34 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 36, 37, 38. In one embodiment, the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 40, 41, 42 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 44, 45, 46. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43. In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ

ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 19, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 19, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 43. In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to

SEQ ID NO. 43, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35. In one embodiment, the multispecific antibody comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In another aspect, the disclosure features a multispecific antibody comprising a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof; and c) a third light chain comprising a first light chain variable region (VL) and a third heavy chain comprising a first heavy chain variable region (VH), wherein the third light chain and the third heavy chain are derived from a third antibody or an antigen-binding fragment thereof, wherein the first antibody, the second antibody and the third antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and wherein the VH from the first light chain and the VL from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers. In one embodiment, the multispecific antibody further includes a modification in the Fc region. In one embodiment the first antibody, the second antibody or the third antibody is an ScFv. In one embodiment, the multispecific antibody has an IC_{50} less than 0.1 µg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.01 µg/ml. In one embodiment, the multispecific antibody has an IC_{80} less than 0.3 µg/ml. In one embodiment, the multispecific antibody has an IC_{80} less than 0.1 µg/ml.

In another aspect, the disclosure features a method of treating or preventing an HIV infection, comprising administering to a subject in need thereof an effective amount of the multispecific antibody of any of the above aspects and embodiments.

In another aspect, the disclosure features a pharmaceutical composition comprising the multispecific antibody of any one of the above aspects and embodiments and a pharmaceutically acceptable carrier.

In another aspect, the disclosure features a nucleic acid encoding the multispecific antibody of any of the above aspects and embodiments. In one embodiment, the disclosure features a vector that comprises the nucleic acid. In another embodiment, the disclosure features a host cell that comprises the vector.

In another aspect, the disclosure features a method for the preparation of a multispecific antibody, comprising the step of culturing the host cell under conditions that allow synthesis of said multispecific antibody. In one embodiment, the method further comprises the step of recovering the multispecific antibody from the host cell culture.

In another aspect, the disclosure features an immunoconjugate comprising the multispecific antibody of any of the above aspects or embodiments, coupled to a cytotoxic agent.

In another aspect, the disclosure features a multispecific antibody, or an antigen-binding fragment thereof or salt thereof, comprising a) a first light chain comprising a first light chain variable region (VL) and/or a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain; b) a second light chain comprising a second light chain variable region (VL) and/or a second heavy chain comprising a second heavy chain variable region (VH), wherein the first light chain and/or first heavy chain binds an epitope on CD4s of HIV-1 and the second light and/or heavy chain binds an epitope of a V1, V2, and/or V3 glycan of HIV-1; wherein the VH from the first light chain and the VH or VL from the second light chain are connected by three or more linkers or wherein the VL from the first light chain and the VH or VL from the second light chain are connected by three or more linkers. In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by a two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers. In another embodiment, the linker is not a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly), the peptide Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-

Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 292), a single Ser, a single Va, the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser, Thr-Val-Ala-Ala-Pro, Gln-Pro-Lys-Ala-Ala, Gln-Arg-Ile-Glu-Gly, Ala-Ser-Thr-Lys-Gly-Pro-Ser, Arg-Thr-Val-Ala-Ala-Pro-Ser, Gly-Gln-Pro-Lys-Ala-Ala-Pro, and His-Ile-Asp-Ser-Pro-Asn-Lys. In another embodiment, the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the envelope protein. In another embodiment, the multispecific antibody, salt or antigen binding fragment further comprisies a IgG-1 like domain covalently linked to the first and/or second light chain. In another further embodiment, the multispecific antibody, salt or antigen binding fragment the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers. In another embodiment, the VH from the first light chain and the VL from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers. In another embodiment, the multispecific antibody, salt or antigen binding fragment further comprises a third antibody light chain that binds to a third epitope. In one embodiment, the third epitope is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV. In anotherembodiment, the third epitope is different from the first epitope and the second epitope.

In another aspect, the disclosure features a multispecific antibody, or an antigen-binding fragment thereof or salt thereof, comprising a variable domain and a constant domain; the variable domain comprising a) a first light chain comprising a first light chain variable region (VL) and/or a first heavy chain comprising a first heavy chain variable region (VH) b) a second light chain comprising a second light chain variable region (VL) and/or a second heavy chain comprising a second heavy chain variable region (VH); the constant domain comprising an IgG-like amino acid sequence; wherein the first light chain and the second light chain bind non- oerlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1); and

wherein the first light chain and the second light chain are connected by from about 3 to about 5 or linkers. In one embodiment, the first and second light chains bind an epiptope that

is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic that shows the construction of a single agent to target multiple independent epitopes by structure-based rational design with superior avidity, potency and breadth, resulting from the crosslinking of protomers of each functional Env trimer due to the additive and potential synergistic effect of individual protomer engagement.

Figure 2A is a schematic that shows conventional bi-NAb construction. **Figure 2B** is a schematic that shows the proposed modular bi-NAb and multi-NAb in single construct strategy.

Figure 3A (left) shows modeling of Env trimer BG505 SOSIP.664 binding to bNAbs VRC01 and PGT121 simultaneously by superimposing individual bNAb Fab-Env complex structure. Only VH-VL regions of Fab are shown. Atomic distance of VRC01 and PGT121 functional domains is shown: 39 Å from VRC01 VH C-terminus to PGT121 VH (upper) and 54 Å from PGT121 VL C-terminus to VRC01 VH N-terminus, respectively; **Figure 3A** (right) shows strategies of engineering VRC01-PGT bi-ScFv by joining the termini of VHs and VLs by 3-5 GGGGS (G4S) linkers in dash lines (magenta) based on the atomic distance of VRC01 and PGT121 functional domains (39 and 54 Å) estimated by the modeling. **Figure 3B** is a schematic presentation of VRC01-PGT121 ScFv and full length IgG. **Figure 3C** shows representative size exclusion chromatography profiles of Bi-ScFv and IgG molecules. dVRC01-5x-PGT121 is shown as an example. Both scFV and IgG molecules migrate as a major single peak, free of aggregates.

Figure 4 depicts a set of graphs that show bi-ScFv and bi-IgG1 of VRC01-PGT121 interaction with HIV-1 Env revealed by Bio-Layer Interferometry (BLI), validating the expected bispecific binding feature. dVRC01-5X-PGT121 scFv and dVRC01-5X-PGT121 IgG were able to bind both CD4bs ligand and Env BG505 SOSIP trimer D368R mutant (VRC01-KO).

Figure 5A depicts a graph that shows the potency and breadth curve of Bi-ScFv molecules tested with selected 20 virus panel. **Figure 5B** is a graph that shows the potency and breadth curve of bi-IgG1 molecules tested with selected 20 virus panel. **Figure 5C** is a table that shows a summary of the virus neutralization parameters of the bispecific antibody

molecules. **Figure 5D** is a table that shows virus neutralization potency displayed with IC_{50} (μg/ml) values.

Figure 6A is a graph that shows potency and breadth curves. **Figure 6B** is a table that shows a summary of the virus neutralization parameters of the bispecific antibody molecules. **Figure 6C** is a table that shows the potency and breadth curve tested with 200 virus panel.

Figure 7 is a table that shows neutralization potency and breadth (IC_{50} value, in μg/ml) of $_{d}$VRC01-PGT bi-ScFv and IgG assessed against a 200-isolate virus panel.

Figure 8 shows modeling of Env trimer binding to bNAbs 35022 and 10E8 simultaneously by superimposing individual bNAb Fab-Env complex structures. The relatively short distance between the variable chains of 35022 and 10E8 (25 Å) suggests that it is feasible to engineer bi-ScFvs of these bNAbs.

Figure 9A is a graph that shows neutralization profiles of bi-ScFvs of 10E8 and 35022. **Figure 9B** is a graph that shows neutralization profiles of bi-NAb (IgGs) of 10E8 and 35022. **Figure 9C** is a table that shows possible synergistic effect of 35022-10E8 bispecific IgG on virus neutralization potency. IC_{50} (μg/ml) of bispecific IgG, 10E8 and 35022 against selected virus isolates from the tested virus panel were displayed. Note that the neutralization potency ($1/IC_{50}$) of the bispecific IgG is substantially higher than the parental.

Figure 10A through D depicts the design of bispecific antibodies. **Figure 10A** shows the structure of HIV-1 JR-FL SOSIP.664 Env trimer (PDB: 5FYK) showing the footprints of bNAbs VRC01 and PGT121 and their proximity in both intra-protomer (left) and inter-protomer (right) binding configurations. PGT122 serves as a surrogate for PGT121. **Figure 10B** shows distances between VRC01 and PGT121 VH/VL termini and two Bi-ScFv molecules of different topology. **Figure 10C** shows a schematic presentation of the Bi-ScFv and Bi-NAb antibody constructs. **Figure 10D** shows a schematic diagram of the molecular configurations of the Bi-ScFv and Bi-NAb antibodies.

Figure 11 A and Figure 11B show the expression and purification of bispecific antibodies. **Figure 11A** shows reduced SDS-PAGE analysis of bispecific antibodies. **Figure 11B** shows analytical size exclusion profiles of bispecific antibodies.

Figure 12A-C Binding characteristics of anti-Env bispecific antibodies. **Figure 12A** is a schematic diagram of the bispecific binding assay via biolayer interferometry (BLI). **Figure 12B** shows BLI response curves of bispecific binding assay. OCTET biosensors were loaded with biotinylated RSC3 (ligand 1) presenting the CD4bs epitope, and then probed sequentially with the bispecific antibody and BG505.SOSIP.664_D368R trimer (ligand 2)

presenting the V3 glycan epitope. As controls, parental IgGs were used in place of the bispecific antibody. **Figure 12C,** depicts representative images of negative stain EM of Bi-ScFv dVRC01-5X-PGT121, in complex with BG505 SOSIP.664 Env at a molar ratio of 0.5:1 and 6:1. BG505 SOSIP trimer and Bi-ScFv dVRC01-5X-PGT121 is denoted with arrow, respectively.

Figure 13 A and B shows negative stain EM of Bi-ScFv/ HIV Env trimer complex. **Figure 13A** shows bi-ScFv, dVRC01-5X-PGT121, in complex with BG505.SOSIP.664 at a ratio of 0.5:1. Left, Raw micrograph; Right, 2D classes of complex. **Figure 13B** shows bi-ScFv, dVRC01-5X-PGT121, in complex with BG505.SOSIP.664 at a ratio of 6:1. Figure 13B left, Raw micrograph; Figure 13B right, 2D classes of complex.

Figure 14 A and B shows the neutralization profile of bispecific antibodies tested with a 20-virus panel. **Figure 14A** is a summary of IC_{50} (μg/ml) for bispecific antibodies tested in 20 virus panel. **Figure 14 B** shows scatter plots of IC_{50} titers in which each virus is represented by an individual circle. († indicates that the IC_{50} was adjusted by a factor of 3 to account for the molarity difference between the lower molecular weight Bi-ScFv and the IgG and Bi-NAb).

Figures 15A through 15D show neutralization breadth and potency of bispecific antibodies. **Figure 15A** shows neutralization breadth of the parental and bispecific antibodies was tested against an HIV-1 pseudovirus panel consisting of Envs of 20 viral strains. Heat maps of IC_{50} titers were generated in Excel. In the heatmaps, each row represents a virus strain while columns represent antibodies. Warmer colors indicate more potent neutralization and blue indicates at 50 μg/ml, antibody virus neutralization is below detection threshold (see legend). Breadths based on IC_{50}s are also summarized. Potency is shown as IC_{50} geometric mean values calculated against sensitive viruses. **Figure 15B** shows neutralization breadth of the parental and bispecific antibodies was tested against a panel of 208 viral strains. Heat maps of IC_{50}, breadth and potency are shown as in (A). **Figure 15C** shows potency-breadth curves comparing the bispecific antibodies to their parental IgGs (left panel) and summary of IC_{50} titers (μg/ml) against VRC01- and dual-resistant viruses that are sensitive to the bispecific antibodies (right panel). († indicates that the IC_{50} was adjusted by a factor of 3 to account for the molarity difference between the lower molecular weight Bi-ScFv and the IgG or Bi-NAb).

Figures 16 A though 16E depict Tri-NAb construct, expression, and characterization. **Figure 16A** shows tri-NAb constructs scheme. **Figure 16B** shows reduced SDS-PAGE analysis of trispecific antibody. **Figure 16C** shows scheme of the trispecific binding assay via

biolayer interferometry (BLI). **Figure 16D** shows BLI curves of trispecific binding assay. OCTET biosensors were loaded with ligand 1 (biotinylated RSC3) specific for VRC01 epitope (CD4bs), followed by trispecific antibody, ligand 2 (BG505.SOSIP.664_D368R) specific for PGT121 epitope (V3 glycan), and ligand 3 (MPER rFc) specific for 10E8 epitope (MPER). Parental IgGs were used as control. **Figure 16E** shows scatter plots of IC_{80} titers in which each virus is represented by an individual circle (Statistical differences in neutralization were evaluated using non-parametric t test (Wilcoxon matched-pairs signed rank test) with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). († indicates that the IC_{80} titer was adjusted by a factor of 3 to account for the molarity difference between the lower molecular weight Bi-ScFv and the IgG, Bi-NAb and Tri-NAb).

Figures 17 A through 17E depict neutralization breadth and potency of trispecific antibodies. **Figure 17A** shows neutralization breadth of the parental, bispecific and trispecific antibodies was tested against a panel of 208 viral strains. Heat maps of IC50, breadth and potency are shown as in Fig.3A. **Figure 17B** shows scatter plots of IC50 titers in which each virus is represented by an individual dot. Statistical differences in neutralization were evaluated using non-parametric t test (Wilcoxon matched-pairs signed rank test) with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **Figure 17C** shows potency-breadth curves comparing the Tri-NAb to the Bi-NAb as well as the parental IgGs (left panel) and summary of IC50 (μg/ml) against viruses that are resistant to Bi-NAb but sensitive to the Tri-NAb (right panel). **Figure 17D** shows comparison of Tri-NAb potency to parental IgGs potencies. Each dot on the graph represents a virus plotted in decreasing order of sensitivity to the parental IgG (black). The potency of the Tri-NAb (green) and the second parental IgG (gray) against the same virus is overlaid with data points below the parental IgG indicating increased potency. **Figure 17E** shows neutralization potency of Bi-NAb (upper panel) and Tri-NAb (lower panel) compared to the parental antibodies. Viruses were grouped by parental antibody sensitivity, with S indicating sensitive and R, resistant. IC50 titer fold change reflecting potency compared to the respective antibodies is indicated in the parentheses next to the multispecifc antibody potency. Fold changes in red indicating improved potency. († indicates that the IC50 was adjusted by a factor of 3 to account for the molarity difference between the lower molecular weight Bi-ScFv and the IgG, Bi-NAb or Tri-NAb).

Figure 18 is a summary of IC50 (μg/ml) for trispecific and bispecific antibodies tested in a 208 virus panel.

Figure 19 is a summary of IC80 (μg/ml) for trispecific and bispecific antibodies tested in a 208 virus panel.

Figure 20 depicts a schematic of a tri and tetraspecific antibody embodiment of the disclosure.

DETAILED DESCRIPTION

The present disclosure is based, at least in part, on the concept of joining single-chain variable fragment (ScFv) domains of two bNAbs, specific for the Env receptor binding site and a conserved Env glycan patch, respectively, to form bispecific ScFvs (Bi-ScFvs). The optimal Bi-ScFv crosslinks adjacent protomers within one HIV-1 Env spike and demonstrates superior neutralization breadth over its parental bNAbs. Furthermore, the present disclosure shows that the combination of this Bi-ScFv with a third bNAb recognizing the Env membrane proximal external region (MPER) resulted in a trispecific bNAb, which displays near-pan neutralization breadth potently. Thus, multispecific antibodies combining functional moieties of Env bNAbs could achieve exceptional neutralization capacity with profoundly augmented avidity. The multispecific 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.

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

The articles “a” and “an” are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element, *e.g.*, a plurality of elements.

The term “including” is used herein to mean, and is used interchangeably with, the phrase "including but not limited to" or “including, without limitation.”

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, an amino acid sequence with a modified amino acid is understood to include the options of an amino acid with a modified sidechain, a an amino acid with a modified backbone, and an amino acid with a modified sidechain and a modified backbone.

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. According to certain embodiments, about means ±10%, ±9%, ±8%, ±7%, ±6%, ±5%, ±4%, ±3%, ±2%, ±1%, ±0.9%, ±0.8%, ±0.7%, ±0.6%, ±0.5%, 0.4%, 0.3%, ±0.2%, +0.1% or +0.05%. According to certain embodiments, about means +5%. When "about" is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers (*e.g.* "at least two") is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "up to" as in "up to 10" is understood as up to and including 10, *i.e.*, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Ranges provided herein are understood to include all individual integer values and all subranges within the ranges.

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 borad neutralizing antibody is an anitbod that inihibits 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 or pharmaceutical composition comprising one ore 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", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivative thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Non-limiting embodiments of which are discussed below.

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 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.

As used herein, "conservative" amino acid substitutions may be defined as set out in Tables A, B, or C below. Anitbodies, antibody-like molecules and derivative, mutants, variants ans salts thereof include those amnio acid sequence wherein conservative substitutions have been introduced by solid state chemistry and/or recombinant modification of nucleic acids that encode amino acid sequences disclosed herein. In some embodiments, the compositions and pharmaceutical compositions of the disclosure comprise, 1, 2, 3, 4, 5 or more conservative amino acid substitions. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table A.

Table A -- Conservative Substitutions I

Side Chain Characteristics	Amino Acid
Aliphatic	
Non-polar	G A P I L V F
Polar - uncharged	C S T M N Q

Polar - charged	D E K R
Aromatic	H F W Y
Other	N Q D E

Alternately, conservative amino acids can be grouped as described in Lehninger, (Biochemistry, Second Edition; Worth Publishers, Inc. NY, N.Y. (1975), pp. 71-77) as set forth in Table B.

Table B -- Conservative Substitutions II

Side Chain Characteristic	Amino Acid
Non-polar (hydrophobic)	
Aliphatic:	A L I V P .
Aromatic:	F W Y
Sulfur-containing:	M
Borderline:	G Y
Uncharged-polar	
Hydroxyl:	S T Y
Amides:	N Q
Sulfhydryl:	C
Borderline:	G Y
Positively Charged (Basic):	K R H
Negatively Charged (Acidic):	D E

Alternately, exemplary conservative substitutions are set out in Table C.

Table C -- Conservative Substitutions III

Original Residue	Exemplary Substitution
Ala (A)	Val Leu Ile Met
Arg (R)	Lys His
Asn (N)	Gln
Asp (D)	Glu
Cys (C)	Ser Thr
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala Val Leu Pro
His (H)	Lys Arg
Ile (I)	Leu Val Met Ala Phe

Leu (L)	Ile Val Met Ala Phe
Lys (K)	Arg His
Met (M)	Leu Ile Val Ala
Phe (F)	Trp Tyr Ile
Pro (P)	Gly Ala Val Leu Ile
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr Phe Ile
Tyr (Y)	Trp Phe Thr Ser
Val (V)	Ile Leu Met Ala

It should be understood that the polypeptides comprising polypeptide sequences associated with the extracellular matrix described herein are intended to include polypeptides bearing one or more insertions, deletions, or substitutions, or any combination thereof, of amino acid residues as well as modifications other than insertions, deletions, or substitutions of amino acid residues.

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, 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 L1, L2 and L3 or H1, 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.

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 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 biomolecules are immunogenic fragments.

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 wild-type 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 be immunogenic and therefore possess a binding avidity for one or a plurality of antigens. In some embodiments, a functional fragment of a polypeptide associated with the extracellular matrix is a polypeptide that comprises 80, 85, 90, 95, 96, 97, 98, or 99% sequence identity of any polypeptide disclosed in Table 1 and has sufficient length to retain at least partial binding affinity to one or a plurality of ligands that bind to the amino acid sequence in Table 1. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table 1 and has a length of at least about 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 in Table 1 and has a length of at least about 50 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 100 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 150 amino acids. In some embodiments, the

fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 200 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 250 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 300 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 350 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 400 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 450 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 500 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 550 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 600 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 650 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 700 amino acids. In some embodiments, the fragment is a fragment of any polypeptide disclosed in Table I and has a length of at least about 750 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 800 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 850 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 900 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 950 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 1000 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of at least about 1050 amino acids. In some embodiments, the fragment is a fragment of any amino acid sequence disclosed in Table I and has a length of no more than the aforementioned alternative number of amino acids in this paragraph.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a

CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, CDR-L2, and CDR-L3 of light chain and CDR-H1, CDR-H2, and CDR-H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub- regions, and FRs represents two or more of the four sub- regions constituting a framework region.

The "variable domain" (variable domain of a light chain (VL), variable domain of a heavy chain (VH)) as used herein denotes each of the pair of light and heavy chains which is involved directly in binding the antibody to the antigen. The domains of variable human light and heavy chains have the same general structure and each domain comprises four framework (FR) regions whose sequences are widely conserved, connected by three "hypervariable regions" (or complementarity determining regions, CDRs). The framework regions adopt a beta-sheet conformation and the CDRs may form loops connecting the beta-sheet structure. The CDRs in each chain are held in their three-dimensional structure by the framework regions and form together with the CDRs from the other chain an antigen binding site. References to "VH" refer to the variable domain of an immunoglobulin heavy chain, including that of an antibody fragment, such as Fv, scFv, dsFv or Fab. References to "VL" refer to the variable domain of an immunoglobulin light chain, including that of an Fv, scFv, dsFv or Fab.

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.*, hCD40). 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 may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. 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 CH1 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 CH1 domains; (iv) a Fv fragment 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 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 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 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).

Full length antibodies comprise immunoglobulin constant regions of one or more immunoglobulin classes. Immunoglobulin classes include IgG, IgM, IgA, IgD, and IgE isotypes and, in the case of IgG and IgA, their subtypes. In a preferred embodiment, an full length antibody of the disclosure has a constant domain structure of an IgG type antibody.

The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.,* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen-binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad, Sci.* 190:382-391 and , Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid

positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The term “multispecific antibody” refers to an antibody or antibody-like molecule, 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. Bispecific antibodies are antibodies which have two different antigen-binding specificities. Trispecific antibodies accordingly are antibodies of the disclosure which have three different antigen-binding specificities. Tetraspecific antibodies according to the disclosure are antibodies which have four different antigen-binding specificities.

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant 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 term "CD4" includes polypeptide molecules that are derived from CD4 include fragments of CD4, generated either by chemical (for example enzymatic) digestion or genetic engineering means. Such a fragment may be one or more entire CD4 protein domains. The extracellular domain of CD4 consists of four contiguous immunoglobulin-like regions (D1, D2, D3, and D4, see Sakihama et al., Proc. Natl. Acad. Sci. 92:6444, 1995; U.S. Pat. No.

6,117,655), and amino acids 1 to 183 have been shown to be involved in gp120 binding. For instance, a binding molecule or binding domain derived from CD4 would include a sufficient portion of the CD4 protein to mediate specific and functional interaction between the binding fragment and a native or viral binding site of CD4. One such binding fragment includes both the D1 and D2 extracellular domains of CD4 (D1D2 is also a fragment of soluble CD4 or sCD4 which is comprised of D1 D2 D3 and D4), although smaller fragments may also provide specific and functional CD4-like binding. The gp120-binding site has been mapped to D1 of CD4. CD4 polypeptides also include "CD4-derived molecules" which encompasses analogs (non-protein organic molecules), derivatives (chemically functionalized protein molecules obtained starting with the disclosed protein sequences) or mimetics (three-dimensionally similar chemicals) of the native CD4 structure, as well as proteins sequence variants or genetic alleles that maintain the ability to functionally bind to a target molecule.

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

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 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 Jan; 79(2):1252-61).

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. 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 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 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, ply-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-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 linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR2 ("amidate"), P(O)R, P(O)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 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. 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. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably 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.

Standard techniques may be used for recombinant DNA, oligonucleotide 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 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.

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.

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 a subject 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 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 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 or administration, the type and/or severity of the particular condition being treated, or the need to modulate the activity of the molecular 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 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 affect 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 tranlated protein is administered to the same subject.

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 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 to another, e.g., one antibody 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 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 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 co-administer 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 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 preferred 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 may 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 animal is a human. For diagnosis of those conditions which are specific for a specific subject, such as a human being, the term “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 suspecte 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. The term “mammal” encompasses both humans and non-humans and includes but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.is used herein to refer to an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.*, a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, a horse, and a whale), or a bird (*e.g.*, a duck or a goose). In an 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 a subject in need thereof, meaning that the subject is is need of the treatment being administered.

The term "salt" refers to acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. Examples of these acids and bases are well known to those of ordinary skill in the art. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable acids may be of utility in the preparation and purification of the compound in question. Salts include those formed from hydrochloric, hydrobromic, sulphuric, phosphoric, citric, tartaric, lactic, pyruvic, acetic, succinic, fumaric, maleic, methanesulphonic and benzenesulphonic acids. In some embodiments, salts of the compositions comprising either an antibody or antibody-like molecule may be formed by reacting the free base, or a salt, enantiomer or racemate thereof, with one or more equivalents of the appropriate acid. In some embodiments, pharmaceutical acceptable salts of the present disclosure refer to derivatives or amino acid sequences comprising at least one basic group or at least one basic radical. In some embodiments, pharmaceutical acceptable salts of the disclosed compositions comprise a free amino group, a free guanidino group, a pyrazinyl radical, or a pyridyl radical that forms acid addition salts. In some embodiments, the pharmaceutical acceptable salts of the present disclosure refer to modified amino acids that are acid addition salts of the subject compounds with (for example) inorganic acids, such as hydrochloric acid, sulfuric acid or a phosphoric acid, or with suitable organic carboxylic or sulfonic acids, for example aliphatic mono- or di-carboxylic acids, such as trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid, hydroxymaleic acid, malic acid, tartaric acid, citric acid or oxalic acid, or amino acids such as arginine or lysine, aromatic carboxylic acids, such as benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxybenzoic acid, salicylic acid, 4-aminosalicylic acid, aromatic-aliphatic carboxylic acids, such as mandelic acid or cinnamic acid, heteroaromatic carboxylic acids, such as nicotinic acid or isonicotinic acid, aliphatic sulfonic acids, such as methane-, ethane- or 2-hydroxyethane-sulfonic acid, or aromatic sulfonic acids, for example benzene-, p-toluene- or naphthalene-2-sulfonic acid. When several basic groups are present mono- or poly-acid addition salts may be formed. The reaction may be carried out in a solvent or medium in which the salt is insoluble or in a solvent in which the salt is soluble, for example, water, dioxane, ethanol, tetrahydrofuran or diethyl ether, or a mixture of solvents, which may be removed in vacuo or by freeze drying. The reaction may also be a metathetical process or it may be carried out on an ion exchange

resin. In some embodiments, the salts may be those that are physiologically tolerated by a patient. Salts according to the present disclsoure may be found in their anhydrous form or as in hydrated crystalline form (i.e., complexed or crystallized with one or more molecules of water). In some embodiments, the compositions or pharmaceutical compositions comprise crystalline forms or lyophilized forms of the antibodies, antibody-like molecules or salts thereof.

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 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).

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.

Humanization and primatization refer to in cases where the tri-specific fusion antibody or the three antibodies forming the tri-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 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 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 retain the specificity and affinity for the antigen.

In specific embodiments, tri-specific fusion antibodies are formed from anti-HIV human or humanized antibodies.

Similarly, a tri-specific fusion antibody or the three 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 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 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 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 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).

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 biological activity is reduced by eighty percent.

Compositions Comprising Anti-HIV-1 Broadly Neutralizing Antibodies (bNAbs)

In some embodiments, the present disclosure involves multimeric anti-HIV-1 broad neutralizing antibodies (or “bNAbs”). In some embodiments, bNAbs target conserved sites of vulnerability on the HIV-1 envelope (env). In some embodiments, the disclosure relates to pharmaceutical compositions or compositions comprising one or a plurality of bNAbs disclosed herein. In some embodiments, if a composition comprises two or more bNAbs, the mixture may be hetergeneous, meaning that there are two different species of bNAbs in the

same composition. In some embodiments, if a composition comprises two or more bNAbs, the mixture may be homogeneous, meaning that there is a single species of bNAb in the same composition. All embodiments of combinations of bNAbs are contemplated by this disclosure. In some embodiments, the pharmaceutical compositions disclosed herein comprises a combination of two, three, four, five, or six or more different species of antibodies disclosed herein.

The disclosure relates to the identification of a sequence orientation of antibody fragments that are capable of neutralizing HIV ENV protein on the surface of a cell, in vitro or in vivo, or in culture. In some embodiments, The disclosure relates to the identification of a sequence orientation of antibody fragments that are sufficient to neutralize or bind up to or at least about 50%, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or up to 100% of viral isolates in culture or in a subject. In some embodiments, the antibody or antibody-like molecule comprises an orientation set forth in Figure 20. In some embodiments, the antibody or antibody-like molecule comprises an orientation in which, beginning on the amino terminius of an arm of the the antibody or antibody-like molecule and following to the carboxy terminus of the molecule, 1, 2, or 3 CDR sequences from the variable light and/or heavy chain of VCOR1 or VCOR7 are fused to 1, 2, or 3 CDR sequences from the variable light and/or heavy chain of PT121 by a linker comprising 3, 4, 5 or more amino acid sequences (GGGGS) or a linker equivalent in angstrom length of 3, 4, or 5 or more amino acid sequences (GGGGS). In some embodiments, the antibody comprises amino acid sequences that are at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous to 1, 2, and/or 3 CDR sequences from the variable light and/or heavy chain of VCOR1 and/or PT12. In some embodiments, the antibody comprises amino acid sequences that are at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous to 1, 2, or 3 CDR sequences from the variable light and/or heavy chain of PT121. In some embodiments, the antibody comprises amino acid sequences that are at least 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous to 1, 2, or 3 CDR sequences from the variable light and/or heavy chain of VCOR1. In some embodiments, the antibody comprises an antibody fragment of Fv_{sc} of VCOR1 fused to an antibody fragment of Fv_{sc} of PT121 by a linker GGGGS that is repeated 3, 4, or 5 times. In some embodiments, the antibody comprises a linker comprising GGGGS repeated no less than 4 times, 5 times, or 6 times between each set of variable light chains specific a for a different epitope. In some embodiments, the antibody comprises a linker comprising GGGGS repeated no less than 4 times, 5 times, or 6 times between each set

of variable heavy chains specific for a different epitope. In some embodiments, the antibody comprises a linker comprising GGGGS repeated no less than 4 times, 5 times, or 6 times between each set of CDR amino acid sequences specific for a different epitope. In some embodiments, the antibody comprises amino acid sequences that are at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous to any one or combination of sequences of Table 1.

In some embodiments, the disclosure relates to an antibody with at least one antibody fragment specific for an CD4s epitope fused to at least one antibody fragment specific for an V1/V2-glycan region epitope by a linker GGGGS that is repeated 3, 4, or 5 times or a linker equivalent in length to an amino acid sequence GGGGS repeated 3, 4, or 5 times. In some embodiments, the antibody has at least three valencies, wherein the first valency comprises or consists of antibody fragment specific for an CD4s epitope; wherein the second valency comprises or consists of antibody fragment specific for a V1/V2-glycan region epitope; and wherein the third valency comprises or consists of an antibody fragment specific for an epitope that is located in the gp41 membrane proximal external region (MPER). In some embodiments, if any one antibody fragment comprises both heavy and light variable chains specific for one of the epiptopes disclosed herein, the heavy and light variable chains of such antibody are separated by a linker that comprises the amino acid sequence GGGGS repeated 1, 2, 3, 4 or more times. n some embodiments, if any one antibody fragment comprises both heavy and light variable chains specific for one of the epiptopes disclosed herein, the heavy and light variable chains of such antibody are separated by a linker that comprises the amino acid sequence GGGGS repeated 3 times. In some embodiments, the linker GGGGS is replaced with a linker of equivalent length such that orientation of and distance between the various variable heavy and light chain antibody fragments remains substantially comparable or identical to the orientation and distance between the various variable heavy and light chain antibody fragments separated by one or more amino acid sequences GGGGS.

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 one or more of the epiptopes disclosed herein, and the constant portion comprising an IgG-like domain. The IgG domain can comprise SEQ ID NO:28 or any amino acid sequence from about 50% to about 99% homolgous to SEQ ID NO:28. In some embodiments, the variable portion of the antibody comprising 1, 2, 3, 4 or more amino acid sequences capable of binding any 1, 2, 3, or 4 or more epitopes disclosed herein, wherein each amino acid

sequence capable of binding an epitope is separated by at least 3, 4, or 5 contiguous amino acid linkers (such as GGGGS). In some embodiments, the variable portion comprises one or more CDR sequences specific for an epitope disclosed herein. In some embodiments, the variable portion comprises at least three or at least four amino acid domains comprising from 1 to about 3 CDR sequences specific for an epitope disclosed herein. In some embodiments, the order in which the CDR sequences are placed or orented within the antivody correlates to its efficacy in neutralizing various isolates of HIV. Any permutation or order of CDR sequences disclosed in Table 1 is contemplated.

Various bNAbs are known in the art and can be used according to this disclosure. In some embodiments, the present disclosure comprises a compostion or cell comprising bispecific, trispecific or tetraspecific anti-HIV bNAbs. Examples include but are not limited to those described in U.S. Patent No. 8673307, WO2014063059, WO2012158948, WO2015/117008, and PCT/US2015/41272, including antibodies 3BNC117, 3BNC60, 12A12, 12A21, NIH45-46, bANC131, 8ANC134, IB2530, INC9, 8ANC195. 8ANC196, 10-259, 10-303, 10-410, 10- 847, 10-996, 10-1074, 10-1121, 10-1130, 10-1146, 10-1341, 10-1369, and 10-1074GM. Additional examples include those described in Klein et al, Nature, 2012. 492(7427): p. 118-22, Horwitz et al, Proc Natl Acad Sci U S A, 2013. 110(41): p. 16538--43, Scheid, et al. 2011. Science, 333 : 1633-1637, Scheid, et al. 2009. Nature, 458:636-640, Eroshkin et al, Nucleic Acids Res. 2014 Jan;42 (Database issue):Dl 133-9, Mascola et al. Immunol Rev. 2013 Jul;254(l):225-44.

Certain bNAbs target conserved sites of vulnerability on the HIV-1 envelope (ENV) such as the CD4 binding site (CD4bs). The b12 monoclonal antibody was for many years considered the prototype and optimal CD4bs bNAb, although it was only able to neutralize about 40% of HIV-1 strains. In 2010, a new group of CD4bs antibodies named VRC01, VRC02, and VRC03 was disclosed. Of these, VRC01 was the most potent and broad. In a large neutralization panel (190 viruses), VRC01 neutralized 91% of viruses with an IC_{50} less than 50 μg/ml and 72% of viruses with an IC50 less than 1 μg/ml (Wu et al., Science, 329(5993):856-861, 2010). Structural analyses have explained VRC01's high potency and breadth: VRC01 partially mimics the CD4 interaction with gp120. Specifically, the majority of the gp120 area targeted by VRC01 is the highly conserved site of initial CD4 attachment in the outer domain of gp120, which allows VRC01 to bypass conformational and glycan masking that impaired previously identified CD4bs bNAbs. Both the heavy and light chain of VRC01 contribute to the binding of gp120, with the CDRH2 providing the primary interaction, and CDRL1, CDRL3, CDRH1, and CDRH3 providing additional contact points.

It has been shown that passive transfer of VRC01 protects against intrarectal or intravaginal simian-HIV (SHIV) challenge in non-human primates.

VRC01 is a monoclonal antibody that specifically binds to gp120 and is neutralizes a broad range of HIV viruses. The amino acid sequences of the variable heavy (VH) chain and variable light (VL) chain of VRC01 are shown in SEQ ID NOs 6 and 2, respectively and have been described in Wu et al., Science, 329(5993):856-861, 2010, and PCT publication WO2012/154312, incorporated by reference herein in its entirety. The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 7, 8, 9, respectively. The light chain CDRs are shown in SEQ ID NOs 3, 4, 5, respectively.

VRC01-like antibodies are described, for example in US20170267748, incorporated by reference herein in its entirety. Generally, these antibodies bind to the CD4 binding surface of gp120 in substantially the same orientation as VRC01, and are broadly neutralizing VRC01-like antibodies, with several of the important contacts between CD4 and gp120 mimicked by the VRC01-like antibodies. Several VRC01-like antibodies are available, including VRC01-like antibodies, heavy chains and light chains disclosed in PCT International Application No. PCT/US2010/050295, filed Sep. 24, 2010, which is incorporated by reference herein and Wu et al., "Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1," Science, 329(5993):856-861, 2010, which is incorporated by reference herein. These include heavy and light chains of the VRC01, VRC02, VRC03, VRCO6, VRC07, 3BNC117, IOMA and N6. In one embodiment, the antibody that binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 is selected from the group consisting of VRC03, VRC06, VRC07, 3BNC117, IOMA, and N6. The amino acid sequences of the heavy and light variable regions of VRC03 are shown in SEQ ID NOs 162 and 158, respectively and have been described in Wu et al., (Science. 2010 Aug 13;329(5993):856-61; PMID 20616233). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 163, 164, 165, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 159, 160, 161, respectively. The amino acid sequences of the heavy and light variable regions of VRC06 are shown in SEQ ID NOs 170 and 166, respectively and have been described in Li et al., (J Virol. 2012 Oct;86(20):11231-41; PMID 22875963). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 171, 172, 173, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 167, 168, 169, respectively. The amino acid sequences of the heavy and light variable regions of VRC07 are shown in SEQ ID NOs 178 and 2, respectively and have been described in Rudicell et al., (J Virol. 2014 Nov;88(21):12669-82;

PMID 25142607). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 179, 180, 181, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 3, 4, 5, respectively. The amino acid sequences of the heavy and light variable regions of 3BNC117 are shown in SEQ ID NOs 186 and182, respectively and have been described in Scheid et al., (Science. 2011 Sep 16;333(6049):1633-7; PMID 21764753). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 187, 188, 189, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 183, 184, 185, respectively. The amino acid sequences of the heavy and light variable regions of IOMA are shown in SEQ ID NOs 202 and 198, respectively and have been described in Gristick et al., (Nat Struct Mol Biol. 2016 Oct;23(10):906-915; PMID 27617431). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 203, 204, 205, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 199, 200, 201, respectively. The amino acid sequences of the heavy and light variable regions of N6 are shown in SEQ ID NOs 194 and 190, respectively and have been described in Huang et al., (Immunity. 2016 Nov 15;45(5):1108-1121; PMID 27851912). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 195, 196, 197, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 191, 192, 193, respectively.

PGT121, PGT122, PGT123, PGT127, PGT128, PGT135, 10-1074 and BG18 are a family of neutralizing monoclonal antibodies that specifically bind to the V1/V2 and V3 regions of HIV-1 Env and can inhibit HIV-1 infection of target cells. PGT121, PGT122, and PGT123 mAbs and methods of producing them are described in, for example, Walker et al., Nature, 477:466-470, 2011, and Int. Pub. No. WO 2012/030904, each of which is incorporated by reference herein. PGT127 and PGT128 are described in, for example Pejchal et al. (Science, 2011 Nov 25, 334 (6059): 1097-103). PGT135 is described, for example, in Kong et al. (Nature Structural and Molecular Biology, 2013 Jul, 20:796-803). In one embodiment, the antibody that binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 is selected from the group consisting of PGT121, PGT122, PGT128, PGT135, 10-1074, and BG18. The amino acid sequences of the heavy and light variable regions of PGT121 are shown in SEQ ID NOs 14 and 10, respectively. The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 15, 16, 17, respectively. The light chain CDRs are shown in SEQ ID NOs 11, 12, 13, respectively. The amino acid sequences of the heavy and light variable regions of 10-1074 are shown in SEQ ID NOs 58 and 54, respectively and have been described in Mouquet et al. ((2012) Proc.Natl.Acad.Sci.USA 109: E3268-E3277). The heavy chain CDRs (CDR1, CDR2,

CDR3) are shown in SEQ ID NOs 59, 60, 61, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 55, 56, 57, respectively. The amino acid sequences of the heavy and light variable regions of BG18 are shown in SEQ ID NOs 66 and 62, respectively and have been described in Freund et al. ((2012) Sci Transl Med. 2017 Jan 18;9(373); PMID 28100831). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 67, 68, 69, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 63, 64, 65, respectively. The amino acid sequences of the heavy and light variable regions of PGT135 are shown in SEQ ID NOs 74 and 70, respectively and have been described in Kong et al. (Nat Struct Mol Biol. 2013 Jul;20(7):796-80; PMID 23708606). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 75, 76, 77, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 71, 72, 73, respectively. The amino acid sequences of the heavy and light variable regions of PGT122 are shown in SEQ ID NOs 82 and 78, respectively and have been described in Julien et al. (PLoS Pathog. 2013;9(5):e1003342; PMID 23658524). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 83, 84, 85, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 79, 80, 81, respectively. The amino acid sequences of the heavy and light variable regions of PGT128 are shown in SEQ ID NOs 90 and 86, respectively and have been described in Lee et al. (Structure. 2015 Oct 6;23(10):1943-51; PMID 26388028). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 91, 92, 93, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 87, 88, 89, respectively..

35022, N123-VRC34.01, 3BC315, and PGT151are broadly neutralizing monoclonal antibody that specifically bind to the gp120/gp41 interface of HIV-1 Env in its prefusion mature (cleaved) conformation, and which can inhibit HIV-1 infection of target cells. PGT151 antibody and methods of producing this antibody are described in, for example, Blattner et al., Immunity, 40, 669-680, 2014, and Falkowska et al., Immunity, 40, 657-668, 2014, each of which is incorporated by reference herein in its entirety). The amino acid sequences of the heavy and light variable regions of the PGT151 mAb are known and have been deposited in GenBank as Nos. KJ700282.1 (PGT151 VH) and KJ700290.1 (PGT151 VL), each of which is incorporated by reference herein in its entirety). In one embodiment, the antibody that binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 is selected from the group consisting of 35022, N123-VRC34.01, 3BC315, and PGT151. The amino acid sequences of the heavy and light variable regions of N123-VRC34.01 are shown in SEQ ID NOs 138 and 134, respectively and have been described in

Kong et al., (Science 352 (6287), 828-833 (2016)). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 139, 140, 141, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 135, 136, 137, respectively. The amino acid sequences of the heavy and light variable regions of 3BC315 are shown in SEQ ID NOs 146 and 142, respectively and have been described in Lee et al. (Nat Commun. 2015 Sep 25;6:8167; PMID 26404402). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 147, 148, 149, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 143, 144, 145, respectively.The amino acid sequences of the heavy and light variable regions of PGT151 are shown in SEQ ID NOs 154 and 150, respectively and have been described in Blattner et al. (Immunity. 2014 May 15;40(5):669-80; PMID 24768348). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 155, 156, 157, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 151, 152, 153, respectively.

10E8, 10E8v4, 10E8v4 S100cF, Dh511.2_k3, Z13, 4E10, and 2F5 are broadly neutralizing monoclonal antibody that primarily targets a HIV Env membrane proximal external region (MPER) helix spanning residues 671-683. In one embodiment, the antibody that binds to an epitope in the MPER of the of the envelope protein of HIV-1 is selected from the group consisting of: 10E8, 10E8v4, 10E8v4 S100cF, Dh511.2_k3, Z13, 4E10, and 2F5. The amino acid sequences of the heavy and light variable regions of 10E8v4 are shown in SEQ ID NOs 98 and 94, respectively and have been described in Kwon et al. (J Virol. 2016 Jun 10;90(13):5899-914; PMID PMC4907239). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 99, 100, 101, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 95, 96, 97, respectively.The amino acid sequences of the heavy and light variable regions of 10E8v4 S100cF are shown in SEQ ID NOs 106 and 94, respectively and have been described in PCT/US2016/060390 and WO2017079479. The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 107, 108, 109, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 95, 96, 97, respectively.The amino acid sequences of the heavy and light variable regions of DH511.2_k3 are shown in SEQ ID NOs114 and 110, respectively and have been described in Williams et al. (Sci Immunol. 2017 Jan 27;2(7); PMID 28783671). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 115, 116, 117, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 111, 112, 113, respectively. The amino acid sequences of the heavy and light variable regions of 4E10 are shown in SEQ ID NOs 122 and 118, respectively and have been described in Rujas et al. (J Virol. 2015

Dec;89(23):11975-89; PMID 26378169). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 123, 124, 125, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 119, 120, 121, respectively. The amino acid sequences of the heavy and light variable regions of 2F5 are shown in SEQ ID NOs 130 and 126, respectively and have been described in Julien et al. J Mol Biol. 2008 Dec 12;384(2):377-92; PMID 18824005). The heavy chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 131, 132, 133, respectively. The light chain CDRs (CDR1, CDR2, CDR3) are shown in SEQ ID NOs 126, 127, 128, respectively.

PGT141, PGT142, PGT143, and PGT145 are family of broadly neutralizing monoclonal antibodies that specifically bind to the V1/V2 domain of the HIV-1 Env ectodomain trimer in its prefusion mature closed conformation, and which can inhibit HIV-1 infection of target cells. PGT141, PGT142, PGT143, and PGT145 mAbs and methods of producing them are described in, for example, Walker et al., Nature, 477:466-470, 2011, and Int. Pub. No. WO2012/030904, each of which is incorporated by reference herein). The amino acid sequences of the heavy and light variable regions of the PGT141, PGT142, PGT143, PGT144, and PGT145 mAbs are known and have been deposited in GenBank as Nos. JN201906.1 (PGT141 VH), JN201923.1 (PGT141 VL), JN201907.1 (PGT142 VH), JN201924.1 (PGT142 VL), JN201908.1 (PGT143 VH), JN201925.1 (PGT143 VL), JN201909.1 (PGT144 VH), JN201926.1 (PGT144 VL), JN201910.1 (PGT145 VH), and JN201927.1 (PGT145 VL), each of which is incorporated by reference herein in its entirety).

In one embodiment, the disclosure features an antibody, or a fragment thereof, or a salt thereof that binds to an epitope that is located in the CD4-binding site (CD4bs). In one embodiment, the disclosure features an antibody, or a fragment thereof, that binds to an epitope that is located in the V1/V2-glycan region. In one embodiment, the disclosure features an antibody, or a fragment thereof, that binds to an epitope that is located in the V3-glycan region. In one embodiment, the disclosure features an antibody, or a fragment thereof, that binds to an epitope that is located in the gp41 membrane proximal external region (MPER). In one embodiment, the disclosure features an antibody, or a fragment thereof, that binds to an epitope that is located the gp120-gp41 interface of the envelope protein of HIV.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, or a salt thereof, comprising any one or combination of the antigen binding regions of any of the antibodies described in Table 1.

Table 1

SEQ ID NO	Description	Sequence
1	VRC01 (VL-3x-VH)-(5X)-PGT121 (VL-3x-VH) ScFv amino acid sequence	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASG YEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDV YSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSG GGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRA VQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGS QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGHHHHHH
2	VRC01 variable light (VL) chain amino acid sequence	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIK
3	VL-CDR1	RTSQYGSLA
4	VL-CDR2	SGSTRAA
5	VL-CDR3	QQYEF
6	VRC01 variable heavy (VH) chain amino acid sequence	QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWM GWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFC TRGKNCDYNWDFEHWGRGTPVIVSS
7	VH-CDR1	DCTLN
8	VH-CDR2	WLKPRGGAVNYARPLQG
9	VH-CDR3	GKNCDYNWDFEH
10	PGT121 variable light (VL) chain amino acid sequence	SDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQDRPS GIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTKWVF GGGTTLTVL
11	VL-CDR1	GEKSLGSRAVQ
12	VL-CDR2	NNQDRPS
13	VL-CDR3	HIWDSRVPTKWV
14	PGT121 variable heavy (VH) chain amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSS
15	VH-CDR1	DSYWS
16	VH-CDR2	YVHKSGDTNYSPSLKS

17	VH-CDR3	TLHGRRIYGIVAFNEWFTYFYMDV
18	dVRC01 (VL(Δ1,2-V3S)-3x-VH)-(5X)-PGT121 (VL-3x-VH) ScFv amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASGYE FIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYS DTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSGGG GSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRAVQ WYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVE AGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGSQM QLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWIGY VHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCART LHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGHHHHHH
19	dVRC01 variable light (VL) chain amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIK
20	PGT121 (VH-3x-VL)-(5X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
21	PGT121 (VH-3x-VL)-(4X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
22	PGT121 (VH-3x-VL)-(3X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRIS CRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVT MTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPV IVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCRTSQYG SLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLE SGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
23	VRC01 (VL-3x-VH)-(5X)-PGT121 (VL-3x-VH) IgG1 amino acid	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASG YEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDV YSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSG

	sequence	GGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRA VQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGS QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
24	dVRC01 (VL(Δ1,2-V3S)-3x-VH)-(5X)-PGT121 (VL-3x-VH) IgG1 amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASGYE FIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYS DTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSGGG GSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRAVQ WYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVE AGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGSQM QLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWIGY VHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCART LHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
25	PGT121 (VH-3x-VL)-(5X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
26	PGT121 (VH-3x-VL)-(4X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGE SMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPL QGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWG RGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCR TSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLT ISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV

		FSCSVMHEALHNHYTQKSLSLSPGK
27	PGT121 (VH-3x-VL)-(3X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRIS CRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVT MTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPV IVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCRTSQYG SLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLE SGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
28	IgG1 Fc (hinge-CH2-CH3)amino acid sequence	PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
29	signal sequence	MGWSCIILFLVATATGVHS
30	35022(VH-3x-VL)-(5X)-10E8(VH-3x-VL) ScFv amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPG EGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLYFCARTGK YYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGGGSSYELT QETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNR PSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFG GGTKLTVLGSGHHHHHH
31	35022 variable light (VL) chain amino acid sequence	QSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTL IIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHN SGCVFGTGTKVSVL
32	VL-CDR1	TGPNSVCCSHKSIS
33	VL-CDR2	EDNERAP
34	VL-CDR3	CSYTHNSGCV
35	35022 variable heavy (VH) chain amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS
36	VH-CDR1	FYHIN
37	VH-CDR2	WISPYSGDKNLAPAFQD

38	VH-CDR3	GLLRDGSSTWLPYL
39	10E8 variable light (VL) chain amino acid sequence	SYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFY GKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSR LSVFGGGTKLTVL
40	VL-CDR1	RGDSLRSHYAS
41	VL-CDR2	GKNNRPS
42	VL-CDR3	SSRDKSGSRLSV
43	10E8 variable heavy (VH) chain amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSS
44	VH-CDR1	NAWMT
45	VH-CDR2	RITGPGEGWSVDYAAPVEG
46	VH-CDR3	TGKYYDFWSGYPPGEEYFQD
47	35022(VH-3x-VL)-(7X)-10E8(VH-3x-VL) ScFv amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGL EWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDS GLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGS GGGGSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAP ILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRD KSGSRLSVFGGGTKLTVLGSGHHHHHH
48	10E8(VH-3x-VL)-(5X)-35O22(VH-3x-VL) ScFv amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQGQLVQSG AELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSG DKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFC AKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGGGSQSVLTQ SASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDN ERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHNSGCVFG TGTKVSVLGSGHHHHHH
49	10E8(VH-3x-VL)-(7X)-35O22(VH-3x-VL) ScFv amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSQGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPE WMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNL KFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSG GGGSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGR

		APTLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCS YTHNSGCVFGTGTKVSVLGSGHHHHHH
50	35022(VH-3x-VL)-(5X)-10E8(VH-3x-VL) IgG1 amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPG EGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLYFCARTGK YYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGGGSSYELT QETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNR PSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFG GGTKLTVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
51	35022(VH-3x-VL)-(7X)-10E8(VH-3x-VL) IgG1 amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGL EWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDS GLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGS GGGGSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAP ILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRD KSGSRLSVFGGGTKLTVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
52	10E8(VH-3x-VL)-(5X)-35022(VH-3x-VL) IgG1 amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQGQLVQSG AELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSG DKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFC AKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGGGSQSVLTQ SASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDN ERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHNSGCVFG TGTKVSVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
53	10E8(VH-3x-VL)-(7X)-35022(VH-3x-VL) IgG1 amino acid	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL

	sequence	FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSQGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPE WMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNL KFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSG GGGSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGR APTLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCS YTHNSGCVFGTGTKVSVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK

In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that binds to comprises a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 6. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 2. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 2, and a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 6. In one embodiment, the antibody, or a fragment thereof, that binds to an epitope that is located in the CD4-binding site (CD4bs).

In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that binds to comprises a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 14. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 10. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 10, and a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 14. In one embodiment, the antibody, or a fragment thereof, that binds to an epitope that is located in the V3- glycan region.

In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that binds to comprises a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 6. In one embodiment, the disclosure

provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 19. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 19, and a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 6. In one embodiment, the antibody, or a fragment thereof,binds to an epitope that is located in the CD4-binding site (CD4bs).

In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that binds to comprises a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 35. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 31. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 31, and a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 35. In one embodiment, the antibody, or a fragment thereof, binds to an epitope that is located in the gp120-gp41 interface.

In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that binds to comprises a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 43. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 39. In one embodiment, the disclosure provides an antibody, or an antigen-binding fragment thereof, that comprises a light chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 39, and a heavy chain having a variable domain comprising an amino acid sequence as set forth in SEQ ID NO. 43. In one embodiment, the antibody, or a fragment thereof, binds to an epitope that is located in the gp41 membrane proximal external region (MPER).

In one embodiment, the present disclosure provides a human antibody or antigen-binding fragment, has a heavy chain variable domain sequence which is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical, or identical, to an amino acid sequence selected from the group consisting of SEQ ID NO. 6,

SEQ ID NO. 14, SEQ ID NO. 35, and SEQ ID NO. 43, and has a light chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical, or identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31, and SEQ ID NO. 39.

In one embodiment, the disclosure features an isolated antibody comprising a heavy chain/light chain variable domain amino acid sequence selected from the group consisting of SEQ ID NO. 6/SEQ ID NO. 2, SEQ ID NO. 14/SEQ ID NO. 10, SEQ ID NO. 6/SEQ ID NO. 19, SEQ ID NO. 35/SEQ ID NO. 31, and SEQ ID NO. 43/SEQ ID NO. 39.

Complementarity determining regions (CDRs) are known as hypervariable regions both in the light chain and the heavy chain variable domains of an antibody. The more highly conserved portions of variable domains are called the framework (FR). Complementarity determining regions (CDRs) and framework regions (FR) of a given antibody may be identified using systems known in the art, such as those described by Kabat *et al. supra;* Lefranc *et al., supra* and/or Honegger and Pluckthun, *supra.* For example, the numbering system described in Kabat *et al.* (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, VA.) is well known to those in the art. Kabat *et al.* defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain amino acid sequence, without reliance on any experimental data beyond the sequence itself.

In certain embodiments, the present disclosure provides an antibody comprising the CDRs of the heavy and light chain variable domains described in Table 1. For example, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region having the CDRs described in an amino acid sequence as set forth in any one of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35, and SEQ ID NO. 43. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a light chain variable region having the CDRs described in an amino acid sequence as set forth in any one of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31, and SEQ ID NO. 39. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a light chain variable region having the CDRs described in an amino acid sequence as set forth in any one of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31, and SEQ ID NO. 39; and a heavy chain variable region

having the CDRs described in an amino acid sequence as set forth in any one of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35, and SEQ ID NO. 43.

In one embodiment, the present disclosure features an antibody, or an antigen-binding fragment thereof, comprising a heavy chain variable domain comprising a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NO. 7, SEQ ID NO. 8 and SEQ ID NO. 9; SEQ ID NO. 15, SEQ ID NO. 16 and SEQ ID NO. 17; SEQ ID NO. 36, SEQ ID NO. 37 and SEQ ID NO. 38; and SEQ ID NO. 44, SEQ ID NO. 45 and SEQ ID NO. 46, and a light chain variable domain comprising a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5; SEQ ID NO. 11, SEQ ID NO. 12 and SEQ ID NO. 13; SEQ ID NO. 32, SEQ ID NO. 33 and SEQ ID NO. 34; and SEQ ID NO. 40, SEQ ID NO. 41 and SEQ ID NO. 42.

In one embodiment, the antibody of the disclosure comprises a heavy chain CDR set / light chain CDR set selected from the group consisting of the heavy chain variable domain CDR set of SEQ ID NO. 7, SEQ ID NO. 8 and SEQ ID NO. 9 / the light chain variable domain CDR set of SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5; the heavy chain variable domain CDR set of SEQ ID NO. 15, SEQ ID NO. 16 and SEQ ID NO. 17/ the light chain variable domain CDR set of SEQ ID NO. 11, SEQ ID NO. 12 and SEQ ID NO. 13; the heavy chain variable domain CDR set of SEQ ID NO. 36, SEQ ID NO. 37 and SEQ ID NO. 38/ the light chain variable domain CDR set of SEQ ID NO. 32, SEQ ID NO. 33 and SEQ ID NO. 34; and the heavy chain variable domain CDR set of SEQ ID NO. 44, SEQ ID NO. 45 and SEQ ID NO. 46/ the light chain variable domain CDR set of SEQ ID NO. 40, SEQ ID NO. 41 and SEQ ID NO. 42.

One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an antigen binding protein.

An antigen binding protein may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the antigen binding protein to specifically bind to a particular antigen of interest.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having the antigen binding regions of any of the antibodies described in Table 1.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having antigen binding regions of antibody VRC01. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof,

comprising a heavy chain variable domain sequence as set forth in SEQ ID NO. 6, and a light chain variable domain sequence as set forth in SEQ ID NO. 2. In one embodiment, the disclosure is directed to an antibody having a heavy chain variable domain comprising the CDRs of SEQ ID NO. 6, and a light chain variable domain comprising the CDRs of SEQ ID NO. 2. In one embodiment, the disclosure features an isolated human antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 6, and comprises a light chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 2. In one embodiment, the disclosure features an antibody, or an antigen-binding portion thereof, having antigen binding regions of antibody VRC01, comprising a heavy chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 9, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 8, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 7; and comprising a light chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 5, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 4, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 3.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having antigen binding regions of antibody PGT121. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain sequence as set forth in SEQ ID NO. 14, and a light chain variable domain sequence as set forth in SEQ ID NO. 10. In one embodiment, the disclosure is directed to an antibody having a heavy chain variable domain comprising the CDRs of SEQ ID NO. 14, and a light chain variable domain comprising the CDRs of SEQ ID NO. 10. In one embodiment, the disclosure features an isolated human antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 14, and comprises a light chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 10. In one embodiment, the disclosure features an antibody, or an antigen-binding portion thereof, having antigen binding regions of

antibody PGT121, comprising a heavy chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 17, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 16, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 15; and comprising a light chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 13, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 12, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 11.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having antigen binding regions of antibody 35022. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain sequence as set forth in SEQ ID NO. 35, and a light chain variable domain sequence as set forth in SEQ ID NO. 31. In one embodiment, the disclosure is directed to an antibody having a heavy chain variable domain comprising the CDRs of SEQ ID NO. 35, and a light chain variable domain comprising the CDRs of SEQ ID NO. 31. In one embodiment, the disclosure features an isolated human antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 35, and comprises a light chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 31. In one embodiment, the disclosure features an antibody, or an antigen-binding portion thereof, having antigen binding regions of antibody 35022, comprising a heavy chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 38, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 37, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 36; and comprising a light chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 34, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 33, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 32.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having antigen binding regions of antibody 10E8. In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain sequence as set forth in SEQ ID NO. 43, and a light chain variable domain sequence as set forth in SEQ ID NO. 39. In one embodiment, the

disclosure is directed to an antibody having a heavy chain variable domain comprising the CDRs of SEQ ID NO. 43, and a light chain variable domain comprising the CDRs of SEQ ID NO. 39. In one embodiment, the disclosure features an isolated human antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 43, and comprises a light chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 39. In one embodiment, the disclosure features an antibody, or an antigen-binding portion thereof, having antigen binding regions of antibody 10E8, comprising a heavy chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 46, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 45, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 44; and comprising a light chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 42, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 41, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 40.

In one embodiment, the present disclosure is directed to an antibody, or an antigen binding fragment thereof, having antigen binding regions of antibody VRC01 VL(Δ1,2-V3S). In one embodiment, the disclosure provides an antibody, or antigen-binding fragment thereof, comprising a heavy chain variable domain sequence as set forth in SEQ ID NO. 43, and a light chain variable domain sequence as set forth in SEQ ID NO. 39. In one embodiment, the disclosure is directed to an antibody having a heavy chain variable domain comprising the CDRs of SEQ ID NO. 43, and a light chain variable domain comprising the CDRs of SEQ ID NO. 39. In one embodiment, the disclosure features an isolated human antibody, or antigen-binding fragment thereof, that comprises a heavy chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 43, and comprises a light chain variable region having an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to the sequence set forth in SEQ ID NO. 39. In one embodiment, the disclosure features an antibody, or an antigen-binding portion thereof, having antigen binding regions of antibody VRC01 VL(Δ1,2-V3S), comprising a heavy chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 46, a CDR2 domain

comprising the amino acid sequence as set forth in SEQ ID NO. 45, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 44; and comprising a light chain variable region comprising a CDR3 domain comprising the amino acid as set forth in SEQ ID NO. 42, a CDR2 domain comprising the amino acid sequence as set forth in SEQ ID NO. 41, and a CDR1 domain comprising the amino acid sequence as set forth in SEQ ID NO. 40.

The antibody of the disclosure may be of an IgG class. The antibody of the disclosure may further be an IgG1 isotype.

Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (VL and VH). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, Prot. Eng. 10:423; Kortt et al., 2001, Biomol. Eng. 18:95-108). By combining different VL and VH-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum et al., 2001, Biomol. Eng. 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Patent 4,946,778; Bird, 1988, Science 242:423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879; Ward et al., 1989, Nature 334:544, de Graaf et al., 2002, Methods Mol. Biol. 178:379-87.

In one embodiment, the present disclosure provides a single chain human antibody, having a variable domain region from a heavy chain and a variable domain region from a light chain and a peptide linker connection the heavy chain and light chain variable domain regions, wherein the heavy chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequences selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35, and SEQ ID NO. 43; and that has a light chain variable domain sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99%, or 100% identical to the amino acid sequence consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31, and SEQ ID NO. 39. Preferably, the single chain antibody has both a heavy chain variable domain region and a light chain variable domain region, wherein the single chain human antibody has a heavy chain/light chain variable domain sequence selected from the group consisting of SEQ

ID NO. 6/SEQ ID NO. 2, SEQ ID NO. 14/SEQ ID NO. 10, SEQ ID NO. 6/SEQ ID NO. 19, SEQ ID NO. 35/SEQ ID NO. 31, and SEQ ID NO. 43/SEQ ID NO. 39.

Techniques are known for deriving an antibody of a different subclass or isotype from an antibody of interest, i.e., subclass switching. Thus, IgG antibodies may be derived from an IgM antibody, for example, and vice versa. Such techniques allow the preparation of new antibodies that possess the antigen-binding properties of a given antibody (the parent antibody), but also exhibit biological properties associated with an antibody isotype or subclass different from that of the parent antibody. Recombinant DNA techniques may be employed. Cloned DNA encoding particular antibody polypeptides may be employed in such procedures, e.g., DNA encoding the constant domain of an antibody of the desired isotype (Lantto et al., 2002, Methods Mol. Biol. 178:303-16).

The present disclosure provides a number of antibodies structurally characterized by the amino acid sequences of their variable domain regions. However, the amino acid sequences can undergo some changes while retaining their high degree of binding to their specific targets. More specifically, many amino acids in the variable domain region can be changed with conservative substitutions and it is predictable that the binding characteristics of the resulting antibody will not differ from the binding characteristics of the wild type antibody sequence. There are many amino acids in an antibody variable domain that do not directly interact with the antigen or impact antigen binding and are not critical for determining antibody structure. For example, a predicted nonessential amino acid residue in any of the disclosed antibodies is preferably replaced with another amino acid residue from the same class. Methods of identifying 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), all of which are incorporated by reference in their entireties herein). Near et al. Mol. Immunol. 30:369-377, 1993 explains how to impact or not impact binding through site-directed mutagenesis. Near et al. only mutated residues that they thought had a high probability of changing antigen binding. Most had a modest or negative effect on binding affinity (Near et al. Table 3) and binding to different forms of digoxin (Near et al. Table 2).

A conservative modification or functional equivalent of a peptide, polypeptide, or protein disclosed in this disclosure (e.g., the hinge region or a heavy chain having the hinge region) refers to a polypeptide derivative of the peptide, polypeptide, or protein, e.g., a protein having one or more point mutations, insertions, deletions, truncations, a fusion protein,

or a combination thereof. It retains substantially the activity to of the parent peptide, polypeptide, or protein (such as those disclosed in this disclosure). In general, a conservative modification or functional equivalent is at least 60% (e.g., any number between 60% and 100%, inclusive, e.g., 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, and 99%) identical to a parent (e.g., one of SEQ ID NOs: 1-53).

In one embodiment, the substitutions made within a heavy or light chain that is at least 95% identical (or at least 96% identical, or at least 97% identical, or at least 98% identical, or at least 99% identical) are conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine.

As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology=# of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4: 11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity

between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs {e.g., XBLAST and NBLAST) can be used. (See www.ncbi.nlm.nih.gov).

Other modifications of the antibody are contemplated herein. For example, the antibody can be linked to one of a variety of nonproteinaceous polymers, for example, polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in, for example, Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

Variant antibodies and salts thereof also are included within the scope of the disclosure. Variants of the sequences recited in the application also are included within the scope of the disclosure. Further variants of the antibody sequences having improved affinity can be obtained using methods known in the art and are included within the scope of the disclosure. For example, amino acid substitutions can be used to obtain antibodies with further improved affinity. Alternatively, codon optimization of the nucleotide sequence can be used to improve the efficiency of translation in expression systems for the production of the antibody. Variants may include non-natural amino acids up to a certain percentage. In some embodiments, the antibody comprises a variant amino acid sequence comprising about

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more percent of non-natural amino acids.

Antibody Modifications

Humanization and Primatization

In cases where the multispecific antibody or the antibodies forming the multispecific 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 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 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 retain the specificity and affinity for the antigen.

In specific embodiments, multispecific antibodies are formed from anti-HIV human or humanized antibodies.

Similarly, a tri-specific fusion antibody or the three 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 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 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.

Affinity Maturation

One or more hypervariable region residues 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 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).

Modifications to the Fc Region

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 cyotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody, improved or decreased internalization and/or recycling, among others.

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

Multispecific bNAbs Comprising Tetra-Glycine-Serine (G4S) Peptide Linkers

As discussed herein, a variety of broadly neutralizing antibodies (bnAbs) have been isolated from HIV-1–infected individuals, but their potential to treat or prevent infection in humans may be limited by the potency or breadth of viruses neutralized. The present disclosure describes combinations of bnAbs that optimize potency and breadth of protection, thus reducing the likelihood of resistance and viral escape. Because multiple antibodies may help to reduce the viral replication that sustains chronic HIV-1 infection, the present disclosure reports the generation of multispecific antibodies designed to increase the potential efficacy of HIV-1 antibodies for prevention or therapy.

In one embodiment, the multispecific antibody is capable of binding two different antigen targets. In one embodiment, the multispecific antibody is capable of binding three different antigen targets. In one embodiment, the multispecific antibody is capable of binding four different antigen targets. In one embodiment, the multispecific antibody is capable of binding five different antigen targets.

In one embodiment, the disclosure features a multispecific antibody that binds two or more non-overlapping epitopes, wherein the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the HIV envelope protein, wherein the multispecific antibody has an IC_{50} less than 0.1 μg/ml, an IC_{50} less than 0.09 μg/ml, an IC_{50} less than 0.08 μg/ml, an IC_{50} less than 0.07 μg/ml, an IC_{50} less than 0.06 μg/ml, an IC_{50} less than 0.05 μg/ml, an IC_{50} less than 0.04 μg/ml, an IC_{50} less than 0.03 μg/ml, anIC_{50} less than 0.02 μg/ml, and IC_{50} less than 0.01 μg/ml.

In one embodiment, the disclosure features a multispecific antibody that binds two or more non-overlapping epitopes, wherein the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the HIV envelope protein, wherein the multispecific antibody has an IC_{80} less than 0.3 μg/ml, an IC_{80} less than 0.2 μg/ml, an IC_{80} less than 0.1 μg/ml.

In one embodiment, the disclosure features a multispecific antibody that binds two or more non-overlapping epitopes, wherein a first antibody binds an epitope in the CD4-binding site of the HIV envelope protein, and the second antibody binds an epitope in the V3- glycan region of the HIV envelope protein, wherein the multispecific antibody has an IC_{50} less than 0.1 μg/ml, an IC_{50} less than 0.09 μg/ml, an IC_{50} less than 0.08 μg/ml, an IC_{50} less than 0.07 μg/ml, an IC_{50} less than 0.06 μg/ml, an IC_{50} less than 0.05 μg/ml, an IC_{50} less than 0.04 μg/ml, an IC_{50} less than 0.03 μg/ml, anIC_{50} less than 0.02 μg/ml, and IC_{50} less than 0.01 μg/ml.

In one embodiment, the disclosure features a multispecific antibody that binds two or more non-overlapping epitopes, wherein a first antibody binds an epitope in the gp120/gp41 interface of the HIV envelope protein, and the second antibody binds an epitope in the MPER of the envelope protein, wherein the multispecific antibody has an IC_{50} less than 0.1 μg/ml, an IC_{50} less than 0.09 μg/ml, an IC_{50} less than 0.08 μg/ml, an IC_{50} less than 0.07 μg/ml, an IC_{50} less than 0.06 μg/ml, an IC_{50} less than 0.05 μg/ml, an IC_{50} less than 0.04 μg/ml, an IC_{50} less than 0.03 μg/ml, anIC_{50} less than 0.02 μg/ml, and IC_{50} less than 0.01 μg/ml.

In one aspect, the disclosure features a multispecific antibody, or an antigen-binding fragment thereof, comprising a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof, wherein the first antibody and the second antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and wherein the VH from the first light chain and the VL from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) proteinone or more linkers.

In one embodiment, the multispecific antibody has an IC_{50} less than 0.1 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.09 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.08 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.07 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.06 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.05 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.04 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.03 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.02 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} less than 0.01 μg/ml. In one embodiment, the multispecific antibody has an IC_{50} between 0.01 and 0.1 μg/ml.

In one embodiment, the multispecific antibody has an IC_{80} less than 0.3 μg/ml. In one embodiment, the multispecific antibody has an IC_{80} less than 0.2 μg/ml. In one embodiment, the multispecific antibody has an IC_{80} less than 0.1 μg/ml. In one embodiment, the multispecific antibody has an IC_{80} between 0.1 and 0.3 μg/ml.

In one embodiment, the linker is a tetra-glycerine-serine (G4S) linker. In some embodiments, linker comprises 1, 2, 3, 4, 5, 6, 7 or more G4S linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by two tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by two tetra-glycine serine (G4S) protein linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by four tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by four tetra-glycine serine (G4S) protein linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by six tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by six tetra-glycine serine (G4S) protein linkers.

In one embodiment, the VH from the first light chain and the VL from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers. In another embodiment, the VL from the first light chain and the VH from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers.

In one embodiment, the linker is not a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly), the peptide Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 292), a single Ser, a single Va, the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser, Thr-Val-Ala-Ala-Pro, Gln-Pro-Lys-Ala-Ala, Gln-Arg-Ile-Glu-Gly, Ala-Ser-Thr-Lys-Gly-Pro-Ser, Arg-Thr-Val-Ala-Ala-Pro-Ser, Gly-Gln-Pro-Lys-Ala-Ala-Pro, and His-Ile-Asp-Ser-Pro-Asn-Lys.

In one embodiment, the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the envelope protein.

In one embodiment, the multispecific antibody, further comprises a third antibody which specifically binds to a third epitope.

The third antibody may be added using knob-in-hole dimerization, as described for example in Merchant et al. (Nat. Biotechnol. 16, 677–681 (1998)).

In one embodiment, the third epitope is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV. In one embodiment, the

third epitope is different from the first epitope and the second epitope.

In one embodiment, the first antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the V3-glycan region of the of the envelope protein of HIV-1.

In one embodiment, the first antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1.

In one embodiment, the antibody that binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 is selected from the group consisting of VRC03, VRC06, VRC07, 3BNC117, IOMA, and N6.

In one embodiment, the antibody that binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 is selected from the group consisting of PGT121, PGT122, PGT128, PGT135, 10-1074, and BG18.

In one embodiment, the first antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1.

In one embodiment, the first antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1.

In one embodiment, the antibody that binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 is selected from the group consisting of 35022, N123-VRC34.01, 3BC315, and PGT151.

In one embodiment, the antibody that binds to an epitope in the MPER of the of the envelope protein of HIV-1 is selected from the group consisting of 10E8, 10E8v4, 10E8v4 S100cF, Dh511.2_k3, Z13, 4E10, and 2F5.

In one aspect, the disclosure features a multispecific antibody, or antigen binding fragment thereof, wherein the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 3, 4, 5 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 7, 8, 9; and the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 11, 12, 13 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 15, 16, 17.

In one aspect, the disclosure features a multispecific antibody, or antigen binding fragment thereof, wherein the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 32, 33, 34 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 36, 37, 38; and the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 40, 41, 42 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 44, 45, 46.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 96% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 96% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 97% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID

NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 97% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 98% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 98% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 96% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 96% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 97% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 97% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 98% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 98% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the second light chain comprises an amino acid sequence that is 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises an amino acid sequence that is 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 19, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95%

identical to SEQ ID NO. 19, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 43.

In one embodiment, the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 43, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35.

In one embodiment, the multispecific antibody comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In one embodiment, the multispecific antibody comprises an amino acid sequence that is 96% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In one embodiment, the multispecific antibody comprises an amino acid sequence that is 97% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In one embodiment, the multispecific antibody comprises an amino acid sequence that is 98% identical to an amino acid sequence selected from the group consisting of SEQ ID

NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In one embodiment, the multispecific antibody comprises an amino acid sequence that is 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

In one embodiment, the multispecific antibody comprises an amino acid sequence set forth in an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

Trispecifc

In another aspect, the disclosure features a multispecific antibody comprising a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof; and c) a third light chain comprising a first light chain variable region (VL) and a third heavy chain comprising a first heavy chain variable region (VH), wherein the third light chain and the third heavy chain are derived from a third antibody or an antigen-binding fragment thereof, wherein the first antibody, the second antibody and the third antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and wherein the VH from the first light chain and the VL from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers.

Tetraspecific

In certain embodiments, the disclosure features a multispecific antibody with four binding sites. In one embodiment, the four binding sites can be arranged by joining two bispecific antibody arms using complementary "Knob-into-holes" Fc fragments. In another embodiment, the four binding sites can be arranged by joining variable VH and VL of four different antibodies by G4S linkers of various length based on structural information, for example 3, 4, 5, 6, 7, or more G4S linkers.

Pentaspecific

In certain embodiments, the disclosure features a multispecific antibody with five binding sites. Antibodies with five binding sites can be arranged in the similar manner as antibodies with four binding sites. In one embodiment, the five binding sites can be arranged by joining one bispecific and one trispecific (VH/VL of three antibodies connected by G4S linkers in tandem) antibody arms using complementary "Knobs-into-holes" Fc fragments; or join variable VH and VL of five different antibodies by G4S linkers of various length based on structural information.

In certain embodiments, the first antibody, the second antibody, the third antibody, the fourth antibody or the fifth antibody is an ScFv.

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. 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)).

Pharmaceutical Formulations

Pharmaceutical formulations of the multispecific antibodies disclosed can be prepared by mixing the antibody with optional pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers include solvents, dispersion media, isotonic agents and the like. The carrier can be liquid, semi-solid, e.g. pastes, or solid carriers. Examples of carriers include water, saline solutions or other buffers (such as phosphate, citrate buffers), oil, alcohol, proteins (such as serum albumin, gelatin), carbohydrates (such as monosaccharides, di saccharides, and other carbohydrates including glucose, sucrose, trehalose, mannose,

mannitol, sorbitol or dextrins), gel, lipids, liposomes, resins, porous matrices, binders, fillers, coatings, stabilizers, preservatives, antioxidants including ascorbic acid and methionine, chelating agents such as EDTA; salt forming counter-ions such as sodium; non-ionic surfactants such as TWEEN, PLURONICS or polyethylene glycol (PEG), or combinations thereof.

The formulation can contain more than one active compound, e.g., one or more multispecifc antibodies, in combination with one or more additional beneficial compound for preventing and treating HIV infections.

The above-described antibodies can be used in combination with one or more anti-retroviral agents for the treatment of HIV latency and/or infection. See, e.g., US 2010/0166806, US 2010/0324034, and US 2012/0203014, which are hereby incorporated in their entirety.

Compositions according to the present disclosure may also be administered in combination with other agents to enhance the biological activity of such agents. Such agents may include any one or more of the standard anti-HIV agents which are known in the art, including, but not limited to, azidothymidine (AZT), dideoxycytidine (ddC), and dideoxyinosine (ddl). Additional agents which have shown anti-HIV effects and may be combined with compositions in accordance to the disclosure include, for example, raltegravir, maraviroc, bestatin, human chorionic gonadotropin (hCG), levamisole, estrogen, efavirenz, etravirine, indomethacin, emtricitabine, tenofovir disoproxil fumarate, amprenavir, tipranavir, indinavir, ritonavir, darunavir, enfuvirtide, and gramicidin.

The active ingredients can be combined with the carrier in any convenient and practical manner, e.g., by admixture, solution, suspension, emulsification, encapsulation, absorption and the like, and can be made in formulations such as tablets, capsules, powder (including lyophilized powder), syrup, suspensions that are suitable for injections, ingestions, infusion, or the like. Sustained-release preparations can also be prepared.

The instant disclosure is related to pharmaceutical compositions of the instant disclosure or the pharmaceutical acceptable salts derived therefrom that comprise analogs that comprise isotopes. In some embodiments, the compositions of the claimed disclosure may contain any isotope described in Cyr and Pearson (Stabilization of radiopharmaceutical compositions using hydrophilic thioethers and hydrophilic 6-hydroxy chromans. Cyr, John E.; Pearson, Daniel A. (Diatide, Inc., USA). PCT Int. Appl. (2002), WO 200260491 A2 20020808), which is herein incorporated by reference. In some embodiments the compositions of the disclosure comprise analog that comprise one or more of the following

isotopes: ^{125}I, ^{131}I, ^{211}At, ^{47}Sc, ^{67}Cu, ^{72}Ga, ^{90}Y, ^{153}Sm, ^{159}Gd, ^{165}Dy, ^{166}Ho, ^{175}Yb, ^{177}Lu, ^{212}Bi,^{213}Bi, ^{68}Ga, ^{99}Tc, ^{111}In, ^{123}I, and ^{3}H.

The pharmaceutical compositions of the instant disclosure or the pharmaceutical acceptable salts derived therefrom may be in a liquid or solid dosage form. Such compositions may include any type of dosage form such as tablets, capsules, powders, liquid formulations, delayed or sustained release, patches, snuffs, nasal sprays and the like. The formulations may additionally include other ingredients such as dyes, preservatives, buffers and anti-oxidants, for example. The physical form and content of the pharmaceutical formulations contemplated are conventional preparations that can be formulated by those skilled in the pharmaceutical formulation field and are based on well established principles and compositions described in, for example, Remington: The Science and Practice of Pharmacy, 19th Edition, 1995; British Pharmacopoeia 2000, each of which is incorpated herein by reference. The compositions of the present disclosure may also include other active agents useful in the treatment of cardiovascular conditions. Solid forms can be prepared according to any means suitable in the art. For example, capsules are prepared by mixing the analog composition with a suitable diluent and filling the proper amount of the mixture in capsules. Tablets are prepared by direct compression, by wet granulation, or by dry granulation. Their formulations usually incorporate diluents, binders, lubricants and disintegrators as well as the compound. Diluents, but are not limited to, include various types of starch, cellulose, crystalline cellulose, microcrystalline cellulose, lactose, fructose, sucrose, mannitol or other sugar alcohols, kaolin, calcium phosphate or sulfate, inorganic salts such as sodium chloride and powdered sugar. Powdered cellulose derivatives are also useful. Non-limiting examples of tablet binders include, but are not limited to, starches, gelatin and sugars such as lactose, fructose, glucose and the like. Natural and synthetic gums are also convenient, including, but are not limited to, acacia, alginates, methylcellulose, polyvinylpyrrolidone and the like. Polyethylene glycol, ethylcellulose and waxes can also serve as binders. A lubricant can be used in a tablet formulation to prevent the tablet and punches from sticking in the die. The lubricant include, but are not limited to, such slippery solids as talc, magnesium and calcium stearate, stearic acid and hydrogenated vegetable oils. Tablets can be coated with sugar as a flavor and sealant, or with film- forming protecting agents to modify the dissolution properties of the tablet. The compounds may also be formulated as chewable tablets, by using large amounts of pleasant-tasting substances such as mannitol in the formulation, as is now well-established in the art.

Also contemplated are liquid formulations and solid form preparations which are intended to be converted, shortly before use, to liquid form preparations. Such liquid forms include, but are not limited to, solutions, suspensions, syrups, slurries, and emulsions. Liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats or oils); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorboc acid). These preparations may contain, in addition to the active agent, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like. The compositions may be in powder form for constitution with a suitable vehicle such as sterile water, saline solution, or alcohol, before use.

The pharmaceutical compositions of the instant disclosure or the pharmaceutical acceptable salts derived therefrom may be in a dosage amount in an effective amount for inducing or increasing the naturally occurring biological activity of the wild-type amnio acid sequence upon which the antibody is derived. The pharmaceutical compositions of the instant disclosure or the pharmaceutical acceptable salts derived therefrom may be in a dosage amount in an effective amount for inducing or increasing the naturally occurring biological activity of the wild-type VIP polypeptide upon which the analog is derived.

In some embodiments, a subject in need thereof is administered from about .0001 to about 3000 milligrams of antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 2000 milligrams of antibody, antibody binding fragment or pharmaceutically salt thereof day. In some embodiments, a subject is administered up to about 1800 milligrams of antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 1600 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 1400 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 1200 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 1000 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered up to about 800 milligrams of

Antibody, antibody binding fragment or pharmaceutically salt thereof per day, per week or per month. In some embodiments, a subject is administered from about 0.0001 milligrams to about 700 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 700 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 600 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 500 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 400 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 300 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 200 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 100 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 50 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 25 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose. In some embodiments, a subject is administered up to about 15 milligrams of Antibody, antibody binding fragment or pharmaceutically salt thereof per dose.

Methods of Treatment and Prevention

In one embodiment, the present disclosure provides a composition comprising a multispecific antibody, or an antigen-binding fragment thereof, as described herein, and a pharmaceutically acceptable carrier. The composition may include a plurality of the antibodies having the characteristics described herein in any combination and can further include antibodies neutralizing to HIV as are known in the art.

It is to be understood that compositions can be a single or a combination of antibodies disclosed herein, which can be the same or different, in order to prophylactically or therapeutically treat the progression of various subtypes of HIV infection. When an antibody or active agent is administered to an animal or a human, it can be combined with one or more pharmaceutically acceptable carriers, excipients or adjuvants as are known to one of ordinary skilled in the art.

Further, with respect to determining the effective level in a patient for treatment of HIV, in particular, suitable animal models are available and have been widely implemented for evaluating the in vivo efficacy against HIV of various therapy protocols. These models include mice, monkeys and cats. Even though these animals are not naturally susceptible to HIV disease, chimeric mice models (for example, SCID, bg/nu/xid, NOD/SCID, SCID-hu, immunocompetent SCID-hu, bone marrow-ablated BALB/c) reconstituted with human peripheral blood mononuclear cells (PBMCs), lymph nodes, fetal liver/thymus or other tissues can be infected with lentiviral vector or HIV, and employed as models for HIV pathogenesis. Similarly, the simian immune deficiency virus (SrV)/monkey model can be employed, as can the feline immune deficiency virus (FIV)/cat model. The pharmaceutical composition can contain other pharmaceuticals, in conjunction with a vector according to the disclosure, when used to therapeutically treat AIDS. These other pharmaceuticals can be used in their traditional fashion (i.e., as agents to treat HIV infection).

According to another embodiment, the present disclosure provides a pharmaceutical composition comprising a multispecific antibody, or an antigen-binding fragment thereof, as described herein, which provides a prophylactic or therapeutic treatment choice to reduce the latent reservoir and infection of the HIV virus. The pharmaceutical compositions of the present disclosure may be formulated by any number of strategies known in the art (e.g., see McGoff and Scher, 2000, Solution Formulation of Proteins/Peptides: In McNally, E.J., ed. Protein Formulation and Delivery. New York, NY: Marcel Dekker; pp. 139-158; Akers and Defilippis, 2000, Peptides and Proteins as Parenteral Solutions. In: Pharmaceutical Formulation Development of Peptides and Proteins. Philadelphia, PA: Talyor and Francis; pp. 145-177; Akers, et al., 2002, Pharm. Biotechnol. 14:47-127). A pharmaceutically acceptable composition suitable for patient administration will contain an effective amount of the multispecific in a formulation which both retains biological activity while also promoting maximal stability during storage within an acceptable temperature range. The pharmaceutical compositions can also include, depending on the formulation desired, pharmaceutically acceptable diluents, pharmaceutically acceptable carriers and/or pharmaceutically acceptable excipients, or any such vehicle commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. The amount of an excipient that is useful in the pharmaceutical composition or formulation of this disclosure is an amount that serves to uniformly distribute the antibody throughout the

composition so that it can be uniformly dispersed when it is to be delivered to a subject in need thereof. It may serve to dilute the antibody or other active agent to a concentration which provides the desired beneficial palliative or curative results while at the same time minimizing any adverse side effects that might occur from too high a concentration. It may also have a preservative effect. Thus, for an active ingredient having a high physiological activity, more of the excipient will be employed. On the other hand, for any active ingredient(s) that exhibit a lower physiological activity, a lesser quantity of the excipient will be employed.

The above described multispecific antibodies and antibody compositions, comprising at least one or a combination of the antibodies described herein, can be administered for the prophylactic and therapeutic treatment of HIV viral infection.

The composition can be a pharmaceutical composition that contains a pharmaceutically acceptable carrier.

According to another embodiment, the present disclosure provides a method of reducing or preventing the establishment of a latent reservoir of HIV infected cells in a subject in need thereof (e.g., a subject infected with HIV or at risk of infection with HIV), thereby treating infection with a HIV infection, comprising administering to the subject a pharmaceutical composition comprising the multispecific antibodies disclosed herein. The compositions of the disclosure can include more than one antibody having the characteristics disclosed (for example, a plurality or pool of antibodies). It also can include other HIV neutralizing antibodies and/or active agent known in the art.

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 in some other way.

Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of HIV-related disease or disorder, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

For in vivo treatment of human and non-human patients, the patient is administered or provided a pharmaceutical formulation including an HIV antibody of the disclosure. When used for in vivo therapy, the antibodies of the disclosure are administered to the patient in therapeutically effective amounts (i.e., amounts that eliminate or reduce the patient's latent viral reservoir). The antibodies are administered to a human patient, in accord with known methods, such as intravenous administration, for example, as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The

antibodies can be administered parenterally, when possible, at the target cell site, or intravenously. In some embodiments, antibody is administered by intravenous or subcutaneous administration. Therapeutic compositions of the disclosure may be administered to a patient or subject systemically, parenterally, or locally. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

For parenteral administration, the antibodies may be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable, parenteral vehicle. Examples of such vehicles include, but are not limited, water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles include, but are not limited to, fixed oils and ethyl oleate. Liposomes can be used as carriers. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, such as, for example, buffers and preservatives. The antibodies can be 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, for example, its therapeutic index, the patient, and the patient's history. Generally, a therapeutically effective amount of an antibody is administered to a patient. In some embodiments, the amount of antibody administered is in the range of about 0.1 mg/kg to about 50 mg/kg of patient body weight. Depending on the type and severity of the infection, about 0.1 mg/kg to about 50 mg/kg body weight (for example, 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. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

Other therapeutic regimens may be combined with the administration of the multispecific antibodies of the present disclosure. The combined administration includes coadministration, 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. Such combined therapy can result in a synergistic therapeutic effect. The parameters for assessing successful treatment and improvement in the disease are also readily measurable by routine procedures familiar to a physician.

Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for an infection if, after receiving a therapeutic amount of an antibody according to the methods of the present disclosure, the patient shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of infected cells or absence of the infected cells; reduction in the percent of total cells that are infected; and/or relief to some extent, one or more of the symptoms associated with the specific infection; reduced morbidity and mortality, and improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

Eliminating the HIV-1 reservoir in chronic infection is key to curing the disease, but direct measurement of the latent reservoir to evaluate therapeutic eradication strategies remains difficult (Siliciano et al, Curr Opin HIV AIDS, 2013. 8(4): p. 318-25). Quantitative viral outgrowth assays and PCR-based assays of integrated DNA yield variable results (Eriksson et al., PLoS Pathog, 2013. 9(2): p. el003174) in part because PCR cannot distinguish between inactive and permanently disabled proviruses, and outgrowth assays underestimate reservoir size (Ho et al., Cell, 2013. 155(3): p. 540-51). To that end, the most effective way to evaluate the reservoir in vivo is to measure viral rebound after terminating therapy as disclosed in the examples below.

V. Methods of Making

Another aspect of the disclosure is a method of generating a multispecific antibody comprising a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof, wherein the first antibody and the second antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and wherein the VH from the first light chain and the VL from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers.

In one embodiment, the multispecific antibody is prepared by recombinant methods. Thus, the disclosure also relates to a method for the preparation of a multispecific antibody according to the disclosure, comprising the steps of transforming a host cell with vectors comprising nucleic acids encoding the first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; the second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof, and in certain embodiments, a third antibody which specifically binds to a third epitope, culturing said host cell under conditions that allow synthesis of said multispecific antibody; and recovering said multispecific antibody from said host cell culture.

Another object of the disclosure is a multispecific antibody produced by a method according to the disclosure.

In other embodiments, the present disclosure features a host cell comprising a vector comprising nucleic acids according to the disclosure encoding the first light chain derived from a first antibody which specifically binds to a first antigen; a vector comprising nucleic acids according to the disclosure encoding the first heavy chain derived from a first antibody which specifically binds to a first antigen; a vector comprising nucleic acids according to the disclosure encoding the second light chain derived from a second antibody which specifically binds to a second antigen; and a vector comprising nucleic acids according to the disclosure encoding the second heavy chain derived from a second antibody which specifically binds to a second antigen.

In other embodiments, the disclosure features a nucleic acid encoding the multispecific antibody according to the disclosure. In one embodiment, the nucleic acid according to the disclosure is an isolated nucleic acid.

In one embodiment the nucleic acid encodes a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof.

Standard recombinant DNA methodologies are used to construct the polynucleotides that encode the polypeptides which form the antibodies, incorporate these polynucleotides into recombinant expression vectors, and introduce such vectors into host cells. See e.g., Sambrook et al., 2001, MOLECULAR CLONING: A LABORATORY MANUAL (Cold Spring Harbor Laboratory Press, 3rd ed.). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Similarly, conventional techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery, and treatment of patients.

Other aspects of the present disclosure relate to isolated nucleic acid molecules comprising a nucleotide sequence encoding any of the antibodies described herein. In some embodiments, the isolated nucleic acid is operably linked to a heterologous promoter to direct transcription of the binding protein-coding nucleic acid sequence. A promoter may refer to nucleic acid control sequences which direct transcription of a nucleic acid. A first nucleic acid sequence is operably linked to a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence of a binding protein if the promoter affects the transcription or expression of the coding sequence.

Examples of promoters may include, but are not limited to, promoters obtained from the genomes of viruses (such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus, Simian Virus 40 (SV40), and the like), from heterologous eukaryotic promoters (such as the actin promoter, an immunoglobulin promoter, from heat-shock promoters, and the like), the CAG-promoter (Niwa et al., Gene 108(2):193-9, 1991), the phosphoglycerate kinase (PGK)-promoter, a tetracycline-inducible promoter (Masui et al., Nucleic Acids Res. 33:e43, 2005), the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, and the promoter of the yeast alpha-mating factors. Polynucleotides encoding binding proteins of the present disclosure may be under the control of a constitutive promoter, an inducible promoter, or any

other suitable promoter described herein or other suitable promoter that will be readily recognized by one skilled in the art.

In some embodiments, the isolated nucleic acid is incorporated into a vector. In some embodiments, the vector is an expression vector. Expression vectors may include one or more regulatory sequences operatively linked to the polynucleotide to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Examples of suitable enhancers may include, but are not limited to, enhancer sequences from mammalian genes (such as globin, elastase, albumin, Į-fetoprotein, insulin and the like), and enhancer sequences from a eukaryotic cell virus (such as SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, adenovirus enhancers, and the like). Examples of suitable vectors may include, for example, plasmids, cosmids, episomes, transposons, and viral vectors (e.g., adenoviral, vaccinia viral, Sindbis-viral, measles, herpes viral, lentiviral, retroviral, adeno-associated viral vectors, etc.). Expression vectors can be used to transfect host cells, such as, for example, bacterial cells, yeast cells, insect cells, and mammalian cells. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art, and can be used to transfect any cell of interest.

Other aspects of the present disclosure relate to a vector system comprising one or more vectors encoding a polypeptide chain of any of the antibodies or antibody binding fragments described herein.

Other aspects of the present disclosure relate to a host cell (e.g., an isolated host cell) comprising one or more isolated polynucleotides, vectors, and/or vector systems described herein. In some embodiments, an isolated host cell of the present disclosure is cultured in vitro. In some embodiments, the host cell is a bacterial cell (e.g., an E. coli cell). In some embodiments, the host cell is a yeast cell (e.g., an S. cerevisiae cell). In some embodiments, the host cell is an insect cell. Examples of insect host cells may include, for example, Drosophila cells (e.g., S2 cells), Trichoplusia ni cells (e.g., HIGH FIVE cells), and Spodoptera frugiperda cells (e.g., Sf21 or Sf9 cells). In some embodiments, the host cell is a mammalian cell. Examples of mammalian host cells may include, for example, human embryonic kidney cells (e.g., 293 or 293 cells subcloned for growth in suspension culture), EXPI293 cells, CHO cells, baby hamster kidney cells (e.g., BHK, ATCC CCL 10), mouse sertoli cells (e.g., TM4 cells), monkey kidney cells (e.g., CV1 ATCC CCL 70), African green monkey kidney cells (e.g., VERO-76, ATCC CRL-1587), human cervical carcinoma cells

(e.g., HELA, ATCC CCL 2), canine kidney cells (e.g., MDCK, ATCC CCL 34), buffalo rat liver cells (e.g., BRL 3A, ATCC CRL 1442), human lung cells (e.g., W138, ATCC CCL 75), human liver cells (e.g., Hep G2, HB 8065), mouse mammary tumor cells (e.g., MMT 060562, ATCC CCL51), TRI cells, MRC 5 cells, FS4 cells, a human hepatoma line (e.g., Hep G2), and myeloma cells (e.g., NS0 and Sp2/0 cells).

Other aspects of the present disclosure relate to a method of producing any of the antibodies described herein. In some embodiments, the method includes a) culturing a host cell (e.g., any of the host cells described herein) comprising an isolated nucleic acid, vector, and/or vector system (e.g., any of the isolated nucleic acids, vectors, and/or vector systems described herein) under conditions such that the host cell expresses the antibody; and b) isolating the antibody from the host cell. Methods of culturing host cells under conditions to express a protein are well known to one of ordinary skill in the art. Methods of isolating proteins from cultured host cells are well known to one of ordinary skill in the art, including, for example, by affinity chromatography (*e.g.*, two step affinity chromatography comprising protein A affinity chromatography followed by size exclusion chromatography).

All patent applciations, issued patents and journal articles disclosed herein are incorporated by reference in their entireties.

EXAMPLES

Example 1. Bi-specific bNAbs targeting the CD4 binding site and V3-base glycan display expanded neutralization breadth

To date, HIV-1 Env bNAbs are classified into five groups depending on their cognate epitopes (1,2): i) PG9/PG16 that recognize the trimer V2 apex; ii) Receptor CD4 binding site (CD4bs) specific bNAbs including VRC01/VRC07 and 3BNC117; iii) 35022 and PGT151 that recognize the gp120/gp41 interface; iv) PGT121/PGT135 that recognize V3 base glycans; and v) 10E8/4E10/2F5 that recognize gp41 membrane proximal external region (MPER) . Many bNAbs demonstrated encouraging protective efficacy as both preventive and therapeutic agents in humanized mouse and simian-human immunodeficiency virus (SHIV) rhesus macaque animal models after passive infusion (11-18). However, the outcome of the most recent clinical trials with bNAbs as therapeutic agents strikingly revealed that the antigenically diverse and persistently evolving HIV-1 Env can rapidly acquire mutations that evade bNAbs administered as single agents (19, 20). The quick onset of escaping virus quasi species in these single bNAb agent therapy trials strongly highlights the need to develop combination therapy strategies to control virus rebound.

The advantage of bNAb combination therapy has been demonstrated by a number of in vitro studies (5, 21). There is a substantial gain of neutralization potency and breadth when two bNAbs targeting independent epitopes are combined to test neutralization capacities against a virus panel consisting of 125 diverse tier 2 and 3 viruses. There is further incremental gain of neutralization potency and breadth by combining 3 or 4 bNAbs of different epitope specificity, resulting in a virtual 100% virus coverage (5). The scientific premise of combination therapy 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 (12, 13). The use of a "single" agent consisting of these multiple functional binding moieties as a means of delivering combination therapy is preferred for both regulatory and manufacturing purposes, in addition to the potential of augmented potency resulting from the increased avidity through bi- or multi- valence Env binding and possible synergistic effect between bNAbs (Figure 1).

Previous bispecific bNAb designs utilizing CrossMab technology to combine two bNAb 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%) (22). The experiments described herein propose to further the crosslinking of Env trimer by structure-based rational design that ensures the optimal accommodation of individual bNAb functional moieties that will orchestrate multi-epitope engagement to achieve superior binding avidity and profoundly enhanced viral inhibition breadth and potency.

As prevention and therapy agents, the bNAb derivatives play major roles in i) inhibiting virus entry by neutralizing the free infectious virus particle, ii) preventing virus cell-to-cell spread, and iii) eliminating 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 interaction between the IgG Fc region and the Fc receptors on effector cells (primarily NK cells) (reviewed in (1)). Thus, the dramatically enhanced viral inhibition breadth and potency associated with the superior binding avidity achieved by Env trimer multi-epitope crosslinking proposed in this study is highly relevant to HIV prevention and therapy efficacy. In addition, the proposed Env trimer multi-epitope crosslinking mechanism will more efficiently combat the emergence of HIV bNAb escaping mutants. Furthermore, modifying the Fc region of bNAb derivatives which will result in bNAb molecules with augmented ADCC effector function, broader effector cell spectra, expanded bio-distribution in HIV reservoir-residing tissues, and longer lasting in vivo half-

lives is also proposed. All these features along with selected combinations and permutations will plausibly be applied in optimizing the next generation of prevention and therapeutic agents.

A goal of the studies described herein is to further the crosslinking of Env trimer by structure-based rational design that ensures the optimal accommodation of individual bNAb functional moieties that will orchestrate multi-epitope engagement to achieve superior binding avidity and profoundly enhanced viral inhibition breadth and potency. Conventional bispecific antibodies are formed by empirical combination of Fabs with distinct binding specificity for different molecules or different epitopes within one molecule (Figure 2A). The empirical combination of Fab functional domains results in compromised biological functions at high frequency, with limited specificities (in most cases, two specificities) combined (22, 23). In contrast, the present studies take a structure-based rational design approach that ensures the optimal accommodation of individual bNAb functional moieties that will orchestrate multi-epitope engagement to achieve superior binding avidity and profoundly enhanced viral inhibition breadth and potency (Figure 2B). The tandem combination of HIV Env binding moieties allows combinations of up to five epitope specificities, which maximizes the possible ways of multi-epitope targeting in one single construct (Figure 2B). This single molecule construct configuration is particularly more amenable than conventional CrossMab or "knob-into-holes" configuration requiring more than one construct when advanced to viral expression vectors for further downstream applications.

As the first step toward generating multi-epitope targeting bNAbs, bi-specific single-chain variable fragment (bi-ScFv) consisting of two bNAb epitope binding moieties based on structural information were engineered. The bNAbs VRC01 (CD4bs-directed) (24) and PGT121 (V3-base glycan-directed) (25), which neutralize approximately 90% and 70% of circulating viruses, were chosen, respectively as a model system based on the following criteria: i) both bNAbs display impressive neutralization potency and breadth; ii) structure biology information is available for rational design; and iii) biological relevance: both VRC01 and PGT121 demonstrated impressive protective efficacy in animal models (11, 18, 26), while VRC01 displayed the ability to control viremia as a therapeutic agent in clinical trial (19).

Figure 3A-C shows bi-ScFv and bi-IgG1 of VRC01-PGT121 design and expression. As illustrated in Figure 3A, the bNAb-Env trimer binding mode and the spatial distance between the bNAb variable chains, VH and VL, were taken into account for optimizing the

bi-ScFv molecular topology and the means to join individual ScFv moieties. The VRC01 CD4bs epitope is adjacent to that of PGT121 on the Env gp140 BG505 SOSIP.664 trimer, suggesting that it is feasible to engineer a bi-ScFv by joining the variable regions of two bNAbs with flexible linkers. Two different approaches were used to construct VRC01-PGT121 bi-ScFv molecules, based on the following atomic parameters of VRC01 and PGT121 functional variable domains: 1) There is approximately 39 Å distance between the C-terminus of VRC01 VH domain and the N-terminus of PGT121 VL domain (Figure 3A, upper), which can be joined by 5 G4S linker using the topology of VRC01 (VL-VH) ScFv-linker-PGT121 (VL-VH) ScFv; and 2) There is approximately 55 Å distance between the N-terminus of VRC01 VH and the C-terminus of PGT121 VL (Figure 3A, lower), which can be bridged by multiple (3 to 5) G4S linkers using the topology of PGT121 (VH-VL) ScFv-linker-VRC01 (VH-VL).

Aa series of VRC01-PGT121 bi-ScFvs and full length IgGs were generated (Figure 3B), with combinations and permutations of VH-VL orientations and linker length for optimization (SEQ ID NOs 1-27). The nomenclature of these constructs was defined by the VH-VL orientations and linker length. For instance, VRC01-5x-PGT121 refers to VRC01(VL-VH)-$(G4S)_5$-PGT121(VL-VH). A construct was also made containing a 2 amino acid deletion at the N-terminus of VRC01 VL to avoid potential steric clash reported previously, which was designated as $_{d}$VRC01-5x-PGT121 (Figure 3B). These bi-ScFvs and full length IgGs were expressed in mammalian 293FreeStyle cells and purified by Ni+ and protein A column, respectively. All the proteins have expected molecular weight, as shown in representative SDS-PAGE gels (Figure 11A), as well as size exclusion chromatography (Figure 3C).

Figure 4 shows bi-ScFv and bi-IgG1 of VRC01-PGT121 interact with HIV-1 Env trimer in a bispecific manner. The binding specificities of these constructs were validated by ELISA binding assay (not shown) as well as Bio-Layer Interferometry (BLI) which demonstrated that both VRC01 and PGT121 binding moieties are functional (Figure 4). As shown in Figure 4, Env CD4bs ligand, RSC3 (Ligand 1), was initially captured to BLI probe, followed by loading the bi-ScFv (upper) and bi-IgG1 (lower) or control antibodies to the probe. The binding signals between the CD4bs ligand RSC3 and the test antibodies were shown as the increased binding response. As expected, only VRC01 and the bi-ScFv or bi-IgG1 demonstrated substantial binding signals. Subsequently, the probe was immersed into buffers containing trimeric Env protein (ligand 2) with CD4bs knockout mutation (VRC01 K/O) but retaining the V3 base glycan epitope for PGT121 recognition. Only the bi-ScFv or

bi-IgG1 which recognizes both CD4bs and V3 base glycan epiotipes displayed binding response signal. Furthermore, it was shown using single molecule electron microscopy (EM) analysis that the VRC01-PGT121 bi-ScFv binds Env trimer in a bi-specific manner (Figure 12C). The well-behaved expression and Env trimer binding phenotypes of the bi-ScFV and IgGs firmly authenticates the rationales of the design.

To assess neutralization capacity of the bi-ScFvs and IgGs, an initial virus panel of high stringency consisting of 20 tier 2 virus strains, including 12 single- and 2 dual-resistant strains was used. The results are shown in Figure 5A-Figure 5D. Figure 5A is a graph that shows the potency and breadth curve of Bi-ScFv molecules tested with selected 20 virus panel. Figure 5B is a graph that shows the potency and breadth curve of bi-IgG1 molecules tested with selected 20 virus panel. Figure 5C is a table that shows a summary of the virus neutralization parameters of the bispecific antibody molecules. Figure 5D is a table that shows virus neutralization potency displayed with IC_{50} (ug/ml) values.

As shown in Figure 5, most of the VRC01-PGT121 bispecific scFvs and IgGs (N=10) display expanded virus neutralization breadth compared with the parental antibody VRC01 and PGT121, respectively. Of the multiple iterations tested, one combination of VRC01/PGT121, namely $_{d}$VRC01-5x-PGT121, containing $(G4S)_5$ linker and a 2 amino acid residue deletion in the N-terminus of VRC01 light chain, was found to display improved coverage over the parental antibodies with 100% coverage of neutralization, and potency comparable to the combination of the individual parental antibodies (Figure 5C and Figure 5D). In IgG1 form, its virus coverage is much higher than PGT121 and slightly higher than VRC01 (Figure 6 and Figure 7), when assayed with a more expanded 200 virus panel. Figure 6A- Figure 6C shows bi-ScFv and bi-IgG1 of VRC01-PGT121 virus neutralization potency and breadth assessed with a 200 virus panel reflecting the worldwide diversified HIV-1 primary isolates. Figure 7 is a Table that shows neutralization potency and breadth (IC_{50} value, in μg/ml) of $_{d}$VRC01-PGT bi-ScFv and IgG assessed against a 200-isolate virus panel.

These results suggest that through rational design and iterative optimization, bi-ScFv-based antibodies can achieve superior neutralization breadth and may be candidate moieties for engineering multifunctional anti-viral agents for the prevention and treatment of HIV-1 infection.

Example 2. Synergistic effect by Bi-specific bNAbs targeting the gp120/gp41 interface and the gp41 MPER

Among the five major bNAb epitope clusters, the gp41 MPER targeted by 10E8, and the gp120/gp41 interface recognized by 35022 (27) are located in proximity on the Env trimer. By superimposing the structures of Env BG505 SOSIP.664 trimer-35022 complex (27) with the more recent fully glycosylated Env trimer-10E8 complex (3), the spatial distance between the variable domains of 35022 and 10E8 (Figure 8) was determined, and a series of bi-ScFvs were designed in similar manners with the VRC01-PGT121 bi-ScFvs described supra. The sequences of these biScFvs and IgG molecules are shown in SEQ ID NOs 30-53.

The engineered 35022-10E8 bi-ScFv and IgG molecules were initially purified and tested for binding to their cognate epitopes on Env protein variants including MPER peptide (specific for 10E8) and BG505 SOSIP.664 (specific for 35022). As expected, all bi-specific molecules display positive binding to both epitopes (not shown).

Next, virus neutralization capacities were tested against a virus panel of high stringency consisting of 24 tier 2 & 3 viruses similar to that were used for testing the bi-ScFVs of VRC01-PGT121. It is of note that this panel of viruses displays a high degree of resistance to 35022, with only 33% virus coverage but high potency for the sensitive strains, while most of these viruses are sensitive to 10E8 neutralization with 92% coverage and good potency (geometric mean IC_{50}=0.5 μg/ml).

Figure 9A-Figure 9C shows bi-ScFv and IgG of 35022-10E8 virus neutralization potency and breadth tested with selected 24 virus panel. The 35022-10E8 bi-ScFvs and IgGs demonstrated unaltered or slightly improved virus coverage (92-96%) (Figure 9A and Figure 9B). Interestingly, substantially increased potencies of the bi-functional molecules was found compared to the parental bNAb 10E8 and 35022 (Figure 9C), likely caused by synergistic effect, which will be validated in subsequent analysis. An expanded 200 virus panel will also be further tested to confirm the neutralization potency and breadth.

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Example 3. Methods

This example describes the general methods used to carry out the experiments in Examples 4-9. The methods used in Examples 4-9 include, but are not limited to the below described methods.

Analysis of Ab structures in complex with SOSIP

The published structure of the two bNAbs PGT122 and VRC01 bound to JR-FL SOSIP.664 (PDB: 5FYK) [40] was used to model bispecific combinations of VRC01 and PGT121 in the program Chimera [41] where PGT122 served as the surrogate for PGT121. Trimers were visualized using the Higher Order Structure-Unit Cell tool. Antibody variable domains were retained for analysis, while antibody residues corresponding to the CL1 and CH1 regions were deleted from the Fab entities. Distances between termini were assessed by selecting the respective terminis' Cβ atoms using the Structure Analysis-Distance tool and measuring the spatial distance.

Antibody production

Bi-NAbs in a ScFv format were designed utilizing tetra-glycine-serine (G_4S) peptide linkers [36, 37]. C-terminal His-tagged Bi-ScFvs DNA sequences were synthesized (GenScript, Piscataway, NJ) and cloned into the pcDNA3.1(-) vector while the Bi-ScFv lacking the C-terminal His tag was subcloned into an IgG1 Fc vector as well as IgG1 Fc vectors carrying knob-and-hole mutations [32]. Bi-NAbs were expressed by transient transfection of either Bi-ScFv or Bi-ScFv-IgG1 Fc plasmids in 293F cells. Tri-NAbs were expressed by transient transfection of the Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ IgG1 Fc "knob", 10E8 HC "hole" and 10E8 LC plasmids in 293F cells. Supernatants were harvested 5 days post transfection, filtrated, followed by affinity purification. Bi-ScFvs with 6-His tag were purified by Complete His-tag purification resin (Sigma-Aldrich). Bi-NAb and Tri-NAb with IgG1 Fc were purified by protein A affinity chromatography. Elutes were dialyzed with phosphate-buffered saline (PBS), and concentrated using an Amicon Ultra 10 kDa molecular weight cut-off concentrator. Antibody purity was analyzed by SDS PAGE.

Protein purification

BG505 SOSIP.664, an avi-tagged version of BG505 SOSIP.664_D368R, and RSC3 core were expressed in 293F cells, purified using a *Galanthus nivalis* (GN)-lectin column as previously described [57], followed by purifications with size-exclusive chromatography (SEC)

with purity confirmed by Blue NativePage. The BirA 500 biotin ligase (AVIDITY AVITAG Technology) was utilized according to the manufacturer's protocol to biotinylate the C-terminal avi-tags of BG505 SOSIP.664_D368R and RSC3.

Ab binding affinity

Biolayer light interferometry (BLI) was performed using an Octet RED96 instrument (ForteBio; Pall Life Sciences). Bispecific binding was confirmed by first capturing biotin labeled RSC3 (ligand 1) at 10 µg/ml onto Streptavidin biosensors for 300 seconds. The biosensors were then submerged in binding buffer (PBS/0.2% TWEEN 20) for a wash for 60 seconds followed by immersion in a solution containing 250 nM of either the parental or Bi-NAb antibodies for 180 seconds and an immediate immersion in a solution containing 300 µg/ml of trimeric BG505 SOSIP.664_D368R (ligand 2) for 300 seconds.

Trispecific binding was confirmed by first capturing 10 µg/ml of biotin labeled RSC3 (ligand 1) onto Streptavidin biosensors for 300 seconds. The biosensors were then submerged in binding buffer for a wash of 60 seconds. Then, the biosensors were immersed in a solution containing 250 nM of either the parental or Bi-NAb antibodies for 180 seconds followed by an immediate immersion in a solution containing 300 µg/ml of trimeric BG505 SOSIP.664_D368R (ligand 2) for 300 seconds. Finally, the biosensors were immersed in a solution containing 50 µg/ml of MPER peptide fused to a rabbit Fc (MPER rFc) for 120 seconds. Baselines were established before and after the loading step. All assays were performed in 1X binding buffer.

HIV-1 neutralization assays

Ab neutralization assays were performed in a single round of infection using HIV-1 Env-pseudoviruses and TZM-bl target cells, as previously described [58, 59]. Neutralization curves were fitted by nonlinear regression using a five-parameter hill slope equation as previously described [58]. The IC_{50} or IC_{80} titers of Abs were reported as the concentration of Ab required to inhibit infection by 50% and 80%, respectively. The IC_{50} or IC_{80} geometric mean (Geomean) indicating mAb neutralization potency was derived from IC_{50} or IC_{80} values against each individual tier 2 virus for each mAb. When IC_{50} or IC_{80} value is >50 µg/mL for certain viruses (no neutralization), a value of 50 µg/mL is designated for calculation. The number of viruses neutralized by mAb (IC_{50} or IC_{80} < 50 µg/mL) out of the total number of tested viruses was used to calculate the neutralization breadth. The virus panel for the neutralization profile covers the major genetic subtypes and circulating recombinant forms and consists almost entirely of primary isolate Envs [45].

Electron microscopy analysis

To confirm bivalent binding, Bi-ScFv/BG505 SOSIP.664 complexes were generated by incubating 6X molar ScFv with BG505 SOSIP.664 overnight at room temperature, followed by purification of complexes by SEC. To specifically confirm crosslinking of the HIV Env trimer, ScFv/BG505 SOSIP.664 complexes were generated by incubating 0.5X molar ScFv with BG505 SOSIP.664 overnight at room temperature, followed by purification of complexes by SEC.

Complexes were then deposited on 400 mesh copper grids and stained with 2% uranyl formate. Negative stain EM images were taken on a 120 kv Tecnai Spirit microscope with a LAB6 filament. Raw micrographs were collected using Leginon [60] and deposited in Appion [61]. DoG picker [62] was performed to select particles in stain. Those particles were stacked and aligned using Iterative MSA/MRA [63]. 2D classes representing the complex are shown in Figure 12 and Figure 13. Images were false colored using Photoshop.

Statistical Analysis

Comparisons of antibody neutralization performance was carried out with one-way ANOVA. Statistical evaluation of difference between two groups was performed via non-parametric t test for paired data, determined with Wilcoxon matched-pairs signed rank test, with a two-tailed p value calculated for significance. Statistical significance was determined as *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. All statistical analysis was performed with GraphPad Prism version 7.

Example 4. Structural analysis of the interactions of HIV-1 Env trimer with bNAbs

For the first step towards generating multi-epitope targeting bNAbs, bispecific single-chain variable fragment (Bi-ScFv) were engineered. This construct consists of two bNAb variable fragments (Fv) connected by a flexible amino acid linker whose length was estimated following Env/bNAb structural information [36, 37]. bNAbs VRC01 (CD4bs-directed) [8] and PGT121 (V3-base glycan-directed) [9], which neutralize approximately 90% and 70% of circulating viruses, respectively, were selected as a model system to determine if a Bi-ScFv could improve breadth and potency over the capacities of the individual bNAbs. To guide the design, a bNAb/Env complex structure [40] was used to assess the distance between the bNAbs Fvs and to determine ideal linker lengths [41].

To determine the distances between the variable domains of VCR01 and PGT121, the PDB structure 5FYK [40] was used, which includes the HIV-1 clade B trimeric Env JR-FL SOSIP.664 bound to bNAbs VRC01 and PGT122. PGT122 is a somatic variant of PGT121 possessing nearly identical Env binding mode [9] that serves as a surrogate for PGT121 in this

study (Figure 10A). Utilizing the distance tool under the structure analysis module in UCSF Chimera, the distances between the C/N -termini of VRC01 and PGT121 VH/VL moieties for all eight termini combinations were examined (**Table 2, shown below**). Table 2 shows a summary of distances between the termini of PGT121 and VRC01 when bound to JR-FL SOSIP.664 trimer (inter-monomer and intra monomer) inspected by Chimera, based on antibody-trimer complex structure (PDB: 5FYK). PGT122 serves as a surrogate for PGT121.

Distance Between PGT121-VRC01 termini			
Inter-Monomer			
Orientation			

Table 2

VRC01	*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	37.171
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	53.470
VH-VL	VL-VH	VAL 106.L CB	VAL 3.V CB	63.459
VL-VH	VL-VH	SER 111.H CB	VAL 3.V CB	96.431
VL-VH	VH-VL	SER 111.H CB	GLN 1.U CB	77.125
Intra-Monomer				
Orientation				
VRC01	*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	78.762
Orientation				
PGT121	*VRC01*	*PGT121 C-terminus*	*VRC01 N-terminus*	*Distance (Å)*
VH-VL	VH-VL	VAL 106.L CB	GLN 1.U CB	96.925
VH-VL	VL-VH	VAL 106.L CB	VAL 3.V CB	94.519
VL-VH	VL-VH	SER 111.H CB	VAL 3.V CB	93.620
VL-VH	VH-VL	SER 111.H CB	GLN 1.U CB	94.350

Table 3, shown below, shows the topology of the bi-ScFv and Bi-nAb.

Table 3

	Bi-ScFv		Bi-nAb	
Topology	VRC01→PGT121	PGT121→VRC01	VRC01→PGT121	PGT121→VRC01

It was found that in general the distance between these two antibody moieties on two separate but adjacent gp120 protomers (inter-protomer distance) is shorter than that within

the same protomer (intra-protomer distance) (Figure 10A and Table 2). While the maximum intra-protomer distance between PGT121 and VRC01 termini is approximately 100 Å, the minimum inter-protomer distance is 37.171 Å (VRC01 $VH_{C\text{-}term}$ to PGT121 $VL_{N\text{-}term}$) and 53.470 Å (PGT121 $VL_{C\text{-}term}$ to VRC01 $VH_{N\text{-}term}$), respectively (Figure 10B and Table 2). The relatively short VRC01 and PGT121 VH/VL N/C termini inter-protomer distance implies the potential to connect their antibody functional moieties by tandem GGGGS (G_4S) linkers without unfavorable steric clash and supports the premise of inter-protomer VRC01/PGT121 Bi-ScFvs to target HIV-1 Env trimer.

Example 5. Design and expression of bispecific antibodies

With the rationale stated herein above, the VRC01/PGT121 inter-protomer binding mode was selected (Figure 10B) and the most favorable molecular topology, including $VRC01_{VL\text{-}VH}$→$PGT121_{VL\text{-}VH}$ and $PGT121_{VH\text{-}VL}$→$VRC01_{VH\text{-}VL}$ respectively, to construct Bi-ScFvs (Figure 10B). First, to derive the individual scFvs, three tandem G_4S linkers were used (designated as 3X linker) [42, 43], with each linker estimated to be briefly 18 Å in length, to connect the cognate VH/VL domains within VRC01 and PGT121, respectively (Figure 10C). 3-5 G_4S linkers, namely 3-5X linkers, were then used to connect the individual VRC01 and PGT121 scFvs to form the Bi-ScFv molecule (Figure 10C). Here, 3-5 G_4S linkers were empirically used to optimize the linkage between individual ScFvs to avoid potential steric hindrance imposed by elements of the HIV-1 Env functional spike. Hence, the nomenclatures of these Bi-ScFv constructs, shown in Figure 10C included: 1) the molecular topology of the whole Bi-ScFv (e.g., VRC01 ScFv at the N-terminus), and 2) the linker length between each ScFv (e.g. 3 tandem G_4S linker). For instance, Bi-ScFv $_{VRC01\text{-}5X\text{-}PGT121}$ represents a Bi-ScFv with $VRC01_{VL\text{-}VH}$→$(G_4S)_5$ linkers→$PGT121_{VL\text{-}VH}$ topology, while Bi-ScFv $_{PGT121\text{-}5X\text{-}VRC01}$ ScFv denotes a Bi-ScFv with $PGT121_{VH\text{-}VL}$→$(G_4S)_5$ linkers→ $VRC01_{VH\text{-}VL}$ topology (Figure 10C).

In a previous study, a truncation of the first two amino acid residues E1 and I2 (ΔE1I2) and a V3S mutation at the N-terminus of VRC01 VL increased potency of HIV neutralization [44] by eliminating a steric clash between the VRC01 VL N-terminus and the V5 region of HIV-1 Env. Therefore, such modifications were incorporated into the VRC01 VL moiety of the Bi-ScFv $_{VRC01\text{-}5X\text{-}PGT121}$, denoted as Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ (Figure 10C). These ScFvs were also fused to the IgG1 Fc via a glycine-serine-glycine linker to support effector functions [39], thus forming Bi-NAbs (Figure 10C and Figure 10D).

Ten bispecific antibodies of the PGT121/VRC01 Bi-ScFvs and Bi-NAbs iterations in a total were expressed in 293F cells and purified by affinity chromatography. All the antibodies expressed well and ran as a homogeneous species at the expected molecular weight on an SDS-PAGE gel (Figure 11A) and size exclusion chromatography (SEC), with negligible aggregation forms (Figure 11B).

Example 6. Binding properties of bispecific antibodies

Bio-Layer Interferometry (BLI) was employed to validate the simultaneous Env trimer engagement of the two arms of the Bi-ScFvs. Env ligands including RSC3 core were used, which selectively displays the CD4bs (VRC01) epitope but not the PGT121 epitope, and the BG505 SOSIP.664_D368R mutant that exclusively exhibits the PGT121 epitope (Figure 12A). In this assay, biotinylated RSC3 was first loaded as the initial ligand (ligand 1), then the parental or bispecific antibodies, followed by the second ligand BG505 SOSIP.664_D368R (ligand 2) (Figure 12A), and the antibody binding signals to each of the Env ligands was assessed. As expected, it was found that only antibodies containing the VRC01 moiety including Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ and Bi-NAb $_{dVRC01\text{-}5X\text{-}PGT121}$ as well as VRC01 Fab and IgG, were able to bind CD4bs ligand, RSC3 (Ligand 1) initially (Figure 12B). Similarly, bispecific antibodies containing the PGT121 moiety bound the CD4bs knockout trimer (VRC01-KO), BG505 SOSIP.664_D368R, which exclusively presents the PGT121 epitope, while the VRC01 Fab or IgG displayed no binding signal (Figure 12B). These data confirm that bispecific antibodies including Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ and Bi-NAb $_{dVRC01\text{-}5X\text{-}PGT121}$ can simultaneously bind both the VRC01 and the PGT121 epitopes, demonstrating that both arms of the bispecific antibody are functional.

As shown in Figure 10, the bispecific antibodies were designed to cross-link adjacent protomers within the same HIV-1 Env trimer by connecting the CD4bs of one Env protomer (protomer 1) with the glycan patch at the V3 base of the adjacent protomer (protomer 2). To further investigate the bivalency of Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$, negative stain electron microscopy (EM) analysis of the Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ and Env trimer BG505 SOSIP.664 complex was performed (Figure 12C, Figure 13A and Figure 13B). The Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ was incubated with the BG505 SOSIP.664 Env trimer at a 1:2 (or 0.5:1) ratio so that each Env trimer would be occupied by a single Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ molecule, in a way that enabled the inter-protomer vs. intra-protomer binding modes depicted in Figure 10A to be distinguished. As expected, both unbound Env trimer (Figure 12C, left panel) and Env trimer bound with single Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ were visualized (Figure 12C, second from

left and middle panel), given the sub-saturation ratio between the Bi-ScFv and Env trimer. Importantly, it was found that each Env protomer was only decorated with one of the Bi-ScFv moieties (Figure 12C, second from left and middle panel), while two adjacent Env protomers were simultaneously decorated by two Bi-ScFv moieties (Figure 12C, second from left and middle panel). The data corroborated the bivalent nature of the binding event between the Bi-ScFv and Env trimer, as predicted by design. The Bi-ScFv crosslinked both the VRC01 (in purple, Figure 12C, middle panel) and PGT121 (in green, Figure 12C, middle panel) epitopes in an inter-protomer mode. When the molar ratio of Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ to BG505 SOSIP.664 trimer was increased to 6:1 to form Bi-ScFv/Env complex, it was observed that all the protomers of Env trimer were fully occupied with Bi-ScFv moieties and each Env trimer bound three Bi-ScFvs (Figure 12C, second from right and right panel). This observation is consistent with the inter-protomer crosslinking binding mode and suggests that three molecules of Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ can fully occupy the total 6 cognate epitopes on each HIV-1 Env trimer.

Example 7. Bispecific antibodies display expanded virus neutralization breadth compared to parental bNAbs

To initially assess the virus neutralization capacity of the bispecific antibodies in comparison to their parental bNAbs, VRC01 and PGT121, a small HIV-1 virus panel (N=20) containing Envs of viruses from diverse clades was selected to perform virus neutralization assay (Figure 14A). This panel included Envs of viruses that were: 1) sensitive to both (dual sensitive) bNAbs (N=6); or 2) resistant to both (dual resistant) bNAbs (N=1); or 3) sensitive to one but resistant to the other parental antibody (N=13) (Figure 14). It is notable that 70% of the selected viruses (N=14) were resistant to at least one of the parental bNAbs, which represented a high bar for the evaluation of neutralization capacity.

Using IC_{50} titers (the concentration of antibody at which 50% of virus entry is inhibited) with a cut-off value set at 50 μg/ml, the neutralization capacity of bispecific antibodies was assessed with this initial virus panel. It was found that all the bispecific antibodies displayed substantially improved neutralization breadth, ranging from 80-90% virus coverage, compared to 60-65% virus coverage of their parental bNAbs (Figure 15A, Figure 14A and Figure 14B). Interestingly, it was observed that the bispecific antibodies with the topology of VRC01$_{VL\text{-}VH}$→PGT121$_{VL\text{-}VH}$ displayed 90% neutralization breadth, which was better than that with the PGT121$_{VH\text{-}VL}$ →VRC01$_{VH\text{-}VL}$ topology (80-85%) (Figure 15A). It was also noted that the five-tandem G_4S linker length (5X) was slightly better than the

shorter variants (4X and 3X) (Figure 15A, Figure 14A and Figure 14B). Finally, the bispecific antibodies, Bi-ScFv $_{dVRC01\text{-}5X\text{-}PGT121}$ and Bi-NAb $_{dVRC01\text{-}5X\text{-}PGT121}$ with the optimal VRC01$_{VL\text{-}VH}$→PGT121$_{VL\text{-}VH}$ topology, the 5X G_4S linker and the N-terminus VRC01 VL modifications ΔE1I2/V3S (Figure 1) displayed the best neutralization breadth (90% virus coverage) (Figure 15A, Figure 14A and Figure 14B).

Subsequently, the top bispecific antibodies, Bi- ScFv$_{dVRC01\text{-}5x\text{-}PGT121}$ and Bi-NAb$_{dVRC01\text{-}5x\text{-}PGT121}$, were tested against a more comprehensive panel of viruses covering the major genetic subtypes and circulating recombinant forms, and containing almost entirely of primary isolate Envs [45]. In this 208 virus panel, the Bi-ScFv$_{dVRC01\text{-}5x\text{-}PGT121}$ and Bi-NAb$_{dVRC01\text{-}5x\text{-}PGT121}$ displayed improved coverage with 94.7% and 95.1% of viruses neutralized, respectively, over the parental antibodies (VRC01=90.4%, and PGT121=64%) (Figure 15B and Figure 15C) and with a potency (IC_{50} geometric mean) comparable to VRC01 (Figure 15B and Figure 15C). Interestingly, both Bi- ScFv$_{dVRC01\text{-}5x\text{-}PGT121}$ and Bi-NAb$_{dVRC01\text{-}5x\text{-}PGT121}$ were able to neutralize three primary isolates with dual resistance to parental bNAbs (Figure 15C, right panel, right panel), suggesting a cooperative effect exerted likely by the inter-protomer crosslinking of two distinct epitopes within the HIV-1 Env trimer.

Example 8. Generation and validation of a trispecific antibody

Previous studies indicated that the HIV-1 gp41 MPER-specific bNAb, 10E8, when combined with gp120-specific bNAbs including the CD4bs bNAb VRC01, displayed an additive, and potentially small synergistic/cooperative effect on neutralization [46]. Here, the MPER-specific 10E8 functional moiety was combined with the top lead bispecific antibodies to improve neutralization breadth and potency further. Knob-into-hole technology [32] was used to generate a heterodimeric Tri-NAb consisting of 10E8 and Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$ moieties (Figure 16A). The 10E8 was placed on one antibody arm with the Fc containing the "knob" mutation, and the Bi-ScFv $_{dVRC01\text{-}5x\text{-}PGT121}$ was fused to the IgG1 Fc containing the "hole" mutation on the other arm. To express the Tri-NAb, 293F cells were co-transfected with the plasmid DNA encoding the heavy and light chain genes of 10E8 and the Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$. The Tri-NAb was then purified via a protein A column and assessed its homogeneity by SDS-PAGE (Figure 16B).

BLI was used to assess the triple specificity of the Tri-NAb, with ligands presenting the epitopes of VRC01, PGT121, and 10E8 (Figure 16C and Figure 16D), respectively. In a BLI OctetRED96 system, the ligands and antibodies were loaded to the biosensor surface sequentially in the following order: 1) biotinylated RSC3 as the initial ligand (ligand 1) to

present VRC01 epitope; 2) the parental bNAbs or Tri-NAb; 3) the second ligand, trimeric BG505.SOSIP.664_D368R with CD4bs/VRC01 epitope knockout to present PGT121 epitope (ligand 2); and finally 4) the third ligand (ligand 3), an MPER peptide fused to a rabbit Fc (MPER rFc) (Figure 16C and Figure 16D) to present the 10E8 epitope (Figure 16D). As expected, it was found that Tri-NAb displayed binding signals for all of the three ligands, while the parental bNAbs only showed binding to ligand 1 with CD4bs epitope (e.g., VRC01) or no binding to any ligands (e.g., PGT121 and 10E8) due to the lack of initial CD4bs ligand 1 engagement in this sequential binding assay. This data confirmed that the Tri-NAb containing the moieties of three bNAbs (VRC01, PGT121, and 10E8) is capable of recognizing all of the three cognate bNAb epitopes on HIV-1 Env molecule.

Example 9. Trispecific antibody displays exceptional neutralization capacity

Next, the neutralization capacity of the Tri-NAb against the comprehensive virus panel consisting of Envs of 208 HIV strains [45] was assessed. The Tri-NAb's neutralization capacity (indicated by IC_{50} titers) was extraordinary as it neutralized all but one virus (99.5%) of the panel, with improved neutralization breadth compared to the parental bNAbs and the Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$ (Figure 17A). Additionally, the Tri-NAb neutralization potency was superior to that of the individual parental bNAbs or the Bi-NAb (Figure 17B, ****p <0.0001, Wilcoxon matched-pairs signed rank test), with an exceptional IC_{50} geometric mean of 0.069 μg/mL, which is at least four-fold lower than that of the VRC01, 10E8, and Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$ antibodies (Figure 17-Figure 17D, Figure 18). The IC_{80} titers of the Tri-NAb corroborated its surpassing neutralization potency over both the parental bNAbs and the Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$ (Figure 16E, ****p <0.0001, Wilcoxon matched-pairs signed rank test), neutralizing 98% of all strains with an IC_{80} geometric mean of 0.298 μg/mL (Figure 16E, Figure 19).

Likely, the gain in neutralization breadth exhibited by the Tri-NAb is attributed to the addition of 10E8 moiety to the Bi-NAb $_{dVRC01\text{-}5x\text{-}PGT121}$ agent (Figure 17C). In this regard, several PGT121/VRC01 dual resistant strains that are sensitive to 10E8 are also sensitive to Tri-NAb neutralization (Figure 17C). The only virus strain that showed resistance to the Tri-NAb, 6471.V1.C16, is also highly resistant to both PGT121 and VRC01, and is only moderately sensitive to 10E8 (IC_{50} = 4.98 μg/mL) (Figure 18). A bivalent 10E8 moiety such as that found in an IgG molecule may be required for neutralizing viruses moderately sensitive to 10E8, which is absent in the trispecific antibody (Figure 18).

To evaluate the impact that each antibody contributed to the Tri-NAb neutralization potency, viruses were grouped by their sensitivities to the parental antibodies and assessed the change in potency of neutralization of the multispecific NAbs from that of the parental antibodies (Figure 17E). Bi-ScFv and Bi-NAb exhibited a 2.1-fold and 1.9-fold increase in neutralization activity compared to VRC01 when measuring activity against VRC01/PGT121 dual sensitive viruses (N=125) accounting for 60% of the 208 tested viruses. With viruses resistant to VRC01 or/and PGT121 (N=73, 40% of the tested viruses), the overall potency of the Bi-ScFv and Bi-NAb decreased compared to the parental bNAbs. When incorporating all viruses in the analysis, the potency of the bispecific antibodies was 1.4- and 1.3-fold greater compared to that of VRC01, respectively (Figure 17E, upper panel).

Of note, the Tri-NAb neutralized viruses sensitive to two antibody moieties (N=194 or 93% of tested viruses) with a greater potency than that of the parental antibodies (Figure 17E, lower panel). However, when the viruses were only sensitive to one of the Tri-NAb's moieties (dual resistant, N=14 or 6.7% of tested viruses), the potency was lower than that of the parental antibody (Figure 17E, lower panel). The Tri-NAb displayed an overall improved potency over VRC01 and 10E8 (4.5-fold and 6.2-fold greater, respectively) for all viruses of the panel (Figure 17E, lower panel). Therefore, the superior neutralization capacity of Tri-NAb over Bi-NAb and individual parental antibodies is profoundly associated with the Env binding avidity (targeting at least two epitopes) in the face of neutralization resistance.

Discussion

Despite the improved breadth and potency displayed by bNAbs recently isolated, bNAb combinations will likely be necessary to cope with the extensive HIV Env diversity present in circulating virus strains. Recently some Bi-NAbs have been developed that combine receptor- or coreceptor-targeting antibodies or domains such as the anti CD4 receptor Fab ibalizumab (iMab) [47, 48], the anti CCR5 coreceptor Fab PRO140 (P140) [49], or the eCD4Ig containing a fusion protein of human CD4 ectodomain and a CCR5-mimetic sulfopeptide [50]. Although these antibody molecules can exhibit broad and potent HIV neutralization, they are different from the Bi- and Tri-NAb molecules described here, in that they target host receptors or use host receptor derivative, whereas the Bi- and Tri-NAb target only viral Env elements. A number of in vitro studies demonstrated that anti-HIV Env bNAb combinations yield superior neutralization results [45, 46]. This is likely due to the cooperative effect when two bNAbs targeting independent epitopes on the Env trimer are combined. Therefore, combining three or four bNAbs with different epitope specificities may be of

significant therapeutic value [46, 51]. Empirical combinations of bNAbs can achieve enhanced potency and breadth over parental bNAbs alone [31, 34, 35] and it has been suggested that some may be capable of crosslinking the Env trimer [31, 34]. Specifically, one study utilized DNA-linkers as "molecular rulers" to construct molecules capable of intra-spike crosslinking with the ultimate goal of enhancing the potency of bNAbs [31] by gain of avidity.

In this study, structure-based antibody rational design was utilized to optimize the multi-epitope engagement of the HIV Env trimer with Bi-ScFvs antibodies. Both the dual engagement of Env epitopes and the enhanced neutralization capacity of the Bi-ScFvs was validated. Furthermore, the experiments described herein confirm that the Bi-NAb engages the CD4bs and the V3 base glycan epitopes through an inter-protomer linkage rather within one Env trimer following the structure-based design (Figure 10 and Figure 11, Table 2).

Neutralization analyses of the Bi-NAbs using a panel of viruses with differential resistance profiles illustrated that those possessing both the VRC01 VL at the N-terminus of the bispecific antibody as well as the VRC01 VL N-terminal mutations (ΔE1I2/V3S) had improved neutralization breadth (Figure 15A, Figure 14A and Figure 14B). Conversely, it was observed that Bi-NAbs with PGT121 at the N-terminus had improved potency but reduced neutralization breadth, suggesting that this molecular configuration may present unfavorable steric constraints. Furthermore, it was observed that molecules with shorter linker lengths of 4X or 3X displayed lower neutralization breadth and potency than those with longer (5x) linkers (Figure 15A-Figure 15C), suggesting that longer linkers may enable the bispecific antibodies to orchestrate individual functional moieties for simultaneously engaging both cognate epitopes.

The Bi-NAb and MPER-specific bNAb 10E8 were combined to form a Tri-NAb with profoundly improved neutralization breadth (Figure 17A-Figure 17C, Figure 16E, Figure 18 and Figure 19). This gain of breadth most likely attributed to the addition of the 10E8 moiety, as PGT121/VRC01 dual resistant viral strains that are sensitive to 10E8 were sensitive to Tri-NAb neutralization (Figure 17C). Consistently, the potency of the Tri-NAb (IC_{50} geomean of 0.069 μg/ml, IC_{80} geometric mean of 0.298 μg/mL) increased in comparison with the Bi-NAb (Figure 17A) and the parental antibodies (Figure 17A-Figure 17C, Figure 17E, Figure 18). Figure 20 is a schematic that shows two arrangements for 4 specific antibody binding sites to be accomodated in one antibody. As shown in Figure 20, the tetra-NAb1 has the configuration of 10E8-5X-35O22/dVRC01-5X-PGT121 and tetra-NAb2 has the configuration of 35O22-5X-10E8/dVRC01-5X-PGT121.

Both Bi- and Tri-NAb outperformed their parental mAbs in neutralization potency when tested with viral isolates that were sensitive to all of the individual bNAbs (Figure 17E); most likely due to the cooperative effect resulted from simultaneous engagement of multiple epitopes within the same Env trimer. Consistently, the Tri-NAb neutralizes these sensitive viruses more potently than the Bi-NAb (Figure 17E). The cooperative effect remains for the Tri-NAb when tested with viruses resistant to only one antibody (single-resistance) (Figure 17E), while this cooperative effect is lost for the Bi-NAb (Figure 17E). When dual neutralization-resistant viruses were tested, the Tri-NAb showed steady neutralization with slightly lower potency compared to the parental mAbs (Figure 17E), while the Bi-NAb only occasionally neutralized the viruses with moderate potency (Figure 17E). The profound improvement of neutralization of the Tri-NAb over Bi-NAb against both sensitive and single-resistant viruses, which represent the majority of the global circulating viruses strongly, highlights the premise of engineering multi-epitope (more than 2 epitopes) targeting antibodies. It can be anticipated that the incorporation of additional bNAb functional moieties to the Tri-NAb will improve its neutralization capacity even further.

The experiments described herein demonstrate the successful combination of three HIV-1 broadly neutralizing antibody specificities into one single trispecific antibody by structure-based rational design. The triple-specificity antibody demonstrated exceptional HIV viral coverage (99.5%) in neutralization assays of a 208 virus panel with remarkable potency (IC_{50} titer geometric mean below 0.1 μg/ml). With these unprecedented neutralization capacities, the Tri-NAb represents an attractive candidate of the next generation of HIV-1 preventive and therapeutic antibody-based agents. Recently, novel potent antibodies of different specificities have been isolated and modified, which exhibit outstanding neutralization capacities [52, 53, 54]. Furthermore, various Fc mutations, especially the "LS" mutations (M428L/N434S) conferring enhanced affinity for neonatal Fc receptor that improves the in vivo half-life and biodistribution of antibody molecules could be incorporated into the multispecific antibody context to potentially improve the utility of passive immunization [44, 55, 56]. Coordinating combinations of additional antibody entities into multi-NAb designs will become more feasible, and may further the development of strategies for HIV-1 infection prevention, remission, and possible eradication in the future.

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SEQUENCE TABLE

SEQ ID NO	Description	Sequence
1	VRC01 (VL-3x-VH)-(5X)-PGT121 (VL-3x-VH) ScFv amino acid sequence	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASG YEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDV YSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSG GGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRA VQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGS QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGHHHHHH
2	VRC01 variable light (VL) chain amino acid sequence	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIK
3	VL-CDR1	RTSQYGSLA
4	VL-CDR2	SGSTRAA
5	VL-CDR3	QQYEF
6	VRC01 variable heavy (VH) chain amino acid sequence	QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWM GWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFC TRGKNCDYNWDFEHWGRGTPVIVSS
7	VH-CDR1	DCTLN
8	VH-CDR2	WLKPRGGAVNYARPLQG
9	VH-CDR3	GKNCDYNWDFEH
10	PGT121 variable light (VL) chain amino acid sequence	SDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQDRPS GIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTKWVF GGGTTLTVL
11	VL-CDR1	GEKSLGSRAVQ
12	VL-CDR2	NNQDRPS
13	VL-CDR3	HIWDSRVPTKWV
14	PGT121 variable heavy (VH) chain amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSS
15	VH-CDR1	DSYWS
16	VH-CDR2	YVHKSGDTNYSPSLKS

17	VH-CDR3	TLHGRRIYGIVAFNEWFTYFYMDV
18	dVRC01 (VL(Δ1,2-V3S)-3x-VH)-(5X)-PGT121 (VL-3x-VH) ScFv amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASGYE FIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYS DTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSGGG GSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRAVQ WYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVE AGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGSQM QLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWIGY VHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCART LHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGHHHHHH
19	dVRC01 variable light (VL) chain amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIK
20	PGT121 (VH-3x-VL)-(5X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
21	PGT121 (VH-3x-VL)-(4X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
22	PGT121 (VH-3x-VL)-(3X)-VRC01 (VH-3x-VL) ScFv amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRIS CRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVT MTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPV IVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCRTSQYG SLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLE SGDFGVYYCQQYEFFGQGTKVQVDIKGSGHHHHHH
23	VRC01 (VL-3x-VH)-(5X)-PGT121 (VL-3x-VH) IgG1 amino acid	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASG YEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDV YSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSG

	sequence	GGGSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRA VQWYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGS QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
24	dVRC01 (VL(Δ1,2-V3S)-3x-VH)-(5X)-PGT121 (VL-3x-VH) IgG1 amino acid sequence	SLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGS TRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKV QVDIKGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRISCRASGYE FIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYS DTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSSGGG GSGGGGSGGGGSGGGGSGGGGSSDISVAPGETARISCGEKSLGSRAVQ WYQHRAGQAPSLIIYNNQDRPSGIPERFSGSPDSPFGTTATLTITSVE AGDEADYYCHIWDSRVPTKWVFGGGTTLTVLGGGGSGGGGSGGGGSQM QLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWIGY VHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCART LHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGSGPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
25	PGT121 (VH-3x-VL)-(5X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQM KKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVN YARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWD FEHWGRGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETA IISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGP DYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
26	PGT121 (VH-3x-VL)-(4X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGE SMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPL QGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWG RGTPVIVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCR TSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLT ISNLESGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV

		FSCSVMHEALHNHYTQKSLSLSPGK
27	PGT121 (VH-3x-VL)-(3X)-VRC01 (VH-3x-VL) IgG1 amino acid sequence	QMQLQESGPGLVKPSETLSLTCSVSGASISDSYWSWIRRSPGKGLEWI GYVHKSGDTNYSPSLKSRVNLSLDTSKNQVSLSLVAATAADSGKYYCA RTLHGRRIYGIVAFNEWFTYFYMDVWGNGTQVTVSSGGGGSGGGGSGG GGSSDISVAPGETARISCGEKSLGSRAVQWYQHRAGQAPSLIIYNNQD RPSGIPERFSGSPDSPFGTTATLTITSVEAGDEADYYCHIWDSRVPTK WVFGGGTTLTVLGGGGSGGGGSGGGGSQVQLVQSGGQMKKPGESMRIS CRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVT MTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPV IVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGETAIISCRTSQYG SLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLE SGDFGVYYCQQYEFFGQGTKVQVDIKGSGPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
28	IgG1 Fc (hinge-CH2-CH3)amino acid sequence	PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
29	signal sequence	MGWSCIILFLVATATGVHS
30	35022(VH-3x-VL)-(5X)-10E8(VH-3x-VL) ScFv amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPG EGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLYFCARTGK YYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGGGSSYELT QETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNR PSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFG GGTKLTVLGSGHHHHHH
31	35022 variable light (VL) chain amino acid sequence	QSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTL IIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHN SGCVFGTGTKVSVL
32	VL-CDR1	TGPNSVCCSHKSIS
33	VL-CDR2	EDNERAP
34	VL-CDR3	CSYTHNSGCV
35	35022 variable heavy (VH) chain amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSS
36	VH-CDR1	FYHIN
37	VH-CDR2	WISPYSGDKNLAPAFQD

38	VH-CDR3	GLLRDGSSTWLPYL
39	10E8 variable light (VL) chain amino acid sequence	SYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFY GKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSR LSVFGGGTKLTVL
40	VL-CDR1	RGDSLRSHYAS
41	VL-CDR2	GKNNRPS
42	VL-CDR3	SSRDKSGSRLSV
43	10E8 variable heavy (VH) chain amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSS
44	VH-CDR1	NAWMT
45	VH-CDR2	RITGPGEGWSVDYAAPVEG
46	VH-CDR3	TGKYYDFWSGYPPGEEYFQD
47	35022(VH-3x-VL)-(7X)-10E8(VH-3x-VL) ScFv amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGL EWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDS GLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGS GGGGSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAP ILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRD KSGSRLSVFGGGTKLTVLGSGHHHHHH
48	10E8(VH-3x-VL)-(5X)-35O22(VH-3x-VL) ScFv amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQGQLVQSG AELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSG DKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFC AKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGGGSQSVLTQ SASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDN ERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHNSGCVFG TGTKVSVLGSGHHHHHH
49	10E8(VH-3x-VL)-(7X)-35O22(VH-3x-VL) ScFv amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSQGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPE WMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNL KFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSG GGGSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGR

		APTLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCS YTHNSGCVFGTGTKVSVLGSGHHHHHH
50	35022(VH-3x-VL)-(5X)-10E8(VH-3x-VL) IgG1 amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES GGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPG EGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLYFCARTGK YYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGGGSSYELT QETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNR PSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFG GGTKLTVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
51	35022(VH-3x-VL)-(7X)-10E8(VH-3x-VL) IgG1 amino acid sequence	QGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWM GWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKF DDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGG GSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAP TLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYT HNSGCVFGTGTKVSVLGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSEVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGL EWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDS GLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGS GGGGSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAP ILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRD KSGSRLSVFGGGTKLTVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
52	10E8(VH-3x-VL)-(5X)-35022(VH-3x-VL) IgG1 amino acid sequence	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSQGQLVQSG AELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPEWMGWISPYSG DKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNLKFDDTGTYFC AKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSGGGGSQSVLTQ SASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGRAPTLIIYEDN ERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCSYTHNSGCVFG TGTKVSVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK
53	10E8(VH-3x-VL)-(7X)-35022(VH-3x-VL) IgG1 amino acid	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLY FCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSSGGGGSGGGGSGGG GSSYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILL

	sequence	FYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSG SRLSVFGGGTKLTVLGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSQGQLVQSGAELKKPGASVKISCKTSGYRFNFYHINWIRQTAGRGPE WMGWISPYSGDKNLAPAFQDRVIMTTDTEVPVTSFTSTGAAYMEIRNL KFDDTGTYFCAKGLLRDGSSTWLPYLWGQGTLLTVSSGGGGSGGGGSG GGGSQSVLTQSASVSGSLGQSVTISCTGPNSVCCSHKSISWYQWPPGR APTLIIYEDNERAPGISPRFSGYKSYWSAYLTISDLRPEDETTYYCCS YTHNSGCVFGTGTKVSVLGSGPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK
54	10-1074 variable light (VL) chain amino acid sequence	SYVRPLSVALGETARISCGRQALGSRAVQWYQHRPGQAPILLIYNNQD RPSGIPERFSGTPDINFGTRATLTISGVEAGDEADYYCHMWDSRSGFS WSFGGATRLTVL
55	VL-CDR1	GRQALGSRAVQ
56	VL-CDR2	NNQDRPS
57	VL-CDR3	HMWDSRSGFSWS
58	10-1074 variable heavy (VH) chain amino acid sequence	QVQLQESGPGLVKPSETLSVTCSVSGDSMNNYYWTWIRQSPGKGLEWI GYISDRESATYNPSLNSRVVISRDTSKNQLSLKLNSVTPADTAVYYCA TARRGQRIYGVVSFGEFFYYYSMDVWGKGTTVTVSS
59	VH-CDR1	NYYWT
60	VH-CDR2	YISDRESATYNPSLNS
61	VH-CDR3	ARRGQRIYGVVSFGEFFYYYSMDV
62	BG18 variable light (VL) chain amino acid sequence	WASSELTQPPSVSVSPGQTARITCSGAPLTSRFTYWYRQKPGQAPVLI ISRSSQRSSGWSGRFSASWSGTTVTLTIRGVQADDEADYYCQSSDTSD SYKMFGGGTKLTVL
63	VL-CDR1	SGAPLTSRFTY
64	VL-CDR2	SRSSQRS
65	VL-CDR3	QSSDTSDSYKM
66	BG18 variable heavy (VH) chain amino acid sequence	EQVQLRESGPGLVKPSETLSLSCTVSQDSRPSDHSWTWVRQSPGKALE WIGDIHYNGATTYNPSLRSRVRIELDQSIPRFSLKMTSMTAADTGMYY CARNAIRIYGVVALGEWFHYGMDVWGQGTAVTVSS
67	VH-CDR1	SDHSWT
68	VH-CDR2	DIHYNGATTYNPSLRS
69	VH-CDR3	NAIRIYGVVALGEWFHYGMDV

70	PGT135 variable light (VL) chain amino acid sequence	EIVMTQSPDTLSVSPGETVTLSCRASQNINKNLAWYQYKPGQSPRLVI FETYSKIAAFPARFVASGSGTEFTLTINNMQSEDVAVYYCQQYEEWPR TFGQGTKVDIK
71	VL-CDR1	RASQNINKNLA
72	VL-CDR2	ETYSKIA
73	VL-CDR3	QQYEEWPRT
74	PGT135 variable heavy (VH) chain amino acid sequence	QLQMQESGPGLVKPSETLSLSCTVSGDSIRGGEWGDKDYHWGWVRHSA GKGLEWIGSIHWRGTTHYKESLRRRVSMSIDTSRNWFSLRLASVTAAD TAVYFCARHRHHDVFMVPIAGWFDVWGPGVQVTVSS
75	VH-CDR1	GGEWGDKDYHWG
76	VH-CDR2	SIHWRGTTHYKESLRR
77	VH-CDR3	HRHHDVFMVPIAGWFDV
78	PGT122 variable light (VL) chain amino acid sequence	TFVSVAPGQTARITCGEESLGSRSVIWYQQRPGQAPSLIIYNNNDRPS GIPDRFSGSPGSTFGTTATLTITSVEAGDEADYYCHIWDSRRPTNWVF GEGTTLIVL
79	VL-CDR1	GEESLGSRSVI
80	VL-CDR2	NNNDRPS
81	VL-CDR3	HIWDSRRPTNWV
82	PGT122 variable heavy (VH) chain amino acid sequence	QVHLQESGPGLVKPSETLSLTCNVSGTLVRDNYWSWIRQPLGKQPEWI GYVHDSGDTNYNPSLKSRVHLSLDKSKNLVSLRLTGVTAADSAIYYCA TTKHGRRIYGVVAFKEWFTYFYMDVWGKGTSVTVSS
83	VH-CDR1	DNYWS
84	VH-CDR2	YVHDSGDTNYNPSLKS
85	VH-CDR3	TKHGRRIYGVVAFKEWFTYFYMDV
86	PGT128 variable light (VL) chain amino acid sequence	TFVSVAPGQTARITCGEESLGSRSVIWYQQRPGQAPSLIIYNNNDRPS GIPDRFSGSPGSTFGTTATLTITSVEAGDEADYYCHIWDSRRPTNWVF GEGTTLIVL
87	VL-CDR1	GEESLGSRSVI
88	VL-CDR2	NNNDRPS
89	VL-CDR3	HIWDSRRPTNWV
90	PGT128 variable heavy (VH) chain amino acid	EPQLQESGPTLVEASETLSLTCAVSGDSTAACNSFWGWVRQPPGKGLE WVGSLSHCASYWNRGWTYHNPSLKSRLTLALDTPKNLVFLKLNSVTAA DTATYYCARFGGEVLRYTDWPKPAWVDLWGRGTLVTVSS

	sequence	
91	VH-CDR1	ACNSFWG
92	VH-CDR2	SLSHCASYWNRGWTYHNPSLKS
93	VH-CDR3	FGGEVLRYTDWPKPAWVDL
94	10E8v4 variable light (VL) chain amino acid sequence	SELTQDPAVSVALKQTVTITCRGDSLRSHYASWYQKKPGQAPVLLFYG KNNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSRL SVFGGGTKLTVL
95	VL-CDR1	RGDSLRSHYAS
96	VL-CDR2	GKNNRPS
97	VL-CDR3	SSRDKSGSRLSV
98	10E8v4 variable heavy (VH) chain amino acid sequence	EVRLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAESVKGRFTISRDNTKNTLYLEMNNVRTEDTGYY FCARTGKYYDFWSGYPPGEEYFQDWGQGTLVIVSS
99	VH-CDR1	NAWMT
100	VH-CDR2	RITGPGEGWSVDYAESVKG
101	VH-CDR3	TGKYYDFWSGYPPGEEYFQD
94	10E8v4 S100cF variable light (VL) chain amino acid sequence	SELTQDPAVSVALKQTVTITCRGDSLRSHYASWYQKKPGQAPVLLFYG KNNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSSRDKSGSRL SVFGGGTKLTVL
95	VL-CDR1	RGDSLRSHYAS
96	VL-CDR2	GKNNRPS
97	VL-CDR3	SSRDKSGSRLSV
106	10E8v4 S100cF variable heavy (VH) chain amino acid sequence	EVRLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWV GRITGPGEGWSVDYAESVKGRFTISRDNTKNTLYLEMNNVRTEDTGYY FCARTGKYYDFWFGYPPGEEYFQDWGQGTLVIVSS
107	VH-CDR1	NAWMT
108	VH-CDR2	RITGPGEGWSVDYAESVKG
109	VH-CDR3	TGKYYDFWFGYPPGEEYFQD
110	DH511.2_k3 variable light (VL) chain amino acid sequence	DIQMTQSPSFLYGSVGDRVTITCRASQNIKDYLNWYQQRPGRAPRLLI YAASNLQSGVPSRFSGSGYGTDFTLIISSLQPEDFATYFCQESYSSTP THIFGLGTKLEK
111	VL-CDR1	RASQNIKDYLN

112	VL-CDR2	AASNLQS
113	VL-CDR3	QESYSSTPTHI
114	DH511.2_k3 variable heavy (VH) chain amino acid sequence	QVQLVQSGGGLVKPGGSLTLSCSASGFFFDNSWMGWVRQAPGKGLEWV GRIRRLKDGATGEYGAAVKDRFTISRDDSRNMLYLHMRTLKTEDSGTY YCTMDEGTPVTRFLEWGYFYYYMAVWGRGTTVIVSS
115	VH-CDR1	NSWMG
116	VH-CDR2	RIRRLKDGATGEYGAAVKD
117	VH-CDR3	DEGTPVTRFLEWGYFYYYMAV
118	4E10 variable light (VL) chain amino acid sequence	EIVLTQSPGTQSLSPGERATLSCRASQSVGNNKLAWYQQRPGQAPRLL IYGASSRPSGVADRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGQSL STFGQGTKVEVK
119	VL-CDR1	RASQSVGNNKLA
120	VL-CDR2	GASSRPS
121	VL-CDR3	QQYGQSLST
122	4E10 variable heavy (VH) chain amino acid sequence	VQLVQSGAEVKRPGSSVTVSCKASGGSFSTYALSWVRQAPGRGLEWMG GVIPLLTITNYAPRFQGRITITADRSTSTAYLELNSLRPEDTAVYYCA REGTTGWGWLGKPIGAFAHWGQGTLVTVSS
123	VH-CDR1	TYALS
124	VH-CDR2	GVIPLLTITNYAPRFQG
125	VH-CDR3	EGTTGWGWLGKPIGAFAH
126	2F5 variable light (VL) chain amino acid sequence	ALQLTQSPSSLSASVGDRITITCRASQGVTSALAWYRQKPGSPPQLLI YDASSLESGVPSRFSGSGSGTEFTLTISTLRPEDFATYYCQQLHFYPH TFGGGTRVDVR
127	VL-CDR1	RASQGVTSALA
128	VL-CDR2	DASSLES
129	VL-CDR3	QQLHFYPHT
130	2F5 variable heavy (VH) chain amino acid sequence	RITLKESGPPLVKPTQTLTLTCSFSGFSLSDFGVGVGWIRQPPGKALE WLAIIYSDDDKRYSPSLNTRLTITKDTSKNQVVLVMTRVSPVDTATYF CAHRRGPTTLFGVPIARGPVNAMDVWGQGITVTISS
131	VH-CDR1	DFGVGVG
132	VH-CDR2	IIYSDDDKRYSPSLNT
133	VH-CDR3	RRGPTTLFGVPIARGPVNAMDV

134	N123-VRC34.01 variable light (VL) chain amino acid sequence	DIQLTQSPSFLSASVGDKVTITCRASQGVRNELAWYQQKPGKAPNLLI YYASTLQSGVPSRFSATGSGTHFTLTVSSLQPEDFATYFCQHMSSYPL TFGGGTKVEIK
135	VL-CDR1	RASQGVRNELA
136	VL-CDR2	YASTLQS
137	VL-CDR3	QHMSSYPLT
138	N123-VRC34.01 variable heavy (VH) chain amino acid sequence	QEVLVQSGAEVKKPGASVKVSCRAFGYTFTGNALHWVRQAPGQGLEWL GWINPHSGDTTTSQKFQGRVYMTRDKSINTAFLDVTRLTSDDTGIYYC ARDKYYGNEAVGMDVWGQGTSVTVSS
139	VH-CDR1	GNALH
140	VH-CDR2	WINPHSGDTTTSQKFQG
141	VH-CDR3	DKYYGNEAVGMDV
142	3BC315 variable light (VL) chain amino acid sequence	QSALTQPASVSASPGQSITISCSGTRSDVGGYDFVSWYQQHPGKVPKL IIYEVTKRPSGIPQRFSGSKSGNTASLTISGLQADDEADYYCCSYANY DKLILGGGTKLTVL
143	VL-CDR1	SGTRSDVGGYDFVS
144	VL-CDR2	EVTKRPS
145	VL-CDR3	CSYANYDKLI
146	3BC315 variable heavy (VH) chain amino acid sequence	QVQLVQSGAEMKDPGASVKVSCRASGYKFTDYYMHWVRQAPGQGLEWV GWVNTNGGFTKYGAKFQGRVTVTRDTSTNTVFLELSRLTFGDTAMYFC ARPMRPVSHGIDYSGLFVFQFWGRGTMVTVSS
147	VH-CDR1	DYYMH
148	VH-CDR2	WVNTNGGFTKYGAKFQG
149	VH-CDR3	PMRPVSHGIDYSGLFVFQF
150	PGT151 variable light (VL) chain amino acid sequence	DIVMTQTPLSLSVTPGQPASISCKSSESLRQSNGKTSLYWYRQKPGQS PQLLVFEVSNRFSGVSDRFVGSGSGTDFTLRISRVEAEDVGFYYCMQS KDFPLTFGGGTKVDLK
151	VL-CDR1	KSSESLRQSNGKTSLY
152	VL-CDR2	EVSNRFS
153	VL-CDR3	MQSKDFPLT
154	PGT151 variable heavy (VH) chain amino acid	RVQLVESGGGVVQPGKSVRLSCVVSDFPFSKYPMYWVRQAPGKGLEWV AAISGDAWHVVYSNSVQGRFLVSRDNVKNTLYLEMNSLKIEDTAVYRC ARMFQESGPPRLDRWSGRNYYYYSGMDVWGQGTTVTVSS

	sequence	
155	VH-CDR1	KYPMY
156	VH-CDR2	AISGDAWHVVYSNSVQG
157	VH-CDR3	MFQESGPPRLDRWSGRNYYYYSGMDV
158	VRC03 variable light (VL) chain amino acid sequence	EIVLTQSPGILSLSPGETATLFCKASQGGNAMTWYQKRRGQVPRLLIY DTSRRASGVPDRFVGSGSGTDFFLTINKLDREDFAVYYCQQFEFFGLG SELEVHR
159	VL-CDR1	KASQGGNAMT
160	VL-CDR2	DTSRRAS
161	VL-CDR3	QQFEF
162	VRC03 variable heavy (VH) chain amino acid sequence	QVQLVQSGAVIKTPGSSVKISCRASGYNFRDYSIHWVRLIPDKGFEWI GWIKPLWGAVSYARQLQGRVSMTRQLSQDPDDPDWGVAYMEFSGLTPA DTAEYFCVRRGSCDYCGDFPWQYWGQGTVVVVSS
163	VH-CDR1	DYSIH
164	VH-CDR2	WIKPLWGAVSYARQLQG
165	VH-CDR3	VRRGSCDYCGDFPWQY
166	VRC06 variable light (VL) chain amino acid sequence	EIVLTQSPATLSLSPGERATLSCRASQGGNSLNWYQKRRGQTPRLLIY DTSRRASDIPEKFVGSGSGTDFSLTITKVGPEDFAVYYCQQFEFFGLG TTLEIN
167	VL-CDR1	RASQGGNSLN
168	VL-CDR2	DTSRRAS
169	VL-CDR3	QQFEF
170	VRC06 variable heavy (VH) chain amino acid sequence	EVQLVESGPVMRKPGSSMKISCATSGYNFRDFSIHWVRFNRRYG FEWIGWIKPMWGAVNYARQLQGRVSMSRLFSQDLYYPDRGTAYLEFSG LTSADTADYF CVRRGSSCPHCGDFHFEHWGQGTAVVVSA
171	VH-CDR1	DFSIH
172	VH-CDR2	WIKPMWGAVNYARQLQG
173	VH-CDR3	RGSSCPHCGDFHFEH
2	VRC07 variable light (VL) chain amino acid sequence	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYS GSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGT KVQVDIK
3	VL-CDR1	RTSQYGSLA

4	VL-CDR2	SGSTRAA
5	VL-CDR3	QQYEF
178	VRC07 variable heavy (VH) chain amino acid sequence	QVQLVQSGAQVKKPGSSVKVSCKASGYEFINCPINWVRQAPRQRPEWM GWMKPRGGAVSYARQLQGRVTMTRDMYTSTAYLELRSLTSEDTAVYYC ARGKYCTARDYYNWDFEHWGPGTPITVSS
179	VH-CDR1	NCPIN
180	VH-CDR2	WMKPRGGAVSYARQLQG
181	VH-CDR3	GKYCTARDYYNWDFEH
182	3BNC117 variable light (VL) chain amino acid sequence	DIQMTQSPSSLSASVGDTVTITCQANGYLNWYQQRRGKAPKLLIYDGS KLERGVPSRFSGRRWGQEYNLTINNLQPEDIATYFCQVYEFVVPGTRL DLK
183	VL-CDR1	QANGYLN
184	VL-CDR2	DGSKLER
185	VL-CDR3	QVYEF
186	3BNC117 variable heavy (VH) chain amino acid sequence	QVQLLQSGAAVTKPGASVRVSCEASGYNIRDYFIHWWRQAPGQGLQWV GWINPKTGQPNNPRQFQGRVSLTRHASWDFDTFSFYMDLKALRSDDTA VYFCARQRSDYWDFDVWGSGTQVTVSS
187	VH-CDR1	DYFIH
188	VH-CDR2	WINPKTGQPNNPRQFQG
189	VH-CDR3	QRSDYWDFDV
190	N6 variable light (VL) chain amino acid sequence	YIHVTQSPSSLSVSIGDRVTINCQTSQGVGSDLHWYQHKPGRAPKLLI HHTSSVEDGVPSRFSGSGFHTSFNLTISDLQADDIATYYCQVLQFFGR GSRLHIK
191	VL-CDR1	QTSQGVGSDLH
192	VL-CDR2	HTSSVED
193	VL-CDR3	QVLQF
194	N6 variable heavy (VH) chain amino acid sequence	RAHLVQSGTAMKKPGASVRVSCQTSGYTFTAHILFWFRQAPGRGLEWV GWIKPQYGAVNFGGGFRDRVTLTRDVYREIAYMDIRGLKPDDTAVYYC ARDRSYGDSSWALDAWGQGTTVVVSA
195	VH-CDR1	AHILF
196	VH-CDR2	WIKPQYGAVNFGGGFRD
197	VH-CDR3	DRSYGDSSWALDA

198	IOMA variable light (VL) chain amino acid sequence	QSALTQPASVSGSPGQSITISCAGSSRDVGGFDLVSWYQQHPGKAPKL IIYEVNKRPSGISSRFSASKSGNTASLTISGLQEEDEAHYYCYSYADG VAFGGGTKLTVL
199	VL-CDR1	AGSSRDVGGFDLVS
200	VL-CDR2	EVNKRPS
201	VL-CDR3	YSYADGVA
202	IOMA variable heavy (VH) chain amino acid sequence	EVQLVESGAQVKKPGASVTVSCTASGYKFTGYHMHWVRQAPGRGLEWM GWINPFRGAVKYPQNFRGRVSMTRDTSMEIFYMELSRLTSDDTAVYYC AREMFDSSADWSPWRGMVAWGQGTLVTVSS
203	VH-CDR1	GYHMH
204	VH-CDR2	WINPFRGAVKYPQNFRG
205	VH-CDR3	EMFDSSADWSPWRGMVA

CLAIMS

What is claimed is:

1. A multispecific antibody, or an antigen-binding fragment thereof, comprising:

a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and

b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof,

wherein the first light chain and the second light chain bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and

wherein the VH from the first light chain and the VL from the second light chain are connected by one or more linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by one or more linkers.

2. The multispecific antibody, or antigen binding fragment thereof, of claim 1, wherein the VH from the first light chain and the VL from the second light chain are connected by a two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers.

3. The multispecific antibody, or antigen binding fragment thereof, of claim 1, wherein the linker is not a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly), the peptide Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 292), a single Ser, a single Va, the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser, Thr-Val-Ala-Ala-Pro, Gln-Pro-Lys-Ala-Ala, Gln-Arg-Ile-Glu-Gly, Ala-Ser-

Thr-Lys-Gly-Pro-Ser, Arg-Thr-Val-Ala-Ala-Pro-Ser, Gly-Gln-Pro-Lys-Ala-Ala-Pro, and His-Ile-Asp-Ser-Pro-Asn-Lys.

4. The multispecific antibody of claim 1, wherein the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the envelope protein.

5. The multispecific antibody of claim 1, wherein the VH from the first light chain and the VL from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by three tetra-glycine serine (G4S) protein linkers.

6. The multispecific antibody of claim 1, wherein the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers.

7. The multispecific antibody of claim 1, wherein the VH from the first light chain and the VL from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers.

8. The multispecific antibody of claim 1, further comprising a third antibody which specifically binds to a third epitope.

9. The multispecific antibody of claim 8, wherein the third epitope is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV.

10. The multispecific antibody of claim 9, wherein the third epitope is different from the first epitope and the second epitope.

11. The multispecific antibody of claim 1, wherein the first antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1.

12. The multispecific antibody of claim 1, wherein the first antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1.

13. The multispecific antibody of claim 11 or 12, wherein the antibody that binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 is selected from the group consisting of: VRC03, VRC06, VRC07, 3BNC117, IOMA, and N6.

14. The multispecific antibody of claim 11 or 12, wherein the antibody that binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 is selected from the group consisting of: PGT121, PGT122, PGT128, PGT135, 10-1074, and BG18.

15. The multispecific antibody of claim 1, wherein the first antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1.

16. The multispecific antibody of claim 1, wherein the first antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1 and the second antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1.

17. The multispecific antibody of claim 15 or 16, wherein the antibody that binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 is selected from the group consisting of: 35022, N123-VRC34.01, 3BC315, and PGT151.

18. The multispecific antibody of claim 15 or 16, wherein the antibody that binds to an epitope in the MPER of the of the envelope protein of HIV-1 is selected from the group consisting of: 10E8, 10E8v4, 10E8v4 S100cF, Dh511.2_k3, Z13, 4E10, and 2F5.

19. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and

CDR3) as set forth in SEQ ID NOs 3, 4, 5 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 7, 8, 9.

20 The multispecific antibody of claim 1, wherein the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 11, 12, 13 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 15, 16, 17.

21. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 32, 33, 34 and the variable domain of the first heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 36, 37, 38.

22. The multispecific antibody of claim 1, wherein the variable domain of the second light chain comprises a light chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 40, 41, 42 and the variable domain of the second heavy chain comprises a heavy chain variable domain comprising a CDR set (CDR1, CDR2, and CDR3) as set forth in SEQ ID NOs 44, 45, 46.

23. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

24. The multispecific antibody of claim 1, wherein the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 2, SEQ ID NO. 10, SEQ ID NO. 19, SEQ ID NO. 31 and SEQ ID NO. 39 and the variable domain of the second heavy chain comprises

an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 6, SEQ ID NO. 14, SEQ ID NO. 35 and SEQ ID NO. 43.

25. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14.

26. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 19, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14.

27. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 2, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6.

28. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 10, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 14, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 19, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 6.

29. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 43.

30. The multispecific antibody of claim 1, wherein the variable domain of the first light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 39, and the variable domain of the first heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 43, and the variable domain of the second light chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 31, and the variable domain of the second heavy chain comprises an amino acid sequence that is 95% identical to SEQ ID NO. 35.

31. The multispecific antibody of claim 1, comprising an amino acid sequence that is 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO. 1, SEQ ID NO. 18, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 30, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 50, SEQ ID NO. 51, SEQ ID NO. 52 and SEQ ID NO. 53.

32. A multispecific antibody comprising:

a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof;

b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof; and

c) a third light chain comprising a first light chain variable region (VL) and a third heavy chain comprising a first heavy chain variable region (VH), wherein the third light chain and the third heavy chain are derived from a third antibody or an antigen-binding fragment thereof.

wherein the first antibody, the second antibody and the third antibody bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and

wherein the VH from the first light chain and the VL from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers.

33. The multispecific antibody of any one of claims 1-32, further including a modification in the Fc region.

34. The multispecific antibody of any one of claims 1-32, wherein the first antibody, the second antibody or the third antibody is an ScFv.

35. The multispecific antibody of any one of claims 1-32, wherein the multispecific antibody has an IC_{50} less than 0.1 µg/ml.

36. The multispecific antibody of any one of claims 1-32, wherein the multispecific antibody has an IC_{50} less than 0.01 µg/ml.

37. The multispecific antibody of any one of claims 1-32, wherein the multispecific antibody has an IC_{80} less than 0.3 µg/ml.

38. The multispecific antibody of any one of claims 1-32, wherein the multispecific antibody has an IC80 less than 0.1 µg/ml.

39. A method of treating or preventing an HIV infection, comprising administering to a subject in need thereof an effective amount of the multispecific antibody of any one of claims 1-32.

40. A pharmaceutical composition comprising (i) the multispecific antibody of any one of claims 1-32 and (ii) a pharmaceutically acceptable carrier.

41. A nucleic acid encoding the multispecific antibody of any one of claims 1-32.

42. A vector comprising the nucleic acid of claim 41.

43. A host cell comprising the vector of claim 42.

44. A method for the preparation of a multispecific antibody, comprising the step of culturing the host cell of claim 43 under conditions that allow synthesis of said multispecific antibody.

45. The method of claim 44 further comprising the step of recovering the multispecific antibody from the host cell culture.

46. An immunoconjugate comprising the multispecific antibody of any one of claims 1-28 coupled to a cytotoxic agent.

47. A multispecific antibody, or an antigen-binding fragment thereof or salt thereof, comprising:

a) a first light chain comprising a first light chain variable region (VL) and/or a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain;

b) a second light chain comprising a second light chain variable region (VL) and/or a second heavy chain comprising a second heavy chain variable region (VH),

wherein the first light chain and/or first heavy chain binds an epitope on CD4s of HIV-1 and the second light and/or heavy chain binds an epitope of a V1, V2, and/or V3 glycan of HIV-1;

wherein the VH from the first light chain and the VH or VL from the second light chain are connected by three or more linkers or wherein the VL from the first light chain and the VH or VL from the second light chain are connected by three or more linkers.

48. The multispecific antibody, salt or antigen binding fragment of claim 47, wherein the VH from the first light chain and the VL from the second light chain are connected by a two or more tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by two or more tetra-glycine serine (G4S) protein linkers.

49. The multispecific antibody, salt or antigen binding fragment of claim 47, wherein the linker is not a single glycine (Gly) residue; a diglycine peptide (Gly-Gly); a tripeptide (Gly-Gly-Gly); a peptide with four glycine residues (Gly-Gly-Gly-Gly); a peptide with five glycine residues (Gly-Gly-Gly-Gly-Gly); a peptide with six glycine residues (Gly-Gly-Gly-Gly-Gly-Gly); a peptide with seven glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly); a peptide with eight glycine residues (Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly), the peptide Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser, the peptide Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 292), a single Ser, a single Va, the dipeptide Arg-Thr, Gln-Pro, Ser-Ser, Thr-Lys, and Ser-Leu; Thr-Lys-Gly-Pro-Ser, Thr-Val-Ala-Ala-Pro, Gln-Pro-Lys-Ala-Ala, Gln-Arg-Ile-Glu-Gly, Ala-Ser-Thr-Lys-Gly-Pro-Ser, Arg-Thr-Val-Ala-Ala-Pro-Ser, Gly-Gln-Pro-Lys-Ala-Ala-Pro, and His-Ile-Asp-Ser-Pro-Asn-Lys.

50. The multispecific antibody, salt or antigen binding fragment of claim 47, wherein the non-overlapping epitopes are located in the CD4-binding site, the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120/gp41 interface of the envelope protein.

51. The multispecific antibody, salt or antigen binding fragment of claim 47 further comprising a IgG-1 like domain covalently linked to the first and/or second light chain.

52. The multispecific antibody, salt or antigen binding fragment of claim 47, wherein the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers.

53. The multispecific antibody, salt or antigen binding fragment of claim 47, wherein the VH from the first light chain and the VL from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by seven tetra-glycine serine (G4S) protein linkers.

54. The multispecific antibody, salt or antigen binding fragment of claim 47 further comprising a third antibody light chain that binds to a third epitope.

55. The multispecific antibody, salt or antigen binding fragment of claim 54, wherein the third epitope is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3-glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV.

56. The multispecific antibody, salt or antigen binding fragment of claim 54, wherein the third epitope is different from the first epitope and the second epitope.

57. A multispecific antibody, or an antigen-binding fragment thereof or salt thereof, comprising a variable domain and a constant domain;

the variable domain comprising:

a) a first light chain comprising a first light chain variable region (VL) and/or a first heavy chain comprising a first heavy chain variable region (VH)

b) a second light chain comprising a second light chain variable region (VL) and/or a second heavy chain comprising a second heavy chain variable region (VH);

the constant domain comprising an IgG-like amino acid sequence;

wherein the first light chain and the second light chain bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1); and

wherein the first light chain and the second light chain are connected by from about 3 to about 5 or linkers.

58. The multispecific antibody, salt or antigen binding fragment of claim 57, wherein the first and second light chains bind an epiptope that is located in the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3- glycan region, the gp41 membrane proximal external region (MPER), or the gp120-gp41 interface of the envelope protein of HIV.

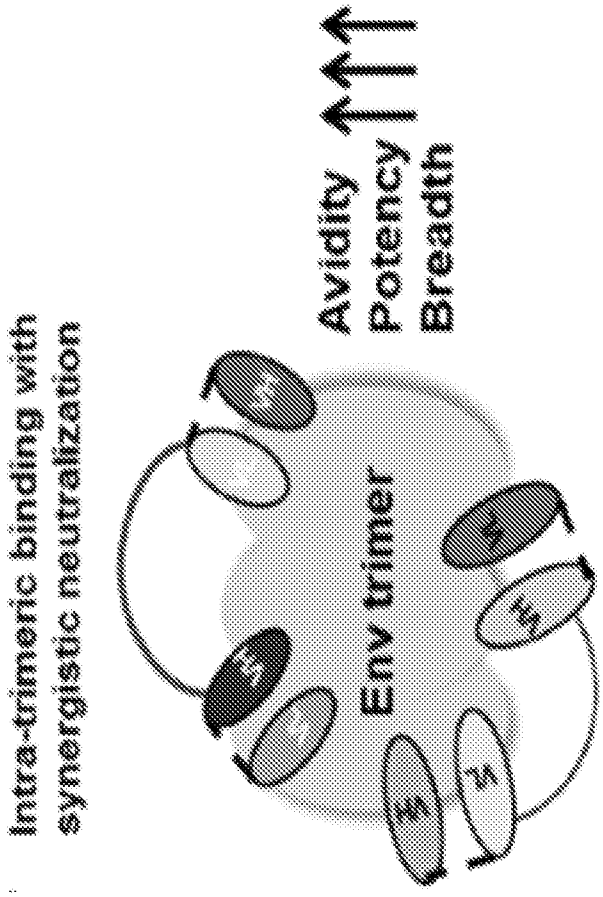

Intra-trimeric binding with
synergistic neutralization
Env trimer
Avidity
Potency
Breadth

FIG. 1

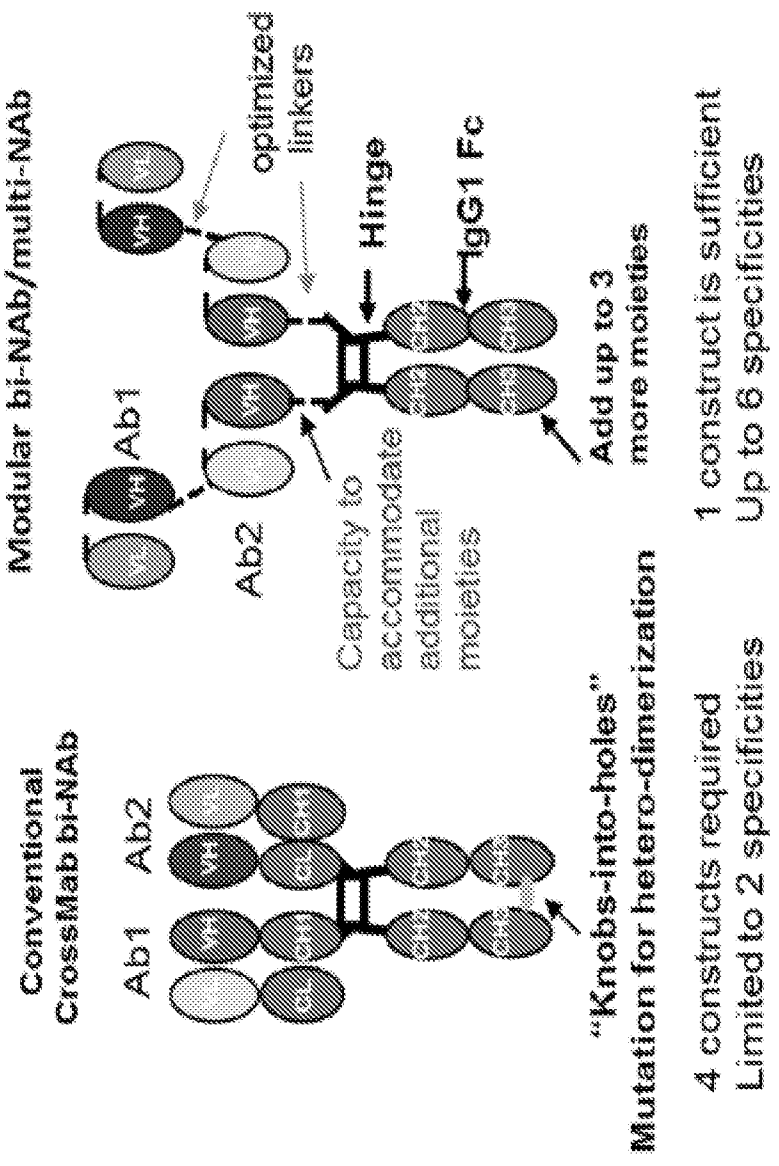

Conventional
CrossMab bi-NAb
Ab1
Ab2
"Knobs-into-holes"
Mutation for hetero-dimerization
4 constructs required
Limited to 2 specificities
Modular bi-NAb/multi-NAb
Ab1
Ab2
Capacity to accommodate additional moieties
optimized linkers
Hinge
IgG1 Fc
Add up to 3 more moieties
1 construct is sufficient
Up to 6 specificities

FIG. 2A

FIG. 2B

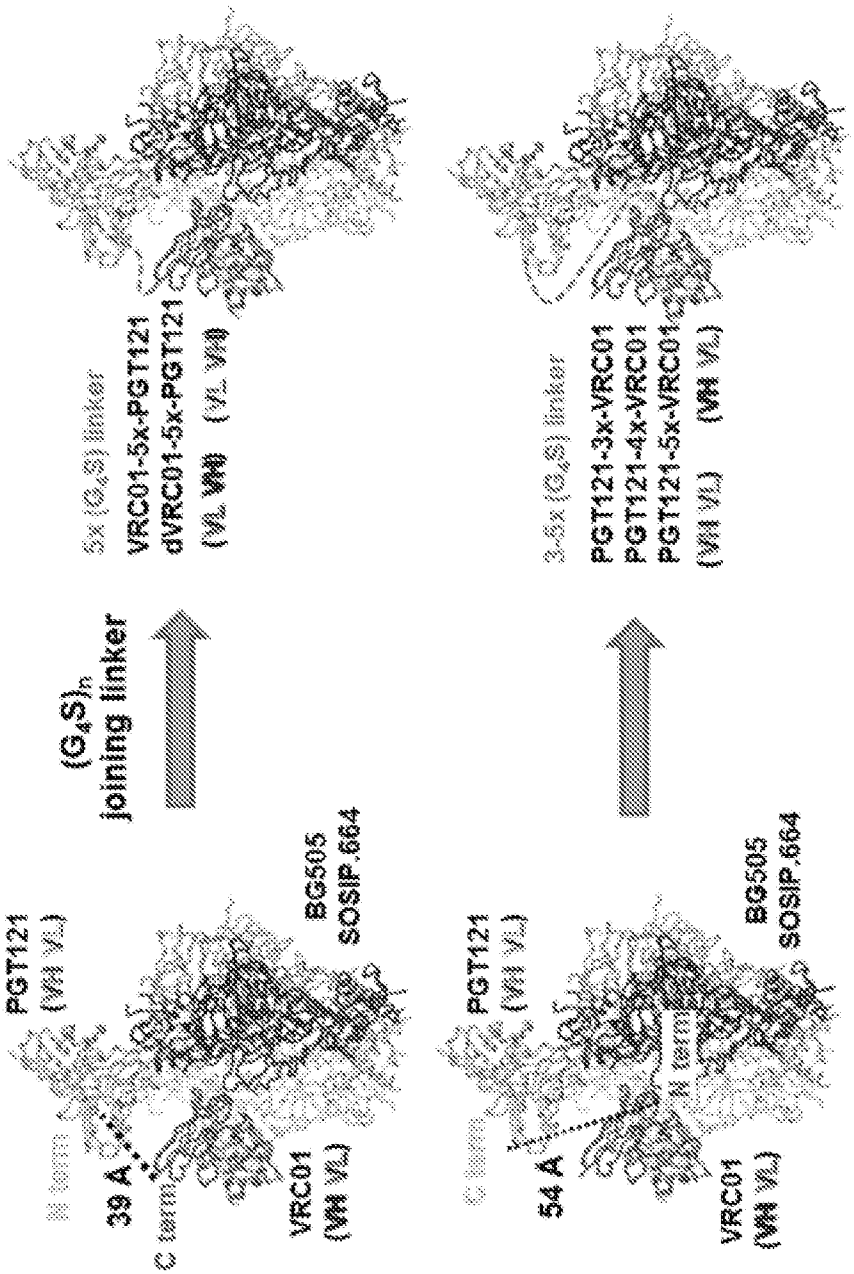

PGT121
(VH VL)
N term
39 Å
C term
VRC01
(VH VL)
BG505
SOSIP.664
(G4S)n
joining linker
5x (G4S) linker
VRC01-5x-PGT121
dVRC01-5x-PGT121
(VL VH) (VL VH)
PGT121
(VH VL)
C term
54 Å
N term
VRC01
(VH VL)
BG505
SOSIP.664
3-5x (G4S) linker
PGT121-3x-VRC01
PGT121-4x-VRC01
PGT121-5x-VRC01
(VH VL) (VH VL)

FIG. 3A

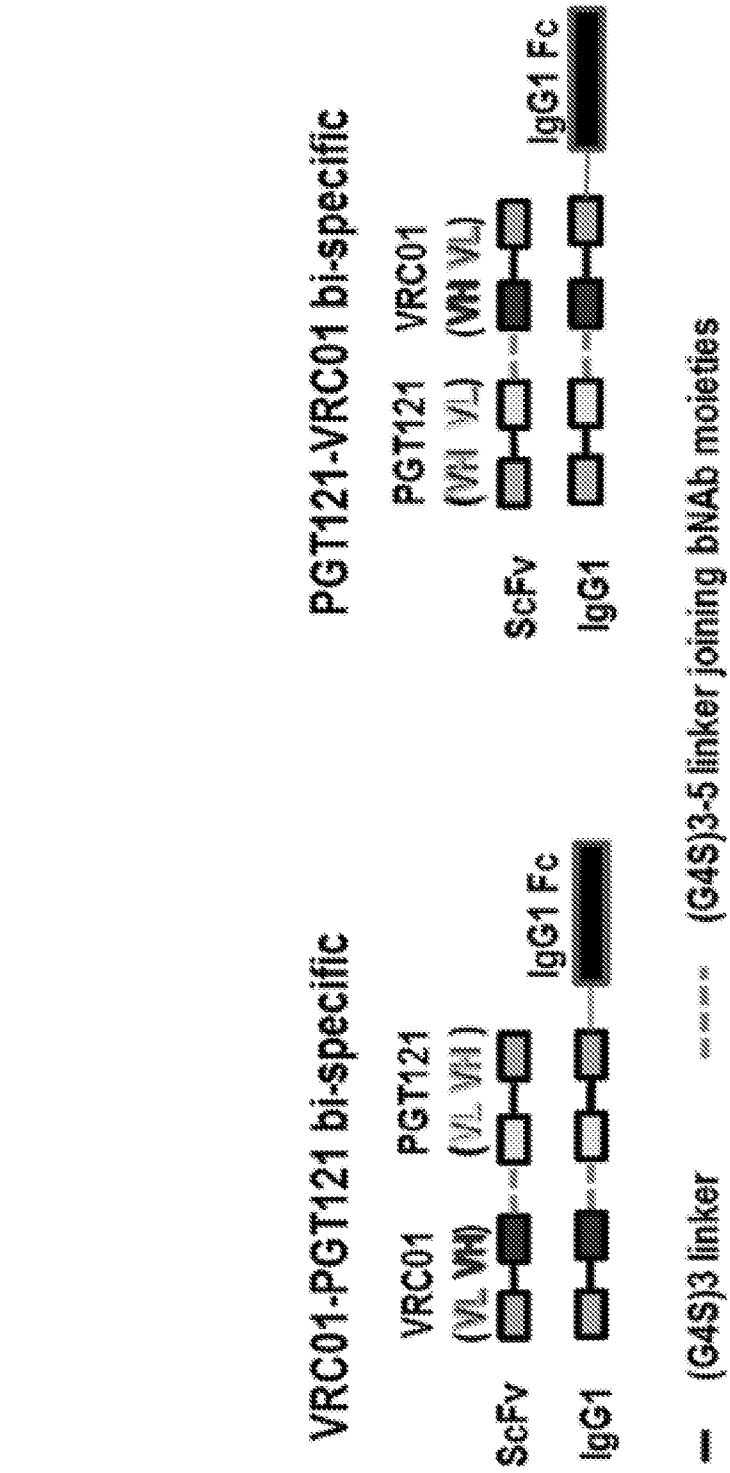

VRC01-PGT121 bi-specific
VRC01 (VL VH)
PGT121 (VL VH)
ScFv
IgG1
IgG1 Fc
PGT121-VRC01 bi-specific
PGT121 (VH VL)
VRC01 (VH VL)
ScFv
IgG1
IgG1 Fc
(G4S)3 linker
(G4S)3-5 linker joining bNAb moieties

FIG. 3B

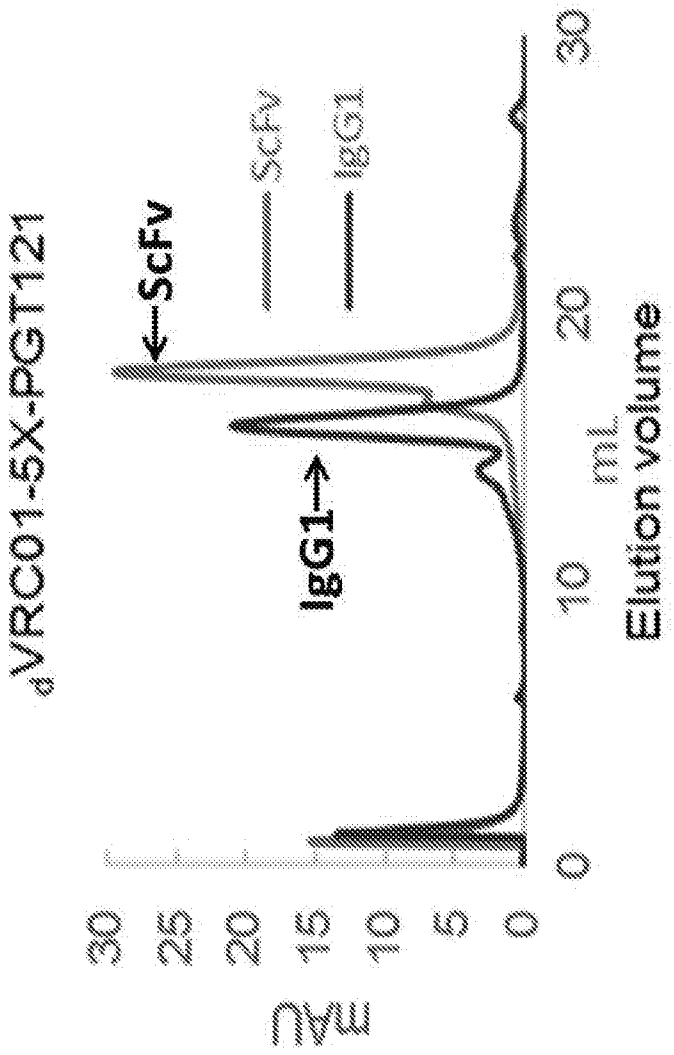

dVRC01-5X-PGT121
←ScFv
IgG1→
ScFv
IgG1
mAU
30
25
20
15
10
5
0
0
10
20
30
mL
Elution volume

FIG. 3C

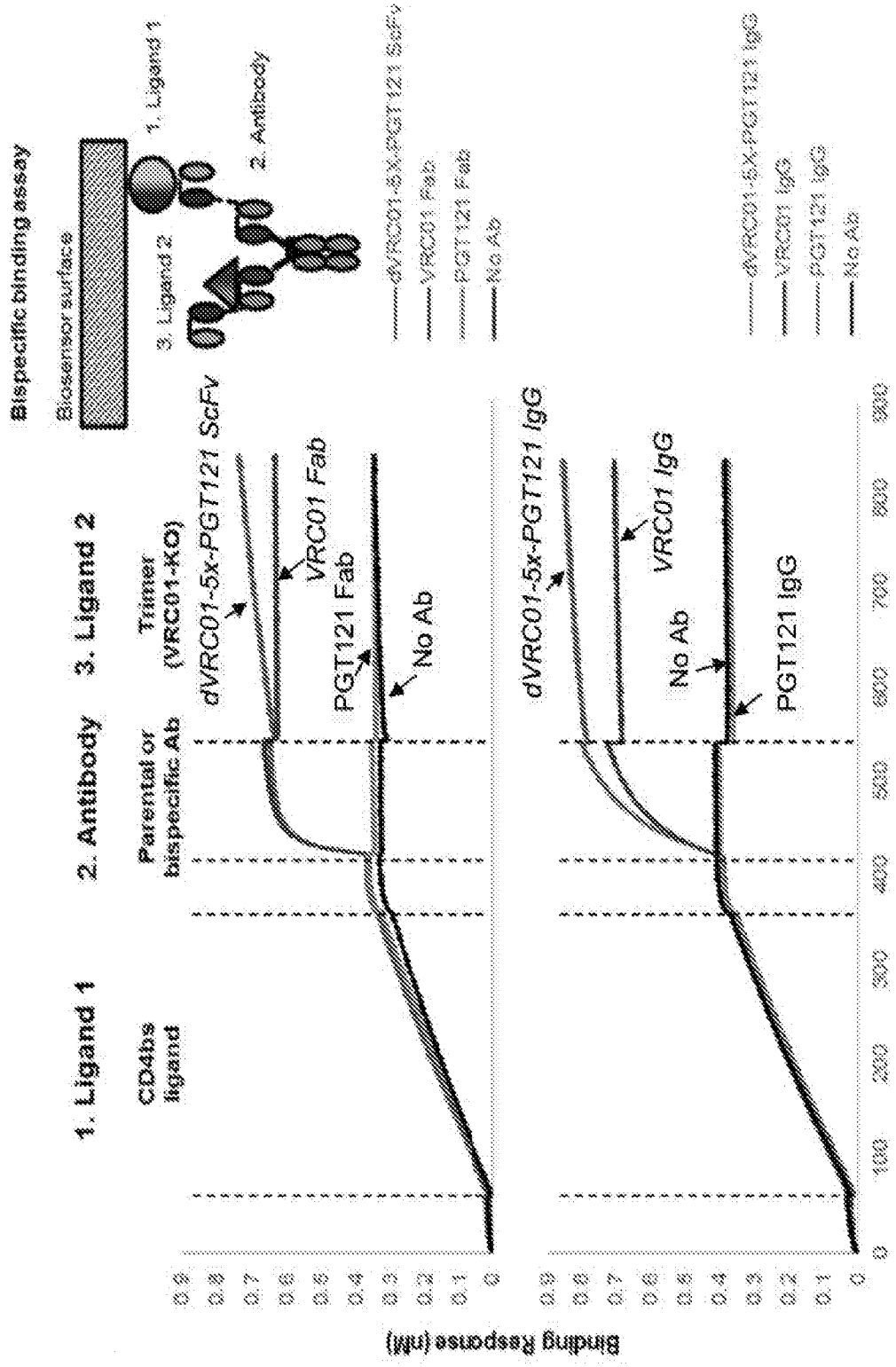

Bispecific binding assay
Biosensor surface
1. Ligand 1
2. Antibody
3. Ligand 2
1. Ligand 1
CD4bs ligand
2. Antibody
Parental or bispecific Ab
3. Ligand 2
Trimer (VRC01-KO)
dVRC01-5x-PGT121 ScFv
VRC01 Fab
PGT121 Fab
No Ab
dVRC01-5x-PGT121 IgG
VRC01 IgG
PGT121 IgG
Binding Response (nm)
Time (sec)

FIG. 4

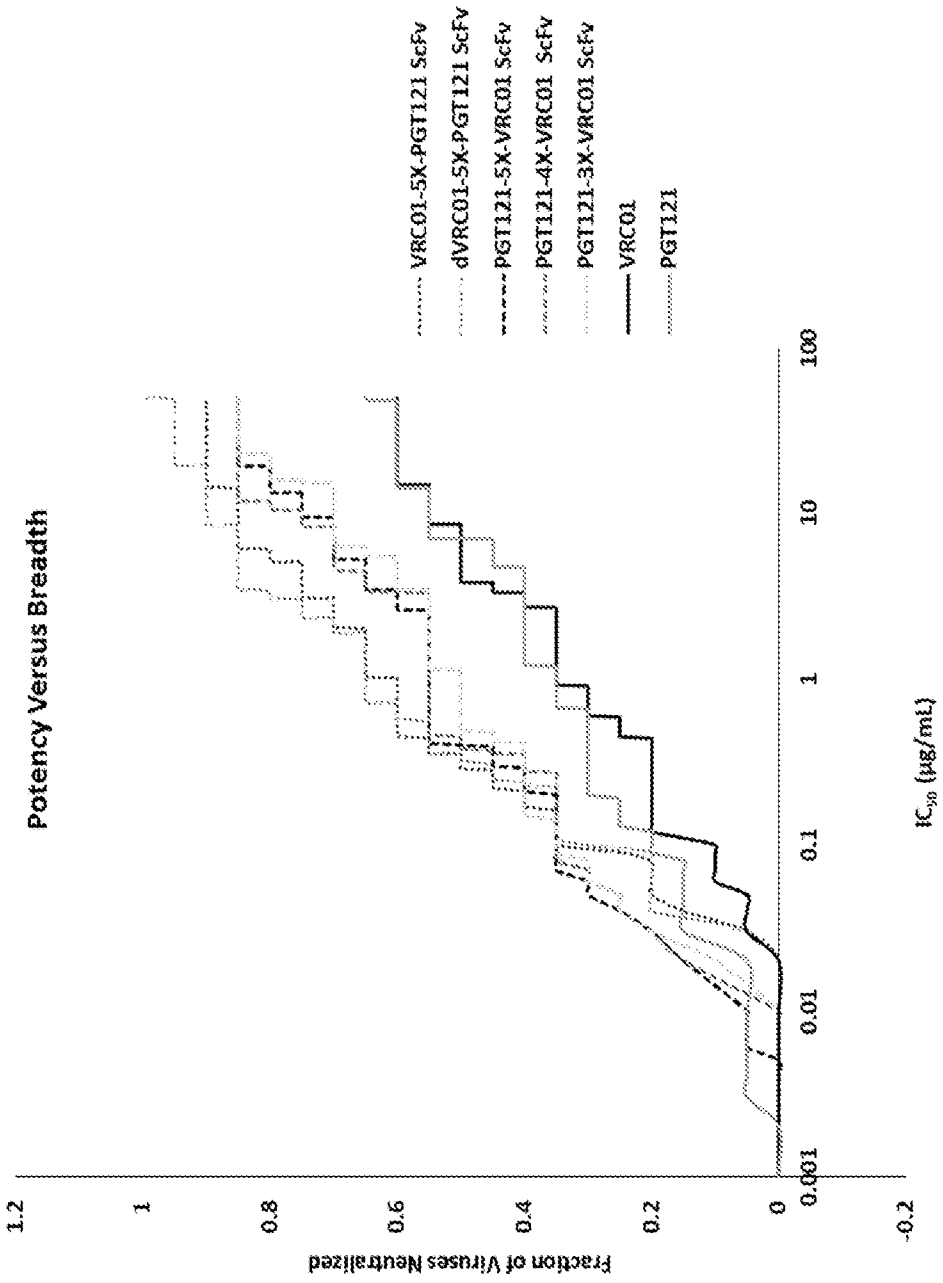

Potency Versus Breadth
Fraction of Viruses Neutralized
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0.001
0.01
0.1
1
10
100
IC50 (µg/mL)
VRC01-5X-PGT121 ScFv
dVRC01-5X-PGT121 ScFv
PGT121-5X-VRC01 ScFv
PGT121-4X-VRC01 ScFv
PGT121-3X-VRC01 ScFv
VRC01
PGT121

FIG. 5A

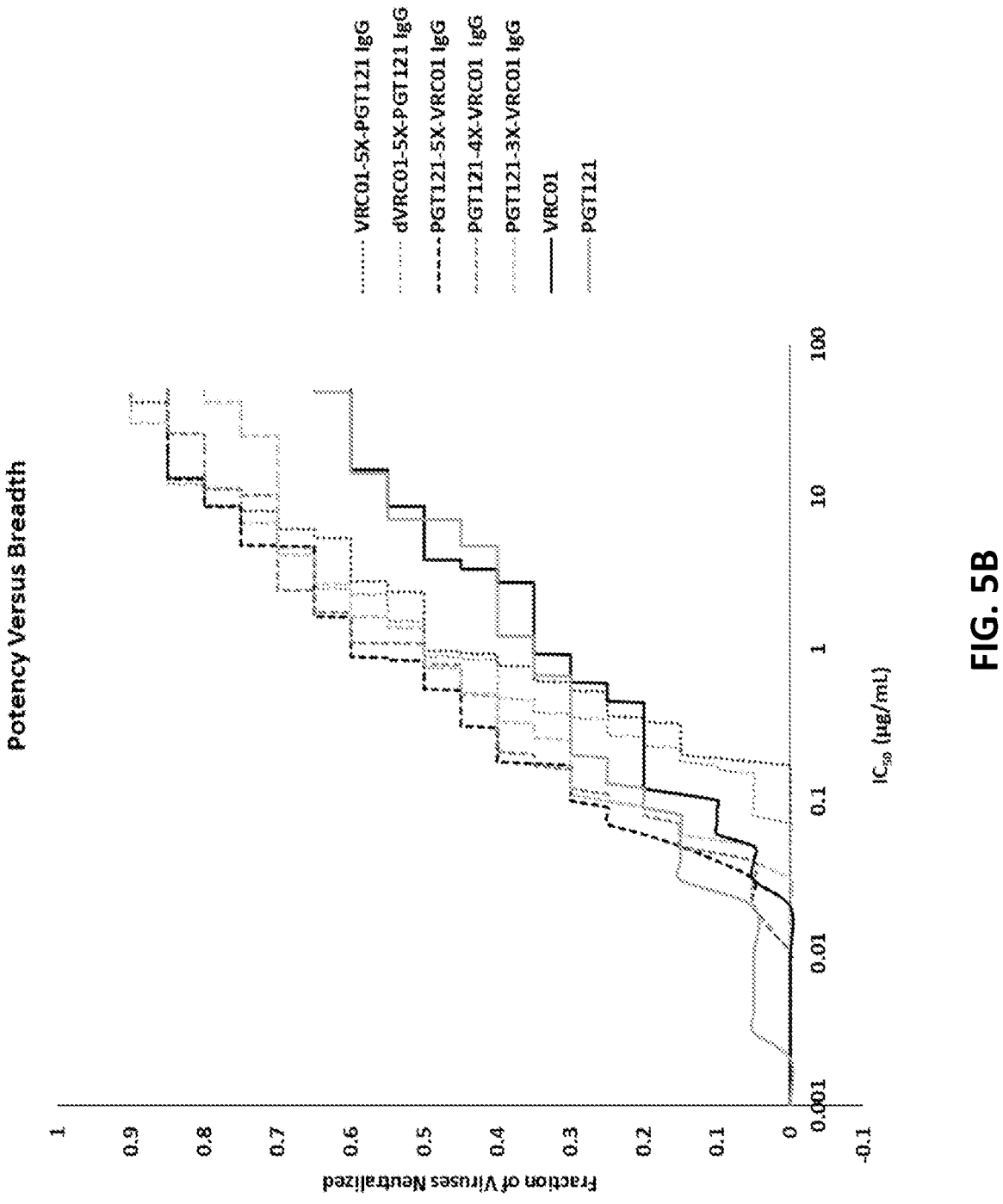

Potency Versus Breadth
Fraction of Viruses Neutralized
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0.001
0.01
0.1
1
10
100
IC50 (µg/mL)
VRC01-5X-PGT121 IgG
dVRC01-5X-PGT121 IgG
PGT121-5X-VRC01 IgG
PGT121-4X-VRC01 IgG
PGT121-3X-VRC01 IgG
VRC01
PGT121

FIG. 5B

	VRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 ScFv	PGT121-5X-VRC01 ScFv	PGT121-4X-VRC01 ScFv	PGT121-3X-VRC01 ScFv	VRC01-5X-PGT121 IgG	dVRC01-5X-PGT121 IgG	PGT121-5X-VRC01 IgG	PGT121-14X-VRC01 IgG	PGT121-3X-VRC01 IgG	VRC01	PGT121
# Viruses	20	20	20	20	20	20	20	20	20	20	20	20
Total VS Neutralized												
IC50 <50ug/ml	18	20	17	17	17	18	18	17	17	16	12	13
IC50 <1ug/ml	12	13	11	11	10	10	10	12	10	10	7	7
% VS Neutralized												
IC50 <50ug/ml	90	100	85	85	85	90	90	85	85	80	60	65
IC50 <1ug/ml	60	65	55	55	50	50	50	60	50	50	35	35
Median IC50	0.251	0.331	0.295	0.357	0.416	0.944	0.864	0.309	0.490	0.414	0.751	0.661
Geometric Mean	0.339	0.504	0.326	0.337	0.419	1.486	1.106	0.392	0.483	0.538	0.697	0.545

FIG. 5C

virus	clade	VRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 ScFv	PGT121-5X-VRC01 ScFv	PGT121-4X-VRC01 ScFv	PGT121-3X-VRC01 ScFv	VRC01	PGT121
Q769.d22.SG3	A	0.160	0.245	2.61	3.37	3.48	0.050	>50
6095.V1.C10.SG3	ACD	0.024	0.030	0.018	0.031	0.034	0.444	1.20
Q168.a2.SG3	AD	0.030	0.038	0.392	0.451	1.13	0.113	>50
BJOX009000.02.4.SG3	AE	0.283	0.352	0.203	0.357	0.416	3.32	7.07
242-14.SG3	AG	>50	19.4	>50	>50	>50	>50	>50
T251-18.SG3	AG	14.3	8.53	9.48	8.28	15.9	8.59	>50
7165.18.SG3	B	0.080	0.106	0.019	0.018	0.021	>50	0.024
AC10.29.SG3	B	0.092	0.097	0.039	0.019	0.016	2.71	0.121
BG1168.01.SG3	B	5.10	2.35	>50	>50	>50	0.911	>50
JRFL.JB.SG3	B	0.048	0.039	0.024	0.022	0.027	0.022	0.029
QH0692.42.SG3	B	2.03	1.89	0.406	0.374	0.480	3.82	7.01
3637.V5.C3.SG3	C	>50	49.4	>50	>50	>50	14.9	>50
CAP210.E8.SG3	C	6.10	3.05	13.3	11.8	22.9	>50	48.2
DU172.17.SG3	C	0.352	0.310	0.067	0.071	0.081	>50	0.089
DU422.01.SG3	C	0.092	0.091	0.045	0.056	0.059	>50	0.195
TZA125.17.SG3	C	3.07	3.44	5.27	3.32	5.51	>50	14.2
ZM214.15.SG3	C	0.444	0.710	0.295	0.274	0.141	0.590	0.661
ZM249.1.SG3	C	0.218	0.225	3.46	4.50	6.29	0.100	>50
57128.vrc15.SG3	D	1.02	0.564	19.3	10.5	15.2	>50	4.74
X2088.c9.SG3	G	0.034	0.029	0.005	0.012	0.012	>50	0.002

Scale

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 5D (1/2)

virus	clade	VRC01-5X-PGT121 IgG	dVRC01-5X-PGT121 IgG	PGT121-5X-VRC01 IgG	PGT121-4X-VRC01 IgG	PGT121-3X-VRC01 IgG	VRC01	PGT121
Q769.d22.SG3	A	0.765	0.461	4.76	10.2	25.0	0.050	>50
6095.V1.C10.SG3	ACD	0.198	0.159	0.035	0.041	0.055	0.444	1.20
Q168.a2.SG3	AD	0.322	0.225	0.309	0.490	0.505	0.113	>50
BJOX009000.02.4.SG3	AE	2.35	1.51	0.175	0.166	0.322	3.32	7.07
242-14.SG3	AG	>50	>50	>50	>50	>50	>50	>50
T251-18.SG3	AG	41.7	30.2	13.0	25.9	41.7	8.59	>50
7165.18.SG3	B	0.528	0.341	0.041	0.047	0.051	>50	0.024
AC10.29.SG3	B	0.600	0.266	0.058	0.090	0.080	2.71	0.121
BG1168.01.SG3	B	6.08	4.61	>50	>50	>50	0.911	>50
JRFL.JB.SG3	B	0.174	0.175	0.090	0.119	0.102	0.022	0.029
QH0692.42.SG3	B	8.02	6.67	0.872	1.08	1.61	3.82	7.01
3637.V5.C3.SG3	C	>50	>50	>50	>50	>50	14.9	>50
CAP210.E8.SG3	C	13.2	11.4	4.69	2.40	4.11	>50	48.2
DU172.17.SG3	C	0.965	0.883	0.178	0.207	0.254	>50	0.089
DU422.01.SG3	C	0.351	0.378	0.068	0.074	0.091	>50	0.195
TZA125.17.SG3	C	8.69	12.1	1.61	1.72	2.64	>50	14.2
ZM214.15.SG3	C	5.31	2.46	0.536	1.07	0.741	0.590	0.661
ZM249.1.SG3	C	0.923	0.844	8.55	11.1	>50	0.100	>50
57128.vrc15.SG3	D	2.76	2.26	0.838	0.789	1.36	>50	4.74
X2088.c9.SG3	G	0.186	0.077	0.016	0.015	0.031	>50	0.002

Scale

<0.001
001-.01
01-.100
100-1.00
1.00-10.0
>10.0

FIG. 5D (2/2)

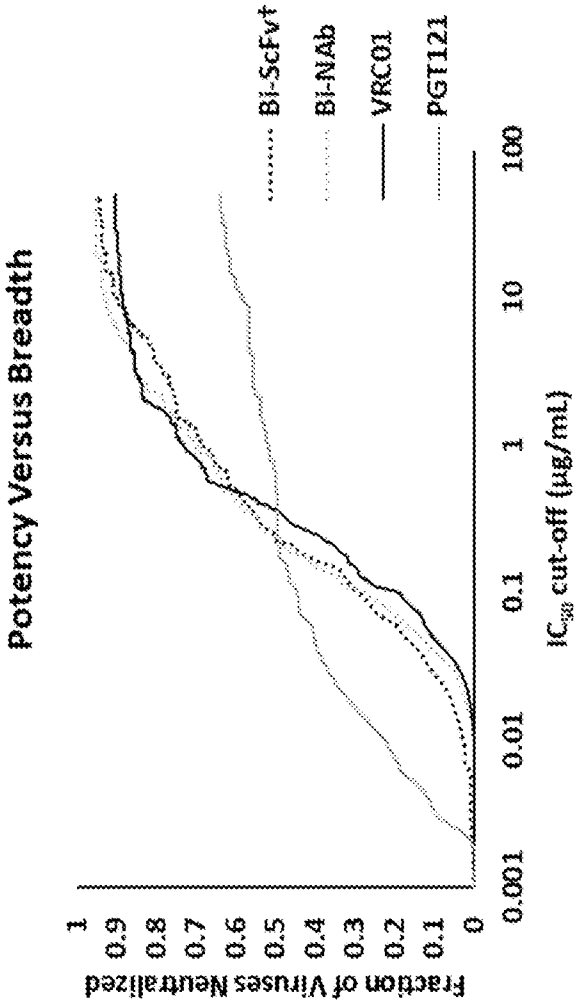

Potency Versus Breadth
Fraction of Viruses Neutralized
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
IC80 cut-off (µg/mL)
Bi-ScFvf
Bi-NAb
VRC01
PGT121

FIG. 6A

	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG	VRC01	PGT121
# Viruses	200	200	200	208
Total VS Neutralized				
IC50 <50ug/ml	193	192	182	131
IC50 <10ug/ml	189	187	178	125
IC50 <1.0ug/ml	157	142	146	108
IC50 <0.1ug/ml	110	57	39	94
IC50 <0.01ug/ml	23	0	0	58
% VS Neutralized				
IC50 <50ug/ml	97	96	91	63
IC50 <10ug/ml	95	94	89	60
IC50 <1.0ug/ml	79	71	73	52
IC50 <0.1ug/ml	55	29	20	45
IC50 <0.01ug/ml	12	0	0	28
Median IC50	0.0700	0.1975	0.2865	0.0130
Geometric Mean	0.1046	0.2986	0.3031	0.0237

FIG. 6B

Virus ID	Clade	Bispecific		Parental	
		dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG	VRC01	PGT121
BJOX025000.01.1	AE	>50	>50	20.2	>25
CNE3	AE	>50	>50	1.79	>25
6322.V4.C1	C	15.2	5.00	>50	>25
6631.V3.C10	C	1.91	2.63	>50	>25
CAP210.E8	C	2.55	4.88	>50	>25
3817.v2.c59	CD	7.23	11.6	>50	>25

FIG. 6C

Virus ID	Clade	dVRC01-SX-PGT121 ScFv	dVRC01-SX-PGT121 IgG1	VRC01	PGT121
0260.v5.c36	A	0.124	0.416	0.523	0.059
0330.v4.c3	A	0.082	0.241	0.066	0.041
0439.v5.c1	A	1.57	2.93	0.228	>50
3365.v2.c30	A	0.078	0.232	0.060	0.059
3415.v1.c1	A	0.354	0.847	0.087	>50
3718.v3.c11	A	0.116	0.295	0.411	1.40
398-F1_F6_20	A	0.015	0.147	0.102	0.032
88201.B42	A	0.015	0.063	0.262	0.063
88539.2B13	A	1.82	1.29	0.105	>50
BG505.W6M.C2	A	0.048	0.134	0.037	0.052
BI369.9A	A	0.087	0.103	0.047	0.035
BS208.B1	A	0.061	0.096	0.027	>50
KER2008.12	A	0.067	0.233	0.487	2.22
KER2018.11	A	0.064	0.271	0.348	>50
KNH1209.18	A	0.014	0.049	0.119	0.032
MB201.A1	A	0.008	0.035	0.241	0.025
MB539.2B7	A	1.94	4.16	0.512	>50
MI369.A5	A	0.056	0.155	0.236	0.022
MS208.A1	A	0.504	0.994	0.174	>50
Q23.17	A	0.060	0.131	0.099	0.054
Q259.17	A	0.013	0.043	0.085	>50
Q769.d22	A	0.175	0.489	0.036	>50
Q769.h5	A	0.094	0.325	0.072	>50
Q842.d12	A	0.030	0.139	0.034	0.016
QH209.14M.A2	A	0.175	0.456	0.026	>50
RW020.2	A	0.026	0.052	0.217	0.032
UG037.8	A	0.104	0.333	0.073	0.065
246-F3.C10.2	AC	0.028	0.092	0.254	>50
3301.V1.C24	AC	0.029	0.065	0.095	0.028
3589.V1.C4	AC	0.127	0.475	0.081	>50
6540.v4.c1	AC	>50	>50	>50	>50
6545.V4.C1	AC	>50	>50	>50	>50

IC_{50} (µg/mL)

- <0.001
- .001-.01
- .01-.100
- .100-1.00
- 1.00-10.0
- >10.0

FIG. 7 (1/7)

Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG1	VRC01	PGT121
0815.V3.C3	ACD	0.031	0.109	0.029	0.020
6095.V1.C10	ACD	0.146	0.331	0.631	37.3
3468.V1.C12	AD	0.015	0.045	0.058	0.042
Q168.a2	AD	0.067	0.141	0.101	>50
Q461.e2	AD	3.28	6.73	0.420	>50
620345.c1	AE	>50	>50	>50	>50
8.IDX009000.02.4	AE	0.228	0.654	1.74	14.7
8.IDX010000.06.2	AE	4.85	18.8	8.40	>50
8.IDX025000.01.1	AE	>50	>50	20.2	>50
8.IDX028000.10.3	AE	0.152	0.285	0.168	>50
C1080.c3	AE	6.87	5.02	2.63	>50
C2101.c1	AE	1.47	1.86	0.269	>50
C3347.c11	AE	1.77	1.73	0.213	>50
C4118.09	AE	2.00	2.26	0.285	>50
CM244.ec1	AE	0.237	0.867	0.116	>50
CNE3	AE	>50	>50	1.79	>50
CNE5	AE	1.01	4.59	0.398	>50
CNE55	AE	1.78	5.74	0.358	>50
CNE56	AE	2.74	6.54	0.525	>50
CNE59	AE	2.83	2.26	0.368	>50
CNE8	AE	0.356	1.14	0.299	>50
MO2138	AE	4.92	7.18	0.898	>50
R1166.c1	AE	4.58	10.6	2.09	>50
R2184.c4	AE	0.791	2.33	0.106	>50
R3265.c6	AE	2.87	6.81	0.382	>50
TH023.6	AE	5.07	1.05	0.546	>50
TH968.8	AE	2.29	3.47	0.390	>50
TH976.17	AE	1.05	2.96	0.299	>50
235-47	AG	0.071	0.246	0.043	0.110
242-14	AG	33.2	>50	>50	>50
263-8	AG	0.250	0.683	0.176	1.23
269-12	AG	0.056	0.239	0.313	0.164

IC_{50} (µg/mL)

- ≤0.01
- 0.01-0.1
- 0.1-1.00
- 1.00-10.0
- >10.0

FIG. 7 (2/7)

Virus ID	Clade	dVRC01-5X-PGT121 5cFv	dVRC01-5X-PGT121 IgG1	VRC01	PGT121
271-11	AG	0.388	0.032	0.059	11.7
928-28	AG	1.16	2.63	0.334	31.0
DJ263.8	AG	0.016	0.020	0.047	0.064
T250-4	AG	2.97	0.028	>50	>50
T251-18	AG	2.86	8.32	4.21	10.8
T253-11	AG	0.217	2.29	0.397	>50
T255-34	AG	0.024	0.089	0.500	>50
T257-31	AG	0.460	1.71	1.72	>50
T266-60	AG	0.534	1.40	1.81	0.160
T278-50	AG	>50	>50	>50	>50
T280-5	AG	0.014	0.050	0.032	0.002
T33-7	AG	0.008	0.180	0.018	>50
3988.25	B	0.021	0.058	0.494	0.032
5768.04	B	0.042	0.108	0.365	0.039
6101.10	B	0.018	0.036	0.035	0.002
6535.3	B	0.255	0.031	1.93	0.035
7163.18	B	0.025	0.196	28.2	0.019
45_01b03	B	0.248	0.031	0.018	0.001
89.6.DG	B	0.124	0.160	0.762	0.016
AC10.29	B	0.179	0.534	1.81	0.028
ADA.DG	B	0.015	0.193	0.470	0.001
Ba1.01	B	0.187	0.028	0.086	0.011
BaL.26	B	0.023	0.260	0.642	0.010
BG1168.01	B	2.03	2.27	0.869	>50
BL01.DG	B	>50	>50	>50	>50
BR07.DG	B	0.162	0.338	1.57	0.064
BX08.16	B	0.059	0.052	0.274	0.002
CAAN.A2	B	0.052	0.104	1.03	0.005
CNE10	B	0.024	0.090	0.565	0.001
CNE12	B	0.023	0.073	0.866	0.002
CNE14	B	0.012	0.048	0.275	0.001
CNE4	B	0.353	0.966	0.910	1.13

IC_{50} (µg/mL)
<50.01
50.1-100
10.1-100
1.01-10.0
>1.0

FIG. 7 (3/7)

Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG1	VRC01	PGT121
CNE57	B	0.050	0.155	0.563	0.008
HO86.8	B	>50	>50	>50	>50
HT593.1	B	0.445	1.10	0.476	>50
HXB2.DG	B	0.003	0.065	0.034	>50
JRCSF.JB	B	0.036	0.278	0.362	0.061
JRFL.JB	B	0.021	0.087	0.028	0.017
MN.3	B	0.009	0.057	0.020	>50
PVO.04	B	0.497	1.28	0.511	0.132
QH0515.01	B	0.452	1.29	1.01	8.70
QH0692.42	B	0.966	1.70	1.54	0.940
REJO.67	B	0.048	0.178	0.075	8.87
RHPA.7	B	0.035	0.110	0.034	0.014
SC422.8	B	0.167	0.458	0.127	0.058
SF162.LS	B	0.0036	0.017	0.207	0.004
SS1196.01	B	0.017	0.063	0.304	0.002
THRO.18	B	1.70	2.60	3.16	>50
TRJO.58	B	0.321	0.820	0.101	4.31
TRO.11	B	0.033	0.138	0.469	0.006
WITO.33	B	0.298	0.893	0.102	0.787
X2278.C2.B6	B	0.064	0.185	0.151	0.007
YU2.DG	B	0.047	0.200	0.076	0.068
8.0X002000.03.2	BC	0.048	0.132	>50	0.018
CH038.12	BC	0.043	0.104	0.447	0.004
CH070.1	BC	0.039	0.036	14.0	0.003
CH117.4	BC	0.008	0.059	0.105	>50
CH119.10	BC	0.070	0.173	0.833	0.023
CH181.12	BC	0.048	0.124	0.487	0.007
CNE15	BC	0.105	0.359	0.141	19.0
CNE19	BC	0.002	0.020	0.247	0.002
CNE20	BC	0.002	0.017	7.39	0.003
CNE21	BC	0.026	0.098	0.274	0.004
CNE40	BC	0.234	0.440	0.433	0.224

IC_{80} (µg/mL)

- <0.001
- 0.001-0.01
- 0.01-0.100
- .100-1.00
- 1.00-10.0
- >10.0

FIG. 7 (4/7)

Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG1	VRC01	PGT121
CNE7	BC	0.055	0.175	0.187	0.032
286.36	C	0.005	0.037	0.223	0.002
288.38	C	0.030	0.085	1.38	0.006
0013095-2.11	C	0.306	0.864	0.086	>50
001428-2.42	C	0.018	0.085	0.014	0.023
0077_V1.C16	C	0.224	0.482	1.13	>50
00836-2.5	C	0.009	0.047	0.122	31.8
0921.V2.C14	C	0.050	0.135	0.230	>50
16055-2.3	C	0.016	0.070	0.100	1.02
16845-2.22	C	2.99	5.21	2.95	9.41
16936-2.21	C	0.011	0.038	0.154	0.003
25710-2.43	C	0.094	0.154	0.487	0.014
25711-2.4	C	0.028	0.103	0.559	0.010
25925-2.22	C	0.060	0.188	0.550	0.024
26191-2.48	C	0.107	0.406	0.183	0.150
3168.V4.C10	C	0.638	1.68	0.129	0.485
3637.V5.C3	C	11.4	18.9	1.97	>50
3873.V1.C24	C	0.322	1.48	2.81	0.015
426c	C	0.419	0.953	1.93	>50
6322.V4.C1	C	15.2	5.00	>50	>50
6471.V1.C18	C	>50	>50	>50	>50
6631.V3.C10	C	1.91	2.63	>50	>50
6644.V2.C33	C	0.021	0.143	0.153	0.018
6785.V5.C14	C	0.059	0.149	0.253	0.019
6838.V1.C35	C	0.002	0.015	0.288	0.119
96ZM651.02	C	0.026	0.070	0.807	0.009
BR025.9	C	0.019	0.033	0.528	0.002
CAP210.E8	C	2.55	4.88	>50	>50
CAP244.D3	C	0.455	1.28	1.34	>50
CAP256.206.C9	C	0.054	0.102	1.07	0.010
CAP45.G3	C	0.166	0.373	6.75	2.08
Ce1176.A3	C	0.051	0.120	1.85	0.016

IC_{50} (µg/mL)

- <0.001
- .001-.01
- .01-.100
- .100-1.00
- 1.00-10.0
- >10.0

FIG. 7 (5/7)

Virus ID	Clade	dVRC01-SX-PGT121 ScFv	dVRC01-SX-PGT121 IgG1	VRC01	PGT121
CE703010217.B6	C	0.009	0.038	0.195	0.002
CNE30	C	0.224	0.609	0.693	0.061
CNE31	C	0.713	2.30	0.772	0.789
CNE53	C	0.050	0.106	0.117	0.022
CNE58	C	0.970	2.32	0.252	>50
DU123.06	C	0.053	0.199	5.70	0.033
DU151.02	C	0.011	0.044	10.5	0.005
DU156.12	C	0.005	0.036	0.077	0.005
DU172.17	C	0.059	0.140	>50	0.104
DU422.01	C	0.057	0.187	>50	0.164
MW965.26	C	0.012	0.051	0.043	0.011
SO18.18	C	0.004	0.023	0.052	0.002
TV1.29	C	0.319	0.473	>50	0.118
TZA125.17	C	2.11	5.14	>50	9.96
TZB0.02	C	0.007	0.035	0.043	0.005
ZA012.29	C	0.023	0.062	0.327	0.005
ZM106.9	C	0.023	0.089	0.264	0.005
ZM109.4	C	0.091	0.351	0.142	13.7
ZM135.10a	C	1.20	2.12	1.40	1.50
ZM176.66	C	0.071	0.221	0.045	13.8
ZM197.7	C	1.14	4.15	0.532	>50
ZM214.15	C	0.857	2.30	0.957	0.682
ZM215.8	C	0.021	0.100	0.362	0.014
ZM233.6	C	0.070	0.268	1.98	4.14
ZM249.1	C	0.486	0.810	0.107	>50
ZM53.12	C	0.0006	0.010	0.702	0.002
ZM55.28a	C	0.137	0.407	0.241	0.070
3326.V4.C3	CD	0.008	0.017	0.107	>50
3337.V2.C6	CD	0.053	0.110	0.105	21.1
3817.v2.c59	CD	7.23	11.6	>50	>50
191821.E8.1	D	0.487	1.57	0.438	>50
231965.c1	D	1.31	1.41	0.392	>50

IC_{80} (µg/mL)

- <0.003
- 0.01 - 0.1
- 0.1 - 100
- 100 - 1.00
- 1.00 - 10.0
- >10.0

FIG. 7 (6/7)

Virus ID	Clade	6VRC01-SX-PGT121 SoFv	6VRC01-SX-PGT121 IgG1	VRC01	PGT121
247-23	D	19.5	18.3	1.63	>50
3016.v5.c45	D	0.043	0.078	0.117	>50
57128.vrc15	D	0.438	0.875	>50	2.16
6405.v4.c34	D	0.092	0.259	1.69	0.019
AD33439M1.vrc4a	D	0.071	0.194	4.42	0.013
AD7412M1.vrc12	D	0.011	0.057	0.101	0.012
MKU3006.ec1	D	3.28	8.54	0.460	>50
UG021.16	D	0.275	0.340	0.451	2.41
UG024.2	D	2.28	0.830	0.219	>50
PC402.c2.11	G	0.024	0.081	0.207	0.024
P1981.C5.3	G	0.025	0.024	0.336	0.034
X1193.c1	G	0.128	0.239	0.124	0.028
X1254.c3	G	0.072	0.188	0.055	0.024
X1632.S2.B10	G	0.088	0.178	0.131	>50
X2088.c9	G	0.016	0.029	>50	0.003
X2131.C1.B5	G	0.023	0.079	0.467	0.010
SIVmac251.30.SG3	NA	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50

IC_{50} (µg/mL)
<0.001
0.01-0.1
0.1-1.00
1.00-10.0
>10.0

FIG. 7 (7/7)

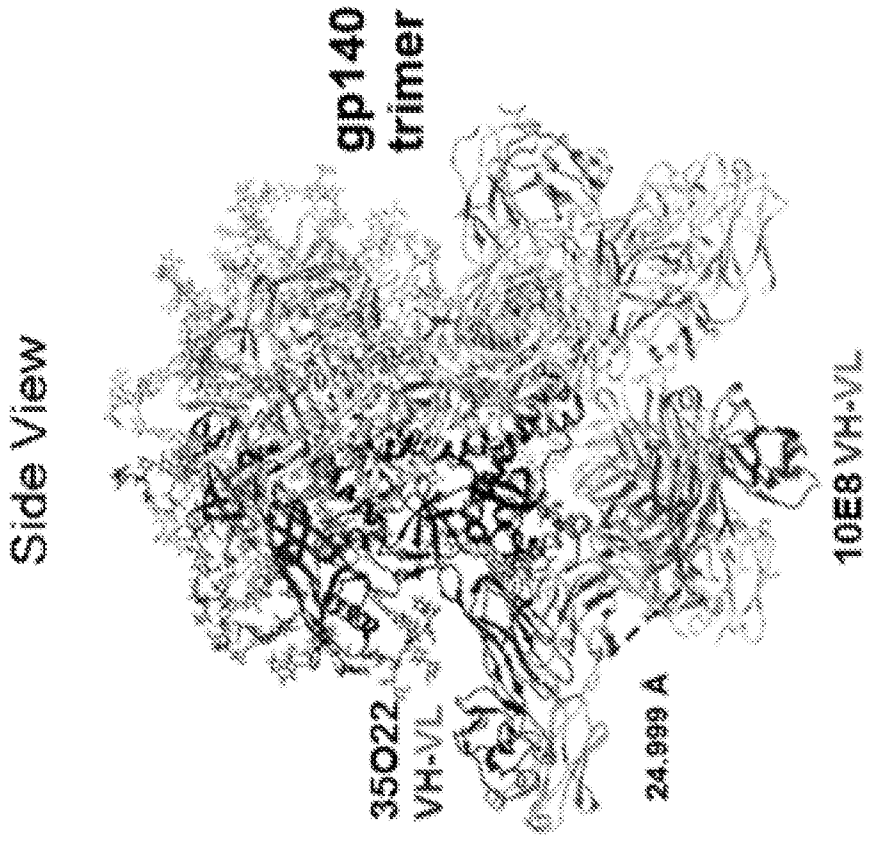


FIG. 8

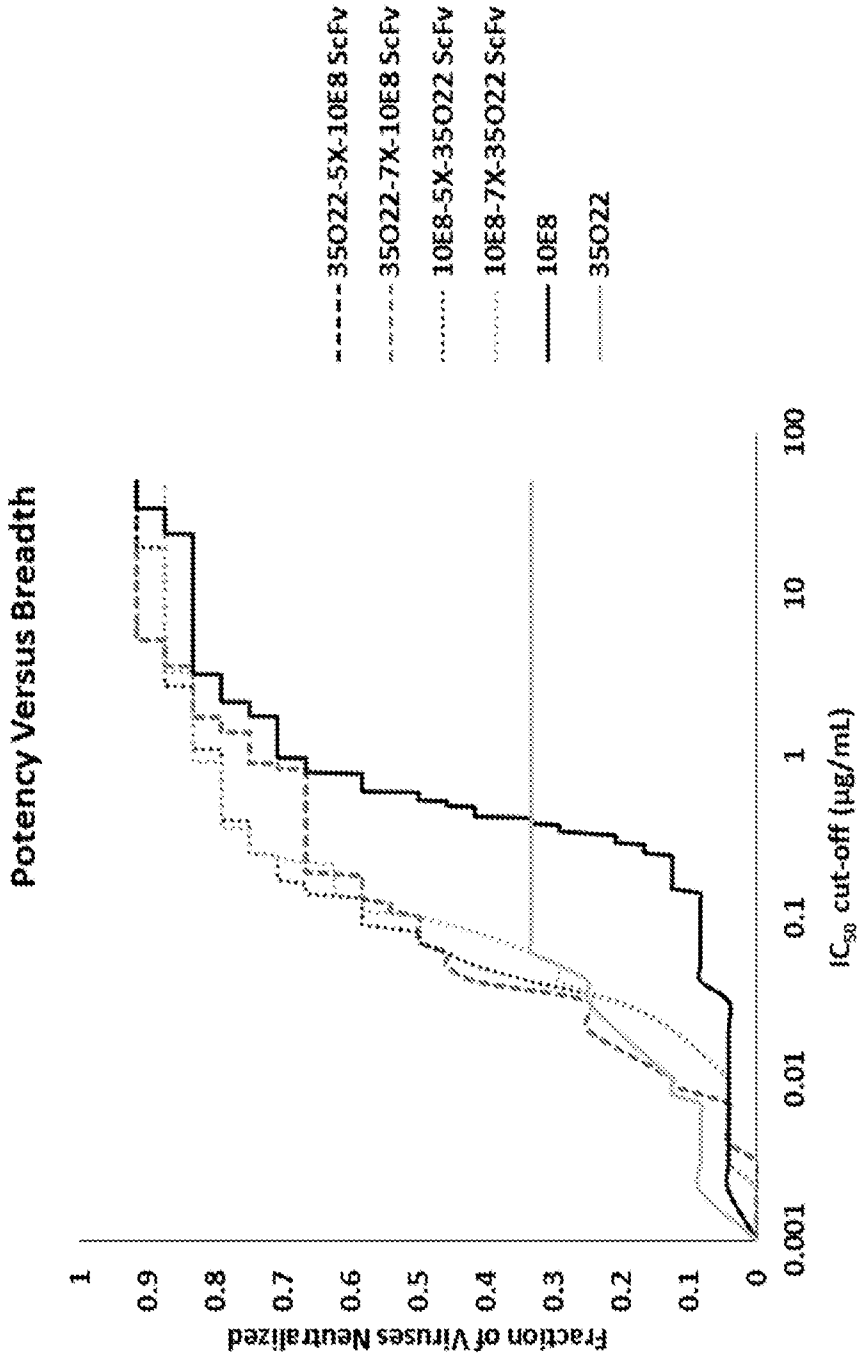

Potency Versus Breadth
Fraction of Viruses Neutralized
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
IC50 cut-off (µg/mL)
35O22-5X-10E8 ScFv
35O22-7X-10E8 ScFv
10E8-5X-35O22 ScFv
10E8-7X-35O22 ScFv
10E8
35O22

FIG. 9A

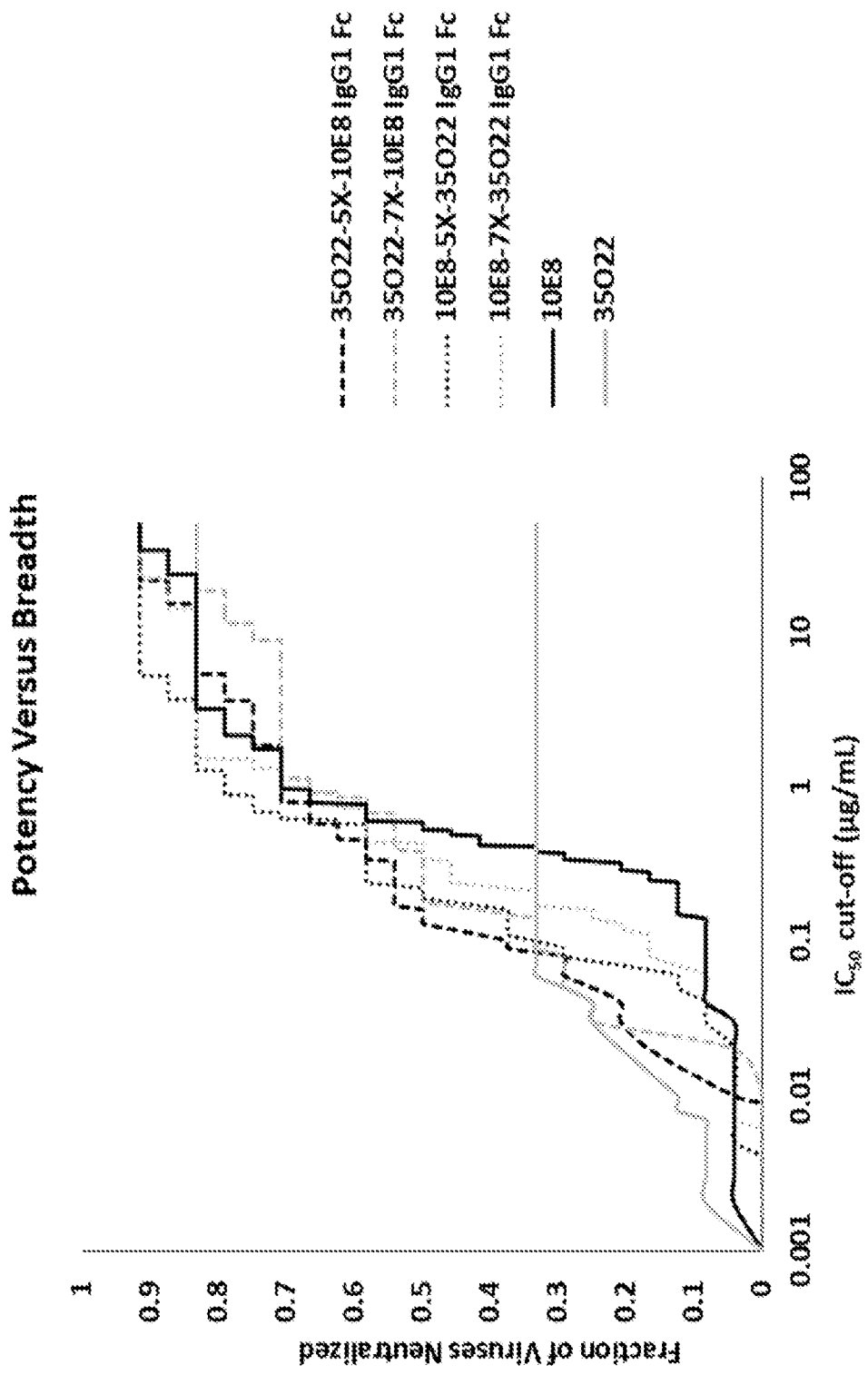

Potency Versus Breadth
Fraction of Viruses Neutralized
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.001
0.01
0.1
1
10
100
IC50 cut-off (µg/mL)
35O22-5X-10E8 IgG1 Fc
35O22-7X-10E8 IgG1 Fc
10E8-5X-35O22 IgG1 Fc
10E8-7X-35O22 IgG1 Fc
10E8
35O22

FIG. 9B

virus	clade	35O22-5X-10E8 IgG1	10E8	35O22
Q769.d22.SG3	A	0.009	0.78	0.053
3589.V1.C4.SG3	AC	0.014	3.19	>50
T251-18.SG3	AG	0.058	0.42	>50
AC10.29.SG3	B	0.089	0.14	>50
QH0692.42.SG3	B	0.086	0.52	>50
ZM106.9.SG3	C	15.30	34.02	>50
ZM214.15.SG3	C	0.042	1.75	>50

FIG. 9C

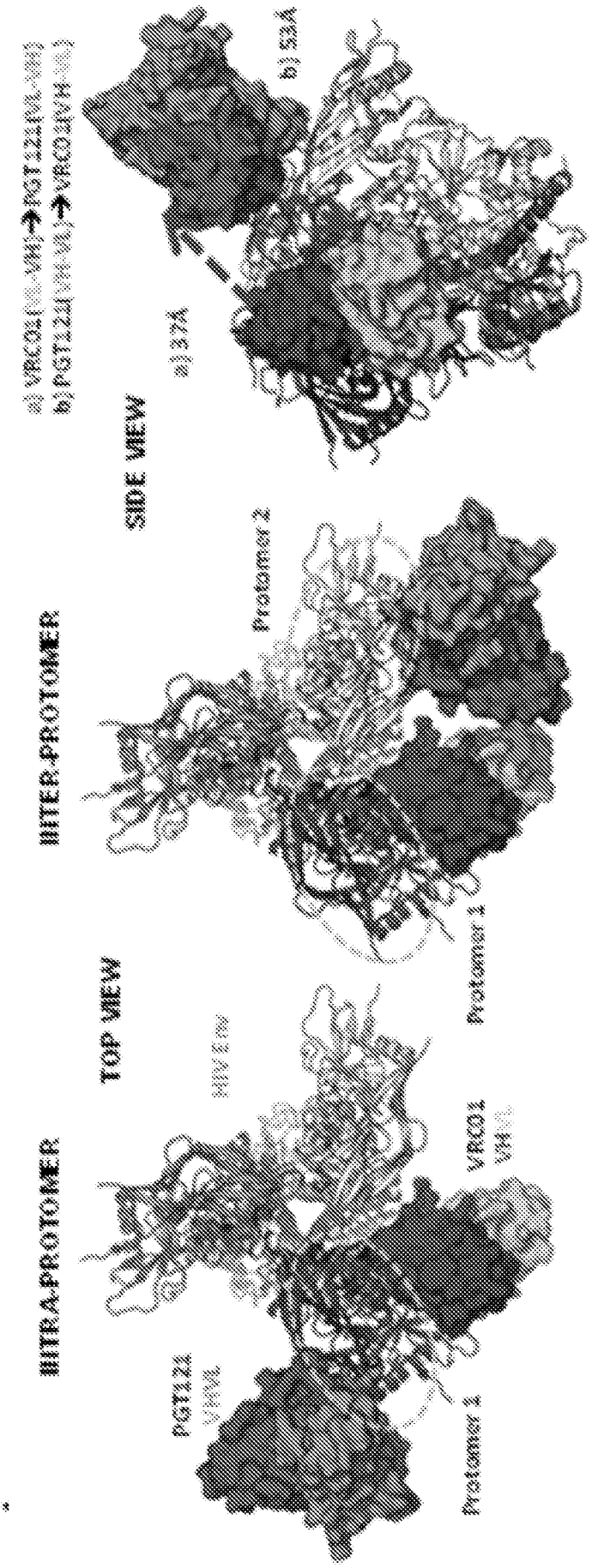


FIG. 10A

FIG. 10B

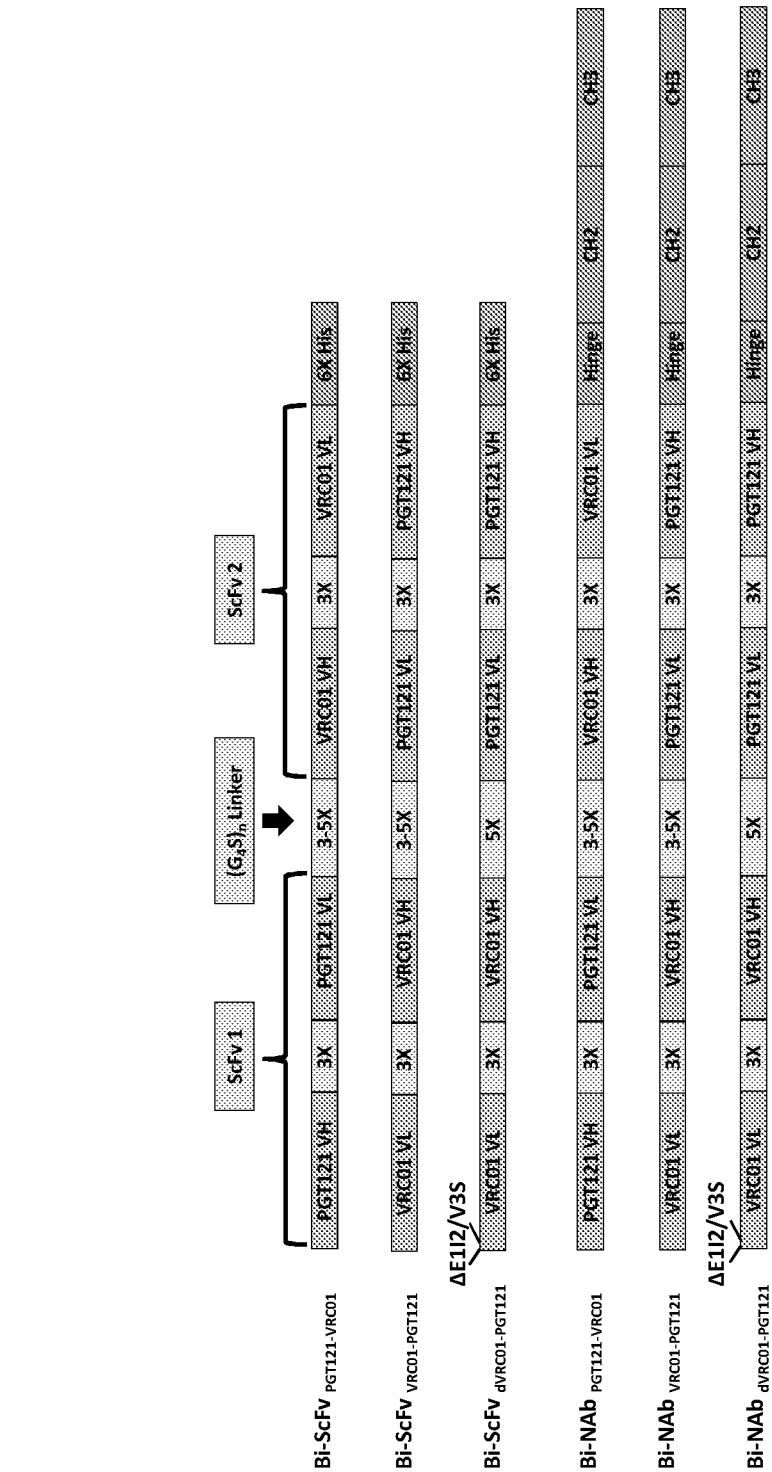

ScFv 1
(G4S)n Linker
ScFv 2
Bi-ScFv PGT121-VRC01
Bi-ScFv VRC01-PGT121
Bi-ScFv dVRC01-PGT121
Bi-NAb PGT121-VRC01
Bi-NAb VRC01-PGT121
Bi-NAb dVRC01-PGT121
ΔE1I2/V3S
PGT121 VH
3X
PGT121 VL
3-5X
VRC01 VH
3X
VRC01 VL
6X His
VRC01 VL
3X
VRC01 VH
3-5X
PGT121 VL
3X
PGT121 VH
6X His
VRC01 VL
3X
VRC01 VH
5X
PGT121 VL
3X
PGT121 VH
6X His
PGT121 VH
3X
PGT121 VL
3-5X
VRC01 VH
3X
VRC01 VL
Hinge
CH2
CH3
VRC01 VL
3X
VRC01 VH
3-5X
PGT121 VL
3X
PGT121 VH
Hinge
CH2
CH3
VRC01 VL
3X
VRC01 VH
5X
PGT121 VL
3X
PGT121 VH
Hinge
CH2
CH3

FIG. 10C

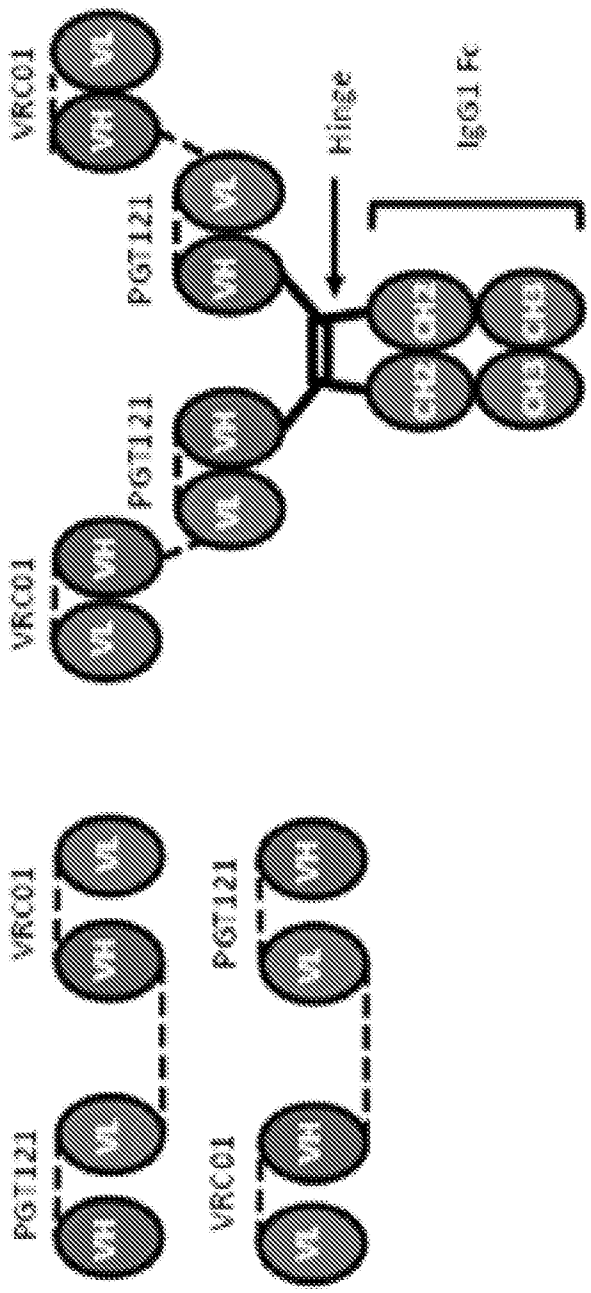

PG121
VL
VH
VRC01
VL
VH
VRC01
VL
VH
PG121
VH
VL
VRC01
VH
VL
PG121
VH
VL
VRC01
VH
VL
Hinge
IgG1 Fc
CH2
CH3
CH2
CH3

FIG. 10D

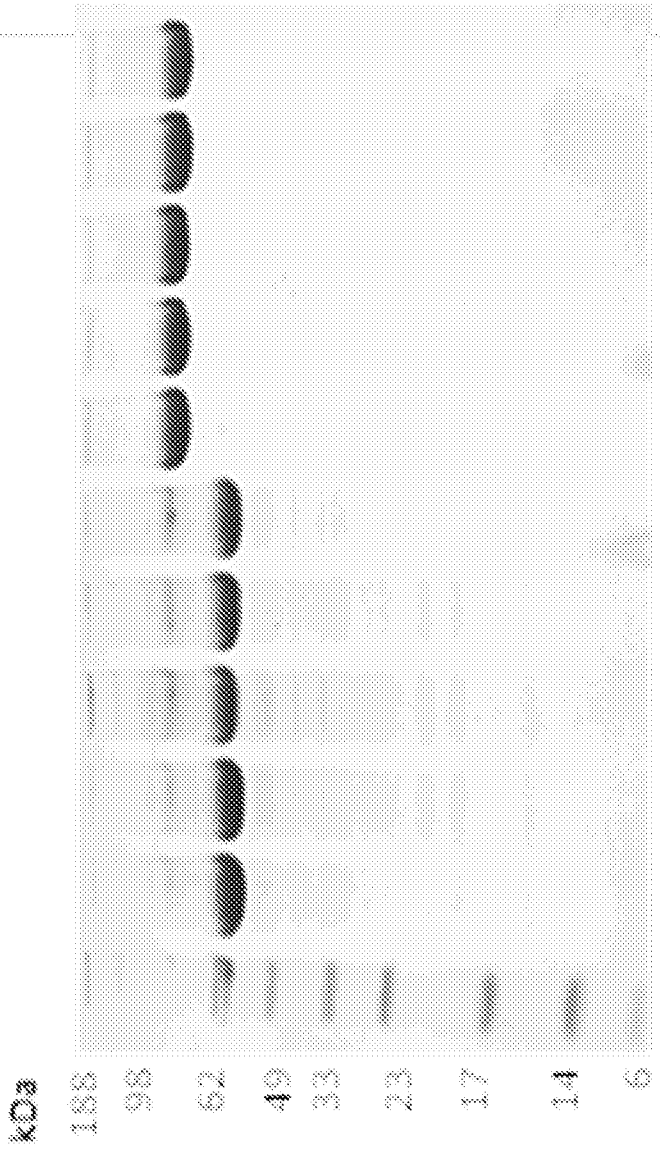


FIG. 11A

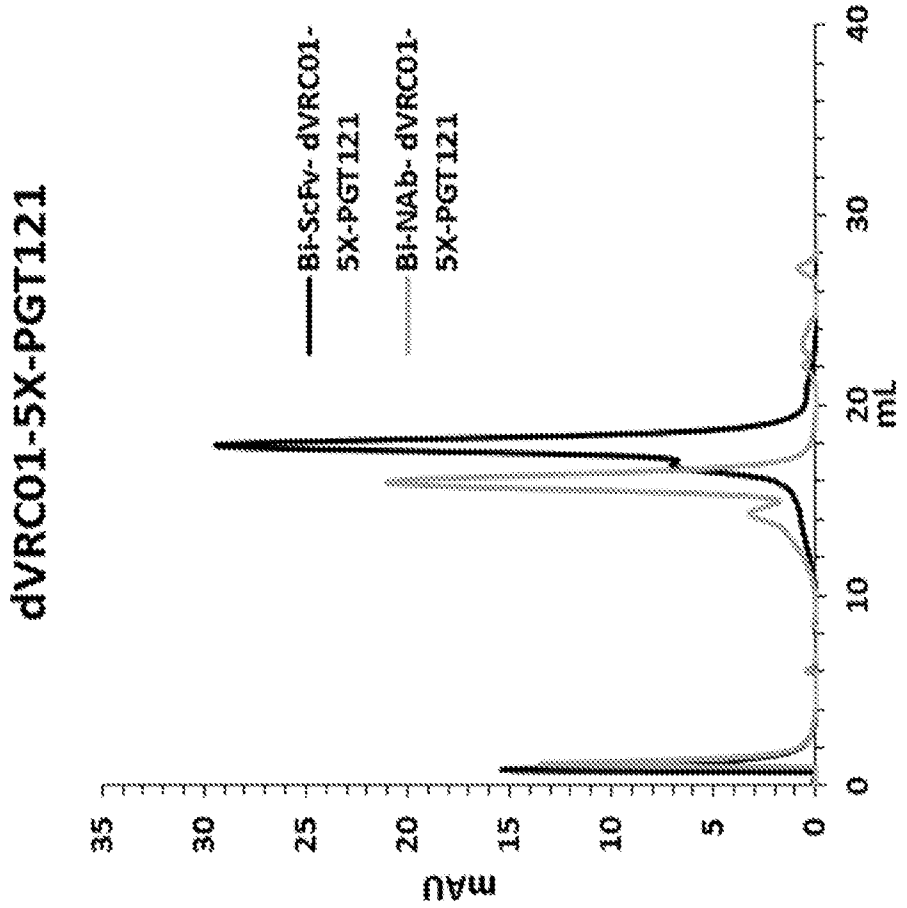

dVRC01-5X-PGT121
Bi-ScFv- dVRC01-5X-PGT121
Bi-NAb- dVRC01-5X-PGT121
mAU
0
5
10
15
20
25
30
35
0
10
20
30
40
mL

FIG. 11B

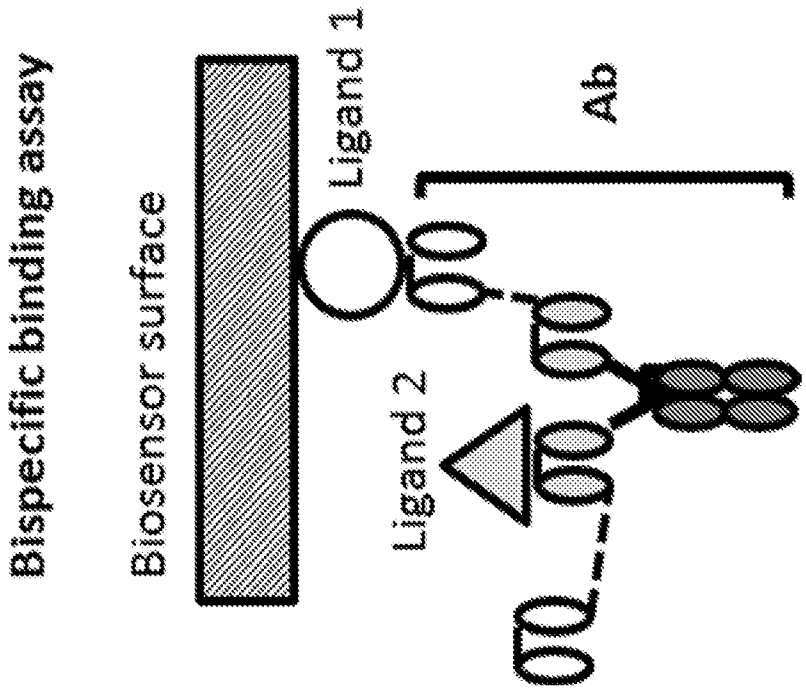

Bispecific binding assay
Biosensor surface
Ligand 1
Ligand 2
Ab

FIG. 12A

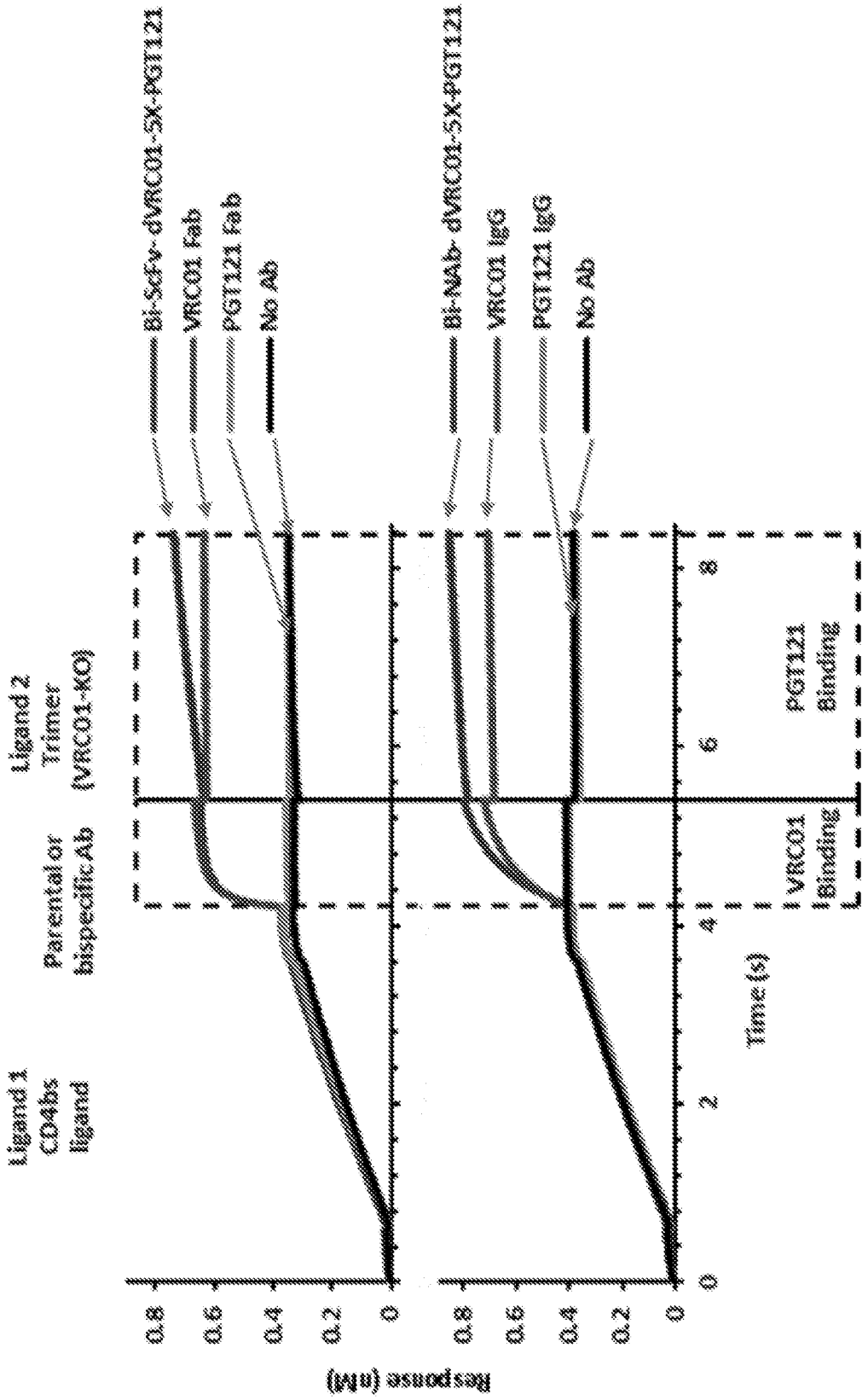

Ligand 1
CD4bs
ligand
Parental or
bispecific Ab
Ligand 2
Trimer
(VRC01-KO)
Bi-ScFv- dVRC01-5X-PGT121
VRC01 Fab
PGT121 Fab
No Ab
Bi-NAb- dVRC01-5X-PGT121
VRC01 IgG
PGT121 IgG
No Ab
VRC01
Binding
PGT121
Binding
Response (nm)
Time (s)
0
0.2
0.4
0.6
0.8
2
4
6
8

FIG. 12B

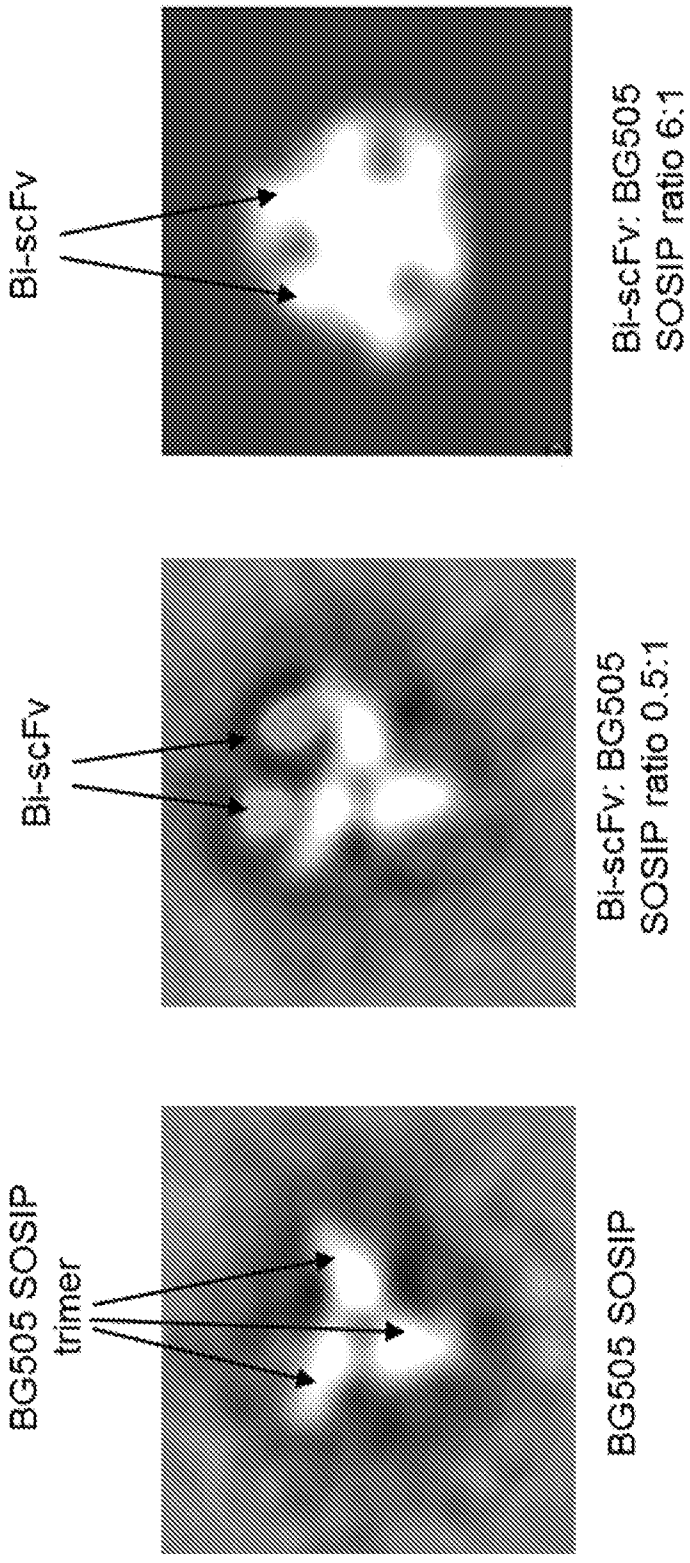

Bi-scFv
Bi-scFv: BG505
SOSIP ratio 6:1
Bi-scFv
Bi-scFv: BG505
SOSIP ratio 0.5:1
BG505 SOSIP
trimer
BG505 SOSIP

FIG. 12C

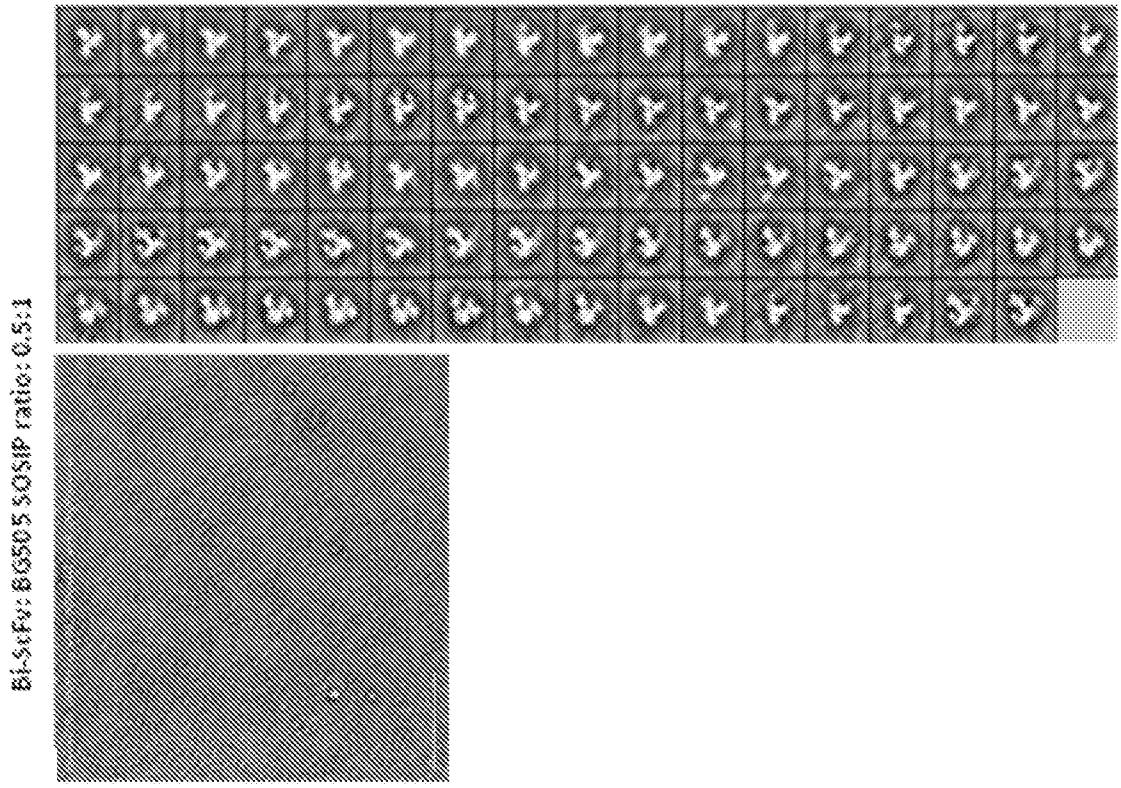


FIG. 13A

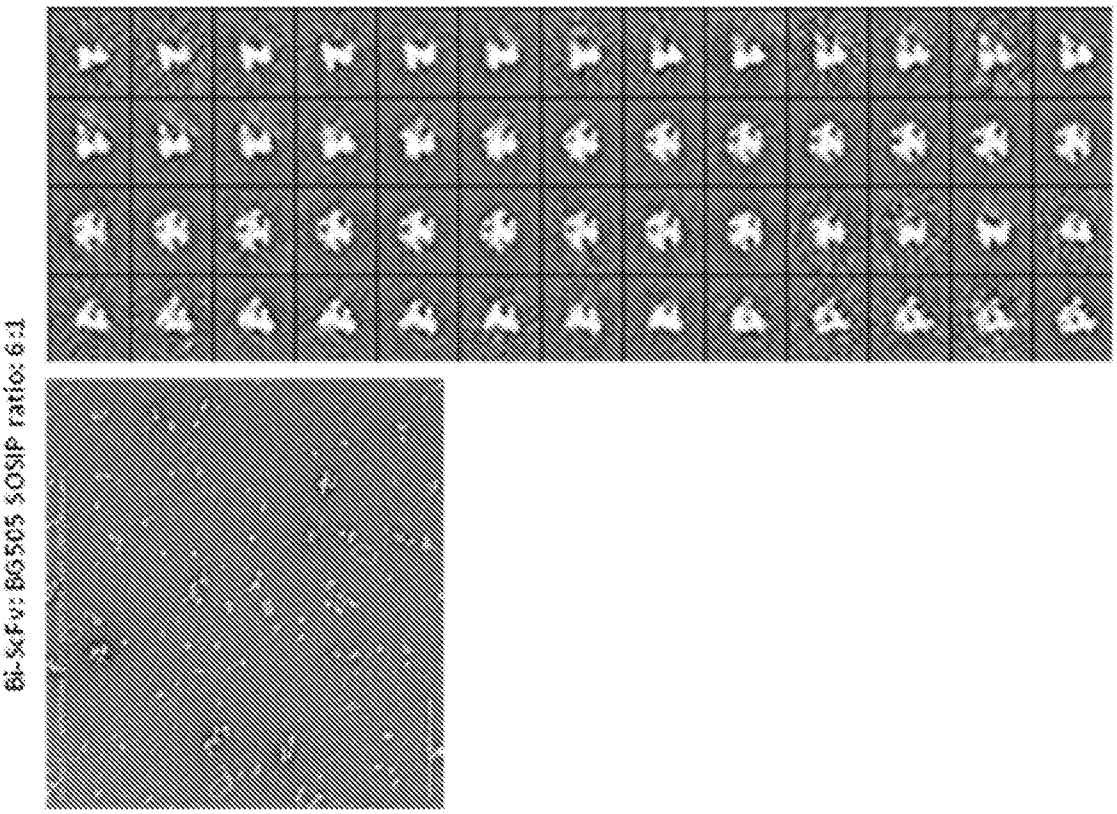


FIG. 13B

virus	clade	VRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 ScFv	PGT121-5X-VRC01 ScFv	PGT121-4X-VRC01 ScFv	PGT121-3X-VRC01 ScFv	VRC01	PGT121
Q769.d22.SG3	A	0.160	0.245	2.61	3.37	3.48	0.050	>50
6095.V1.C10.SG3	ACD	0.024	0.030	0.018	0.031	0.034	0.444	1.20
Q168.a2.SG3	AD	0.030	0.038	0.392	0.451	1.13	0.113	>50
BJOX009000.02.4.SG3	AE	0.283	0.352	0.203	0.357	0.416	3.32	7.07
242-14.SG3	AG	>50	19.4	>50	>50	>50	>50	>50
T251-18.SG3	AG	14.3	8.53	9.48	8.28	15.9	8.59	>50
7165.18.SG3	B	0.080	0.106	0.019	0.018	0.021	>50	0.024
AC10.29.SG3	B	0.092	0.097	0.039	0.019	0.016	2.71	0.121
BG1168.01.SG3	B	5.10	2.35	>50	>50	>50	0.911	>50
JRFL.JB.SG3	B	0.048	0.039	0.024	0.022	0.027	0.022	0.029
QH0692.42.SG3	B	2.03	1.89	0.406	0.374	0.480	3.82	7.01
3637.V5.C3.SG3	C	>50	49.4	>50	>50	>50	14.9	>50
CAP210.E8.SG3	C	6.10	3.05	13.3	11.8	22.9	>50	48.2
DU172.17.SG3	C	0.352	0.310	0.067	0.071	0.081	>50	0.089
DU422.01.SG3	C	0.092	0.091	0.045	0.056	0.059	>50	0.195
TZA125.17.SG3	C	3.07	3.44	5.27	3.32	5.51	>50	14.2
ZM214.15.SG3	C	0.444	0.710	0.295	0.274	0.141	0.590	0.661
ZM249.1.SG3	C	0.218	0.225	3.46	4.50	6.29	0.100	>50
57128.vrc15.SG3	D	1.02	0.564	19.3	10.5	15.2	>50	4.74
X2088.c9.SG3	G	0.034	0.029	0.005	0.012	0.012	>50	0.002

Scale

<0.001
0.01-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 14A (1/2)

virus	clade	VRC01-5X-PGT121 IgG	dVRC01-5X-PGT121 IgG	PGT121-5X-VRC01 IgG	PGT121-4X-VRC01 IgG	PGT121-3X-VRC01 IgG	VRC01	PGT121
Q769.d22.SG3	A	0.765	0.461	4.76	10.2	25.0	0.050	>50
6095.V1.C10.SG3	ACD	0.198	0.159	0.035	0.041	0.055	0.444	1.20
Q168.a2.SG3	AD	0.322	0.225	0.309	0.490	0.505	0.113	>50
BJOX009000.02.4.SG3	AE	2.35	1.51	0.175	0.166	0.322	3.32	7.07
242-14.SG3	AG	>50	>50	>50	>50	>50	>50	>50
T251-18.SG3	AG	41.7	30.2	13.0	25.9	41.7	8.59	>50
7165.18.SG3	B	0.528	0.341	0.041	0.047	0.051	>50	0.024
AC10.29.SG3	B	0.600	0.266	0.058	0.090	0.080	2.71	0.121
BG1168.01.SG3	B	6.08	4.61	>50	>50	>50	0.911	>50
JRFL.JB.SG3	B	0.174	0.175	0.090	0.119	0.102	0.022	0.029
QH0692.42.SG3	B	8.02	6.67	0.872	1.08	1.61	3.82	7.01
3637.V5.C3.SG3	C	>50	>50	>50	>50	>50	14.9	>50
CAP210.E8.SG3	C	13.2	11.4	4.69	2.40	4.11	>50	48.2
DU172.17.SG3	C	0.965	0.883	0.178	0.207	0.254	>50	0.089
DU422.01.SG3	C	0.351	0.378	0.068	0.074	0.091	>50	0.155
TZA125.17.SG3	C	8.69	12.1	1.61	1.72	2.64	>50	14.2
ZM214.15.SG3	C	5.31	2.46	0.536	1.07	0.741	0.590	0.661
ZM249.1.SG3	C	0.923	0.844	8.55	11.1	>50	0.100	>50
57128.vrc15.SG3	D	2.76	2.26	0.838	0.789	1.36	>50	4.74
X2088.c9.SG3	G	0.186	0.077	0.016	0.015	0.031	>50	0.002

Scale

<.001
.001-.01
.01-.100
100-1.00
1.00-10.0
>10.0

FIG. 14A (2/2)

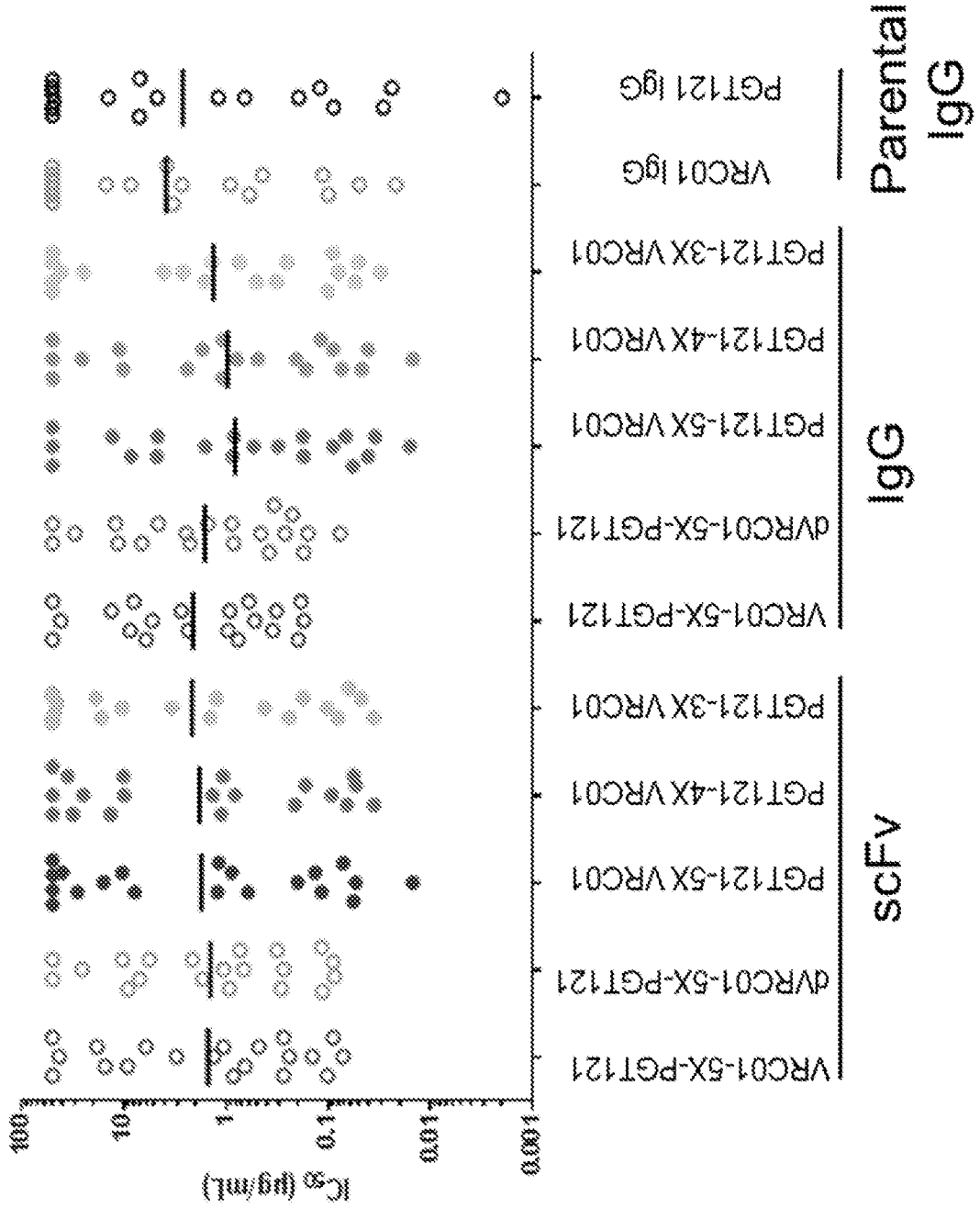

IC50 (µg/mL)
100
10
1
0.1
0.01
0.001
VRC01-5X-PGT121
dVRC01-5X-PGT121
PGT121-5X VRC01
PGT121-4X VRC01
PGT121-3X VRC01
VRC01-5X-PGT121
dVRC01-5X-PGT121
PGT121-5X VRC01
PGT121-4X VRC01
PGT121-3X VRC01
VRC01 IgG
PGT121 IgG
scFv
IgG
Parental IgG

FIG. 14B

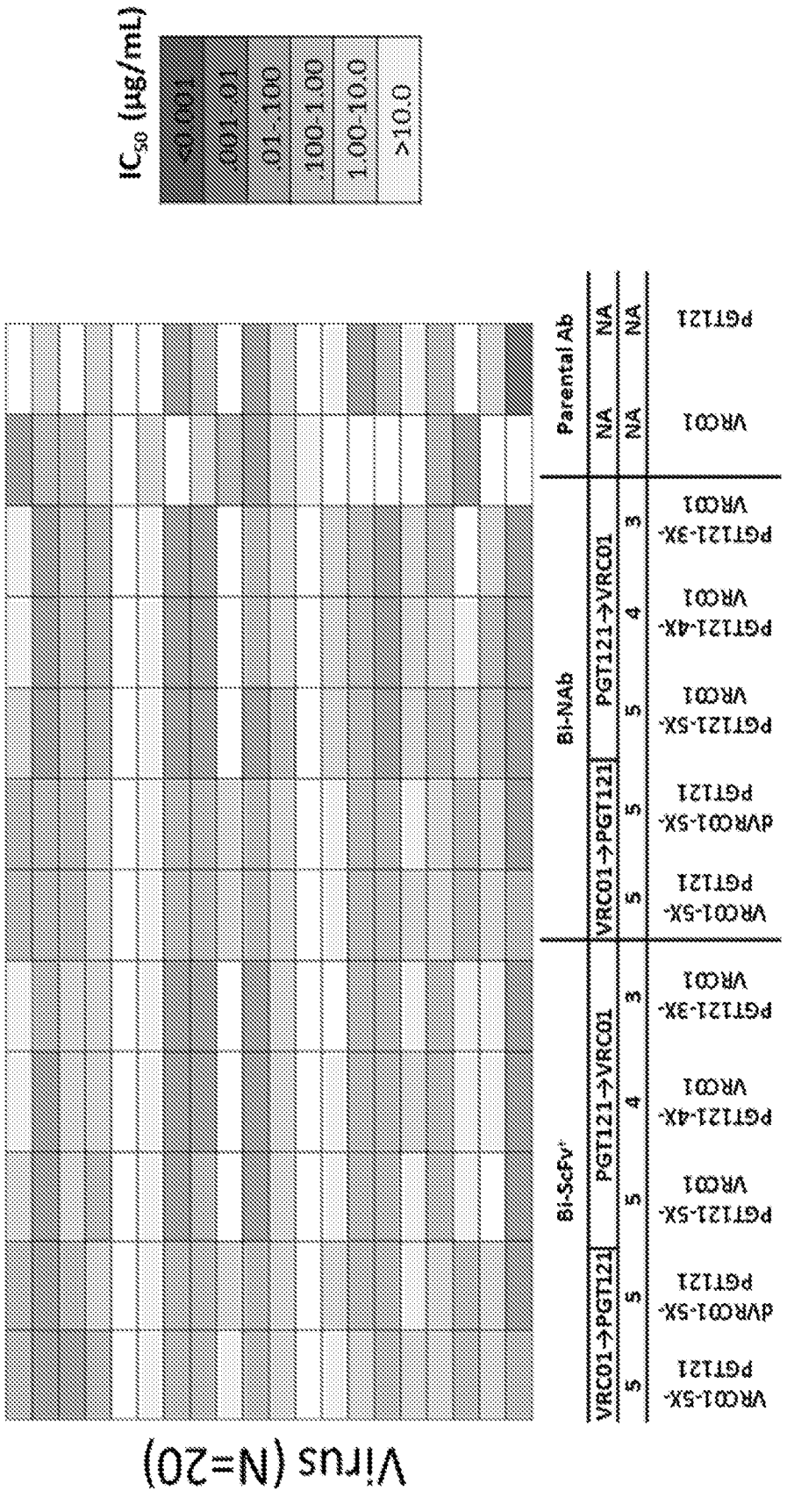

IC50 (µg/mL)
<.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0
Virus (N=20)
Bi-ScFv'
VRC01→PGT121
5
VRC01-5X-PGT121
dVRC01-5X-PGT121
PGT121→VRC01
4
PGT121-4X-VRC01
PGT121-5X-VRC01
3
PGT121-3X-VRC01
Bi-NAb
VRC01→PGT121
5
VRC01-5X-PGT121
dVRC01-5X-PGT121
PGT121→VRC01
4
PGT121-4X-VRC01
PGT121-5X-VRC01
3
PGT121-3X-VRC01
Parental Ab
NA
VRC01
PGT121

FIG. 15A

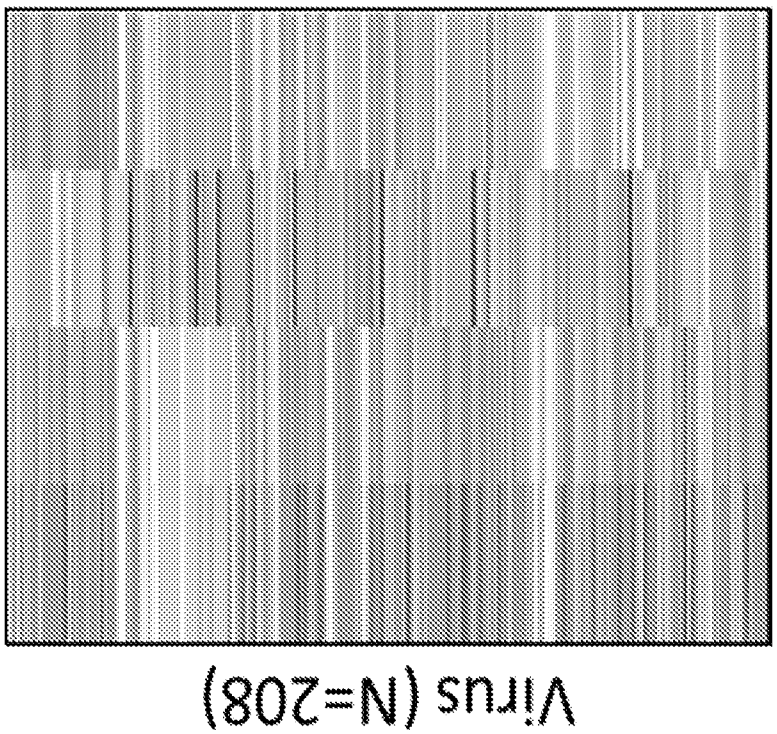
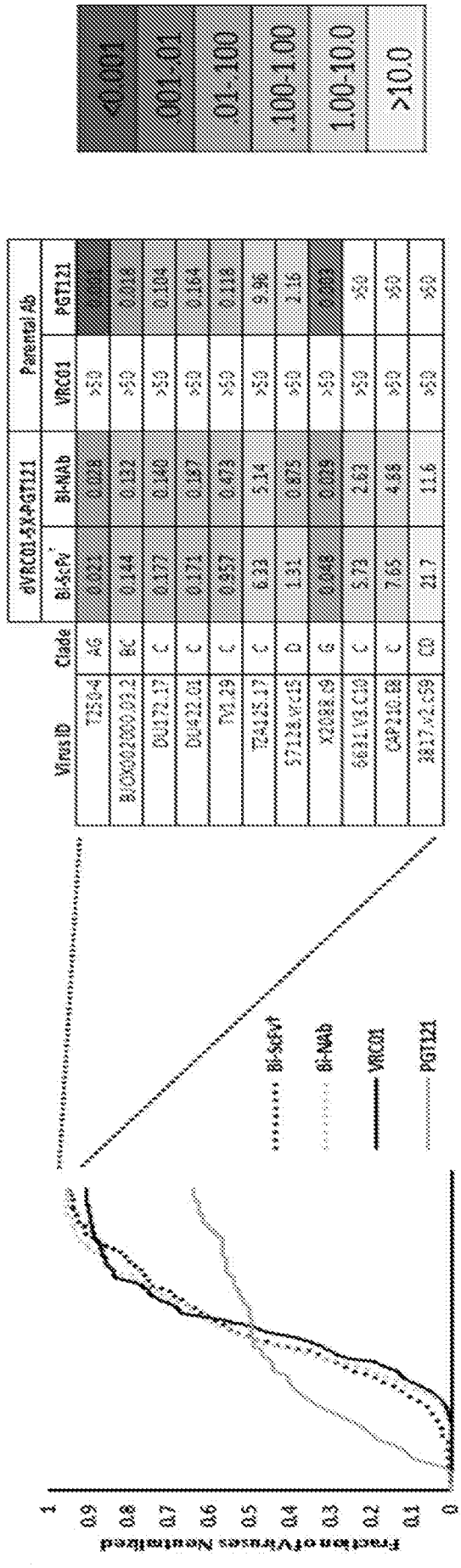


FIG. 15B



Virus ID	Clade	ΔVRC01-5X-PGT121		Parental Ab	
		Bi-ScFv'	Bi-NAb	VRC01	PGT121
T250-4	AG	0.023	0.024	>50	0.01
BJOX002000.02.2	BC	0.144	0.132	>50	0.018
DU172.17	C	0.117	0.146	>50	0.108
DU422.01	C	0.111	0.187	>50	0.164
TV1.29	C	0.057	0.073	>50	0.116
T2A125.17	C	0.23	0.14	>50	9.96
57128.vrc12	D	1.31	0.873	>50	2.15
X2088.09	G	0.048	0.024	>50	0.03
6631.V3.C10	C	5.73	2.83	>50	>50
CAP210.08	C	7.65	4.68	>50	>50
3817.v2.c59	CD	21.7	11.6	>50	>50

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 15C

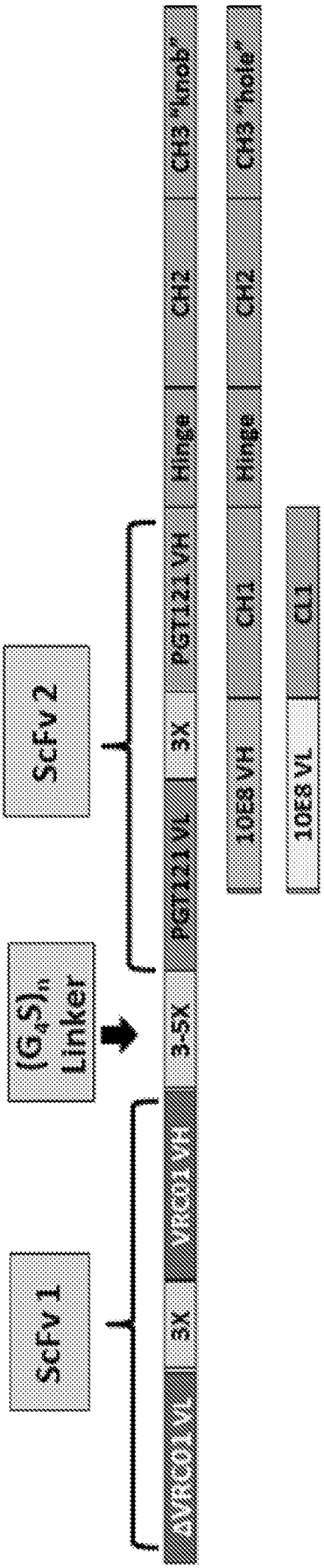

ScFv 1
ScFv 2
$(G_4S)_n$ Linker
AVR601 VL
3X
VRC01 VH
3-5X
PGT121 VL
3X
PGT121 VH
Hinge
CH2
CH3 "knob"
10E8 VH
CH1
Hinge
CH2
CH3 "hole"
10E8 VL
CL1

FIG. 16A

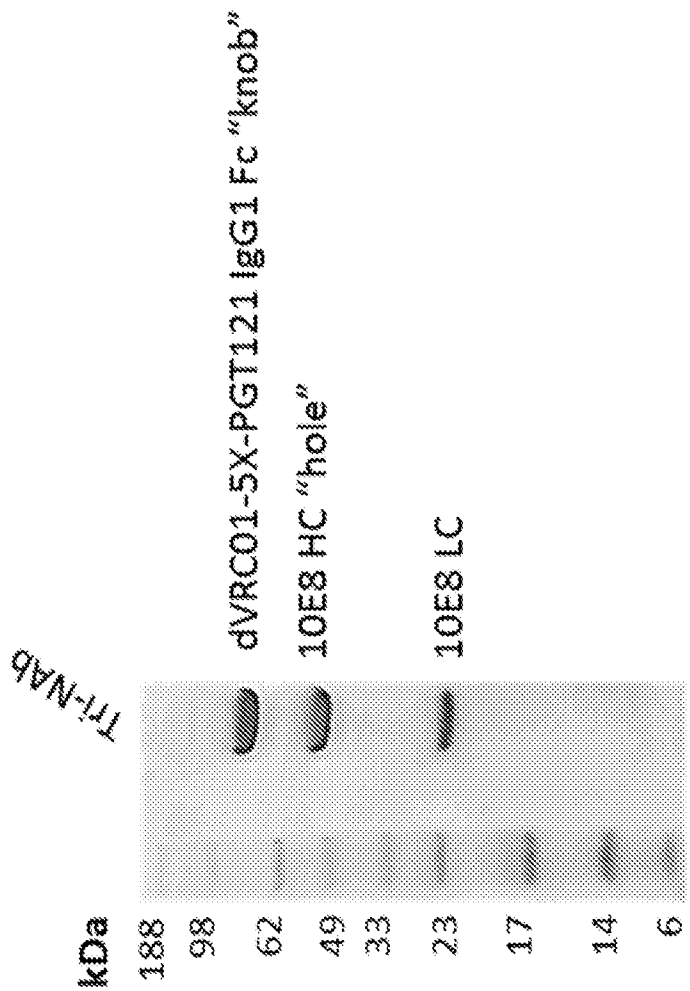

kDa
188
98
62
49
33
23
17
14
6
Tri-NAb
dVRC01-5X-PGT121 IgG1 Fc "knob"
10E8 HC "hole"
10E8 LC

FIG. 16B

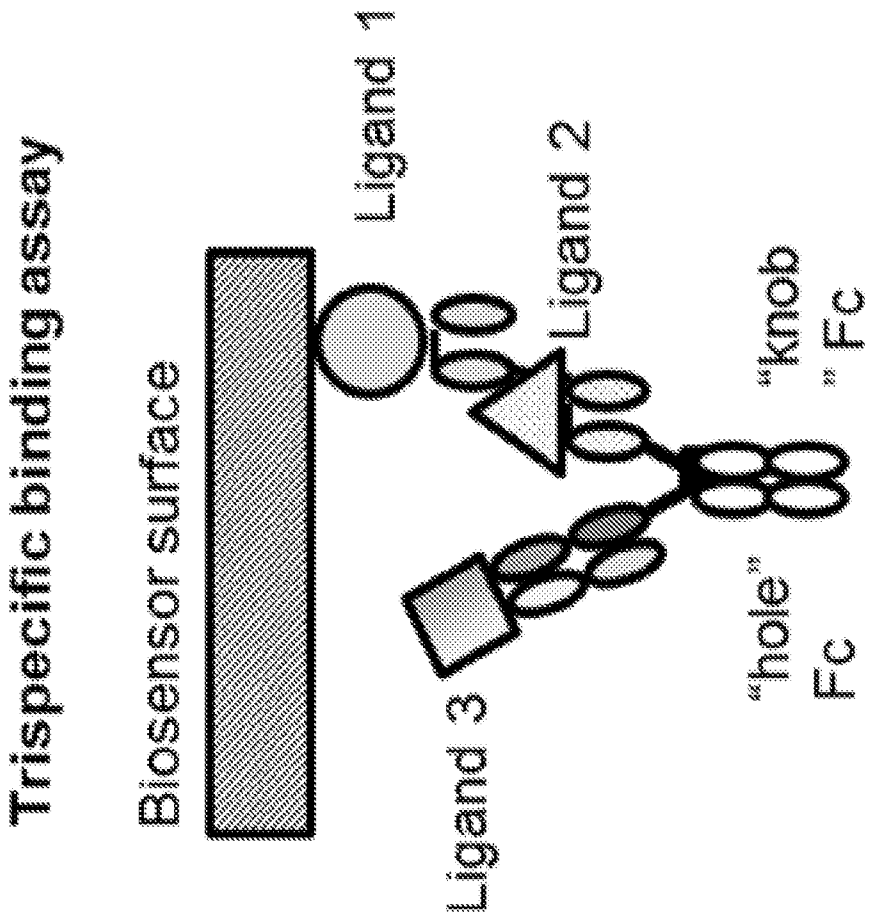

Trispecific binding assay
Biosensor surface
Ligand 1
Ligand 2
Ligand 3
"knob" Fc
"hole" Fc

FIG. 16C

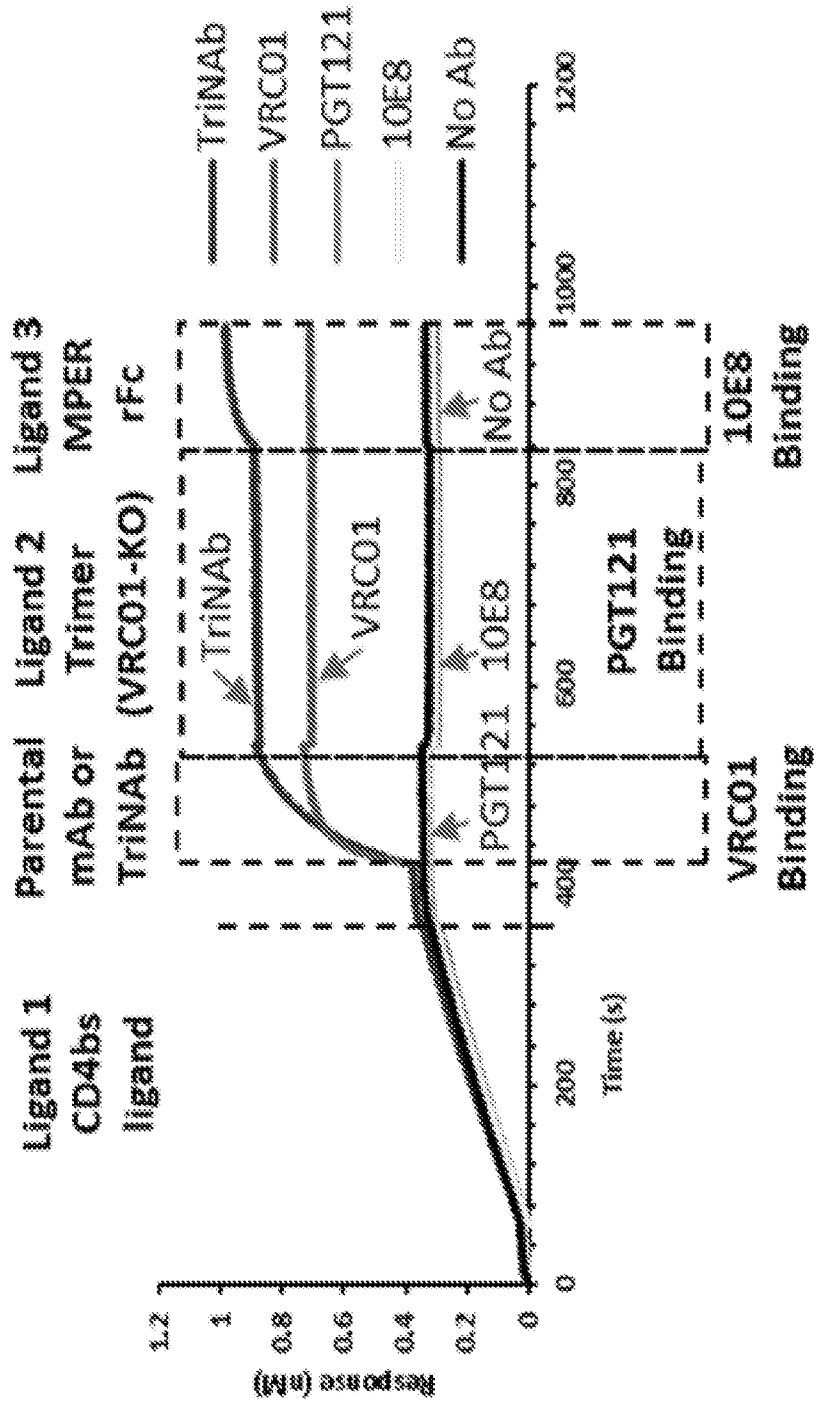

Ligand 1
CD4bs
ligand
Parental
mAb or
TriNAb
Ligand 2
Trimer
(VRC01-KO)
Ligand 3
MPER
rFc
TriNAb
VRC01
PGT121
10E8
No Ab
PGT121
Binding
VRC01
Binding
10E8
Binding
Response (nM)
Time (s)
0
0.2
0.4
0.6
0.8
1
1.2
200
400
600
800
1000
1200

FIG. 16D

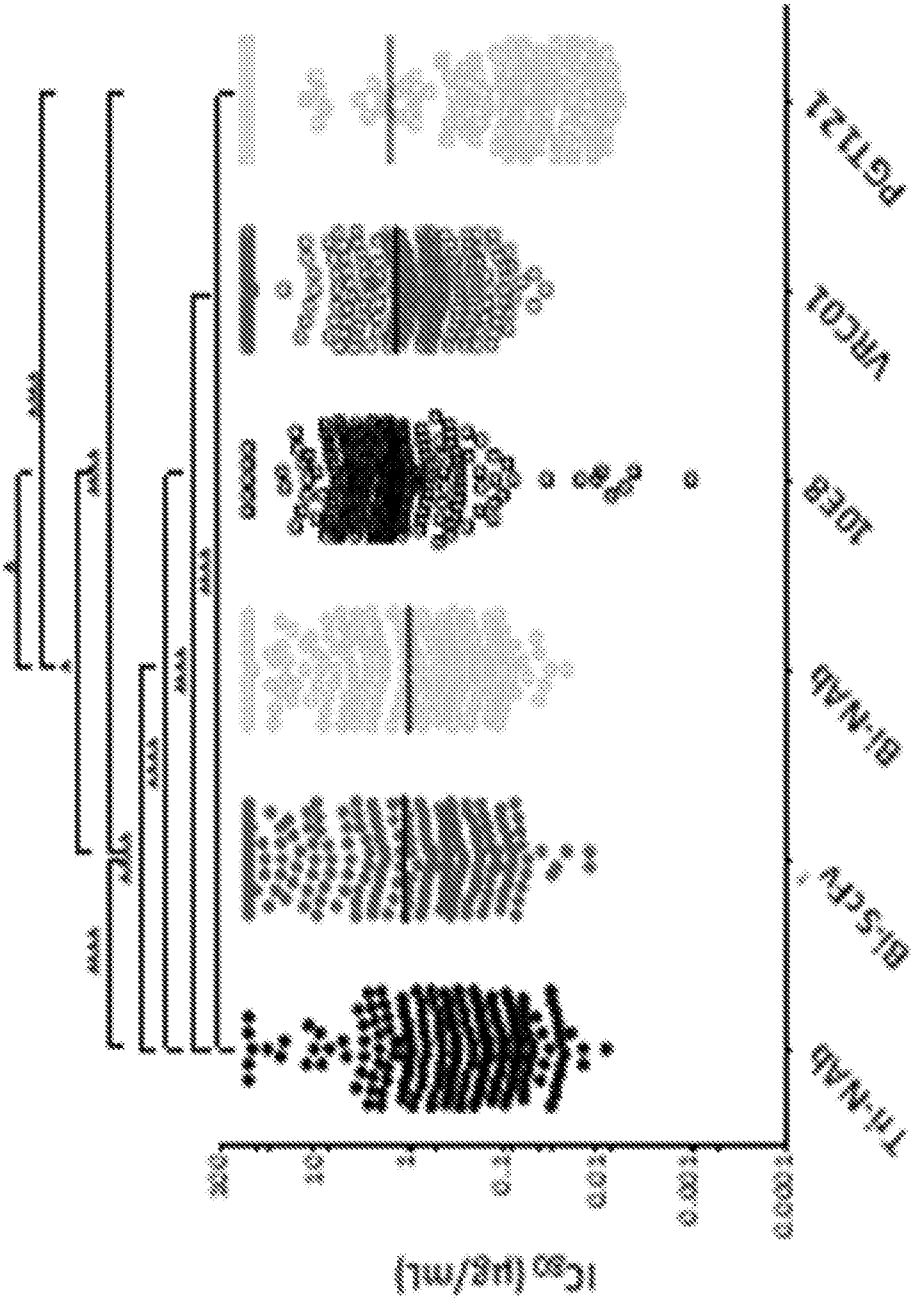

Th-hAb
Bl-5cFv
Bl-hAb
3DE8
VHCD1
PGT121
IC50 (μg/mL)
0.001
0.01
0.1
1
10
100
1000
***
***
***
***
***
***

FIG. 16E

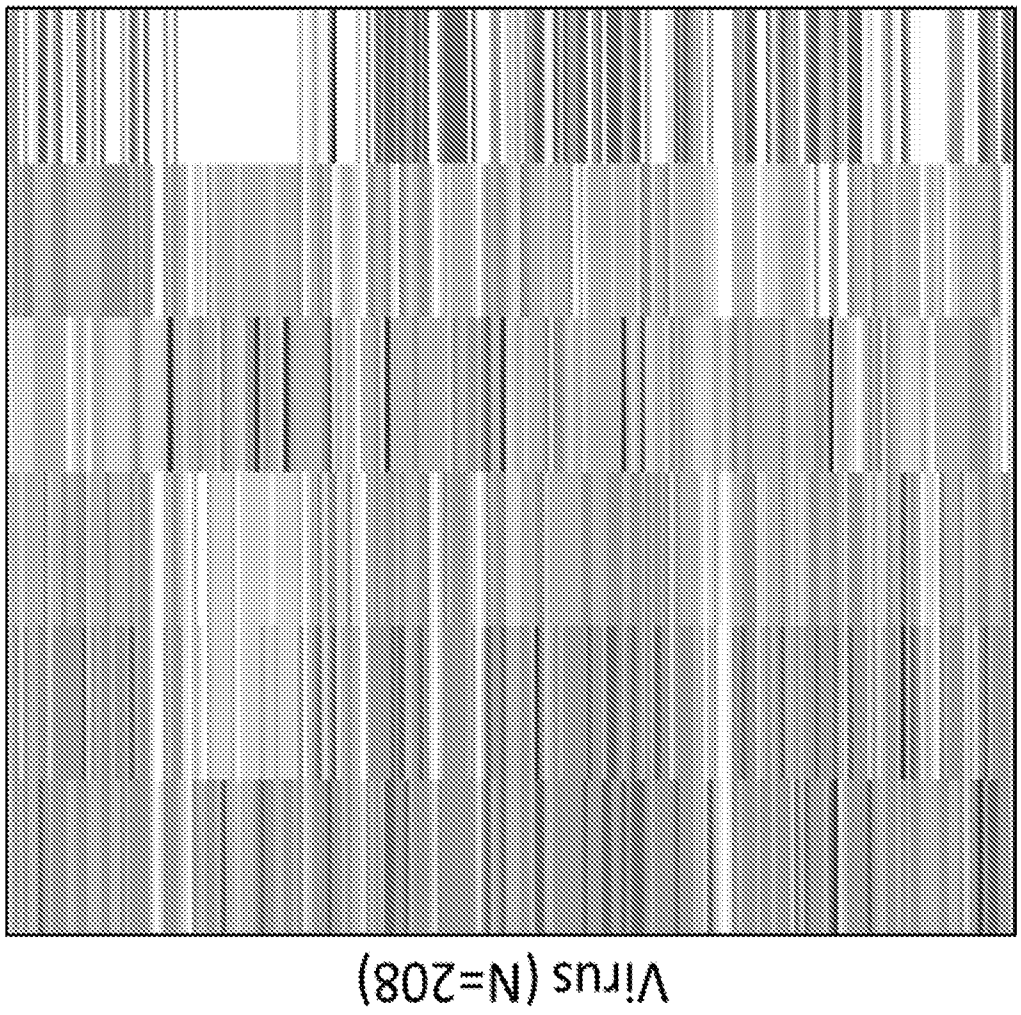


FIG. 17A

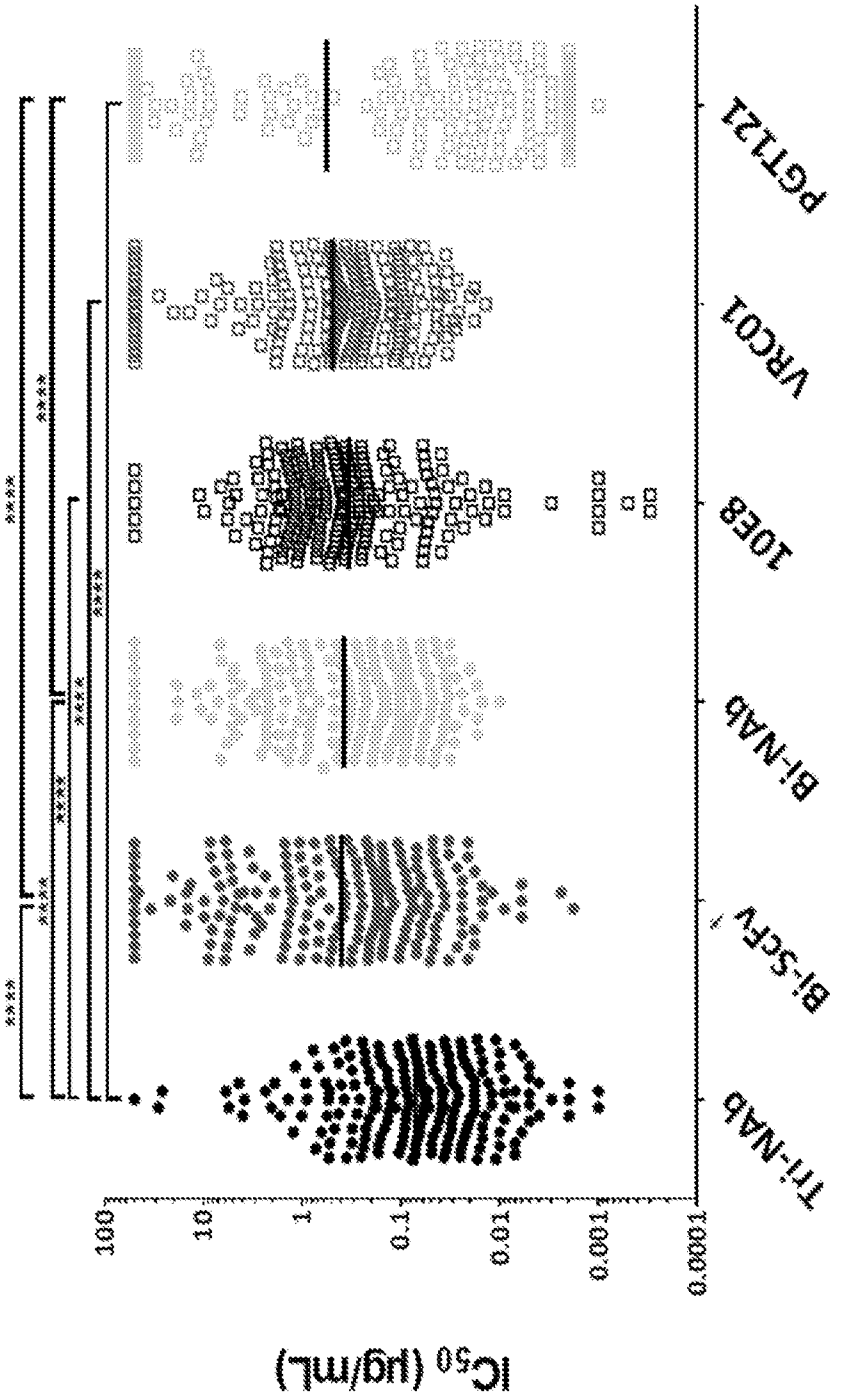
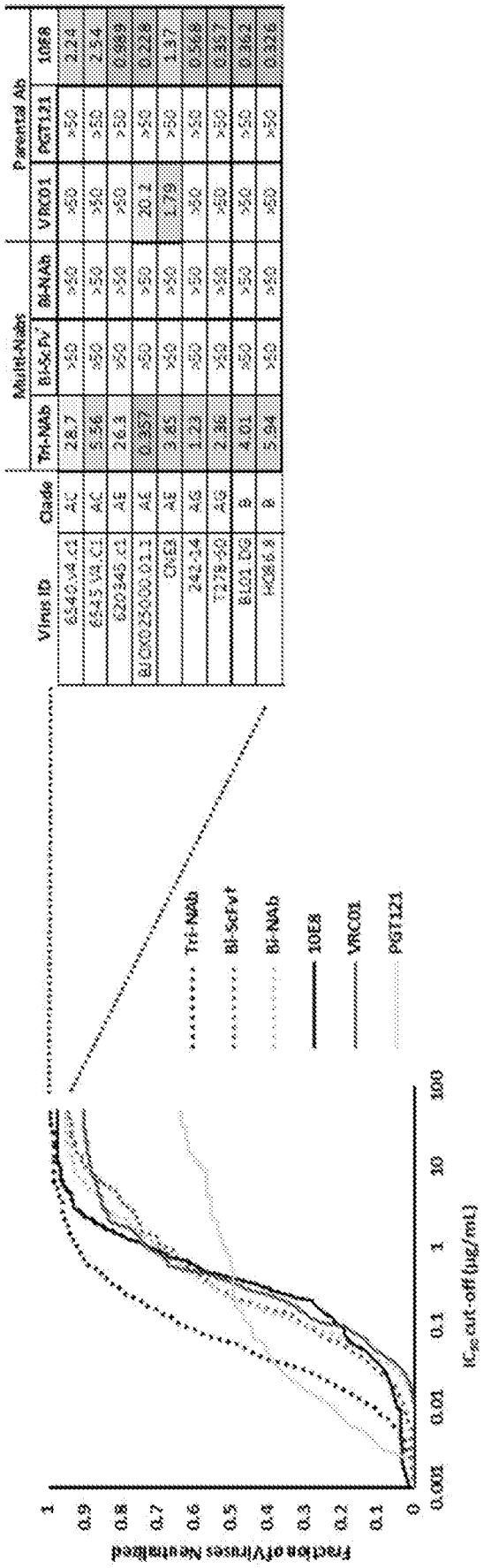

IC50 (μg/mL)
100
10
1
0.1
0.01
0.001
0.0001
Tri-NAb
Bi-SCFv
Bi-NAb
10E8
VRC01
PGT121
****
***

FIG. 17B



Virus ID	Clade	Multi-Nabs			Parental Ab		
		Tri-NAb	Bi-ScFv	Bi-NAb	VRC01	PGT121	10E8
6540.v4.c1	AC	28.7	>50	>50	>50	>50	2.23
6545.v4.c1	AC	5.56	>50	>50	>50	>50	2.54
620345.c1	AE	26.3	>50	>50	>50	>50	1.359
BJOX025000.01.1	AE	0.657	>50	>50	20.1	>50	0.226
CNE8	AE	3.45	>50	>50	1.79	>50	1.37
242.04	AG	1.23	>50	>50	>50	>50	0.536
7278.60	AG	2.36	>50	>50	>50	>50	0.317
6101.D0	B	4.01	>50	>50	>50	>50	0.842
HXB6.8	B	5.84	>50	>50	>50	>50	0.326

FIG. 17C

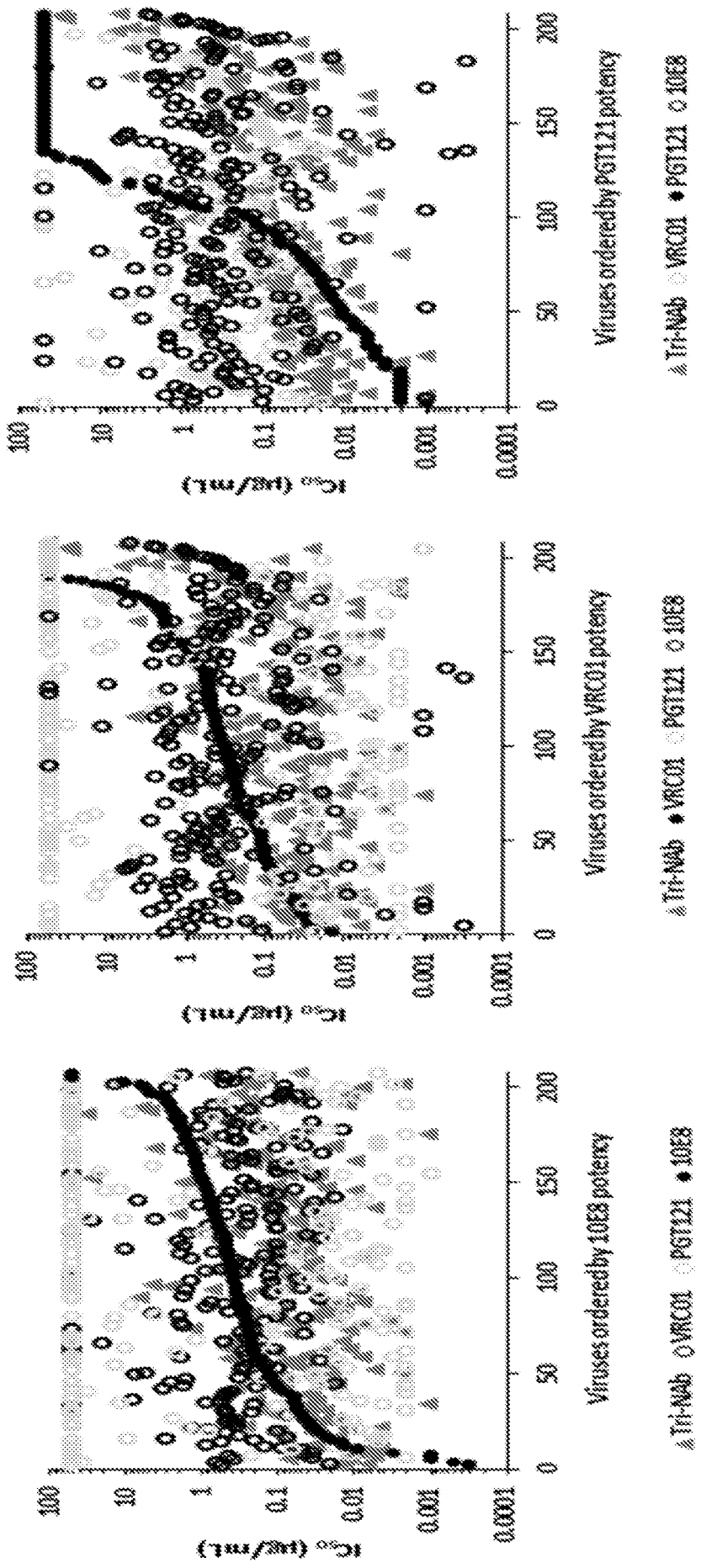

100
10
1
0.1
0.01
0.001
0.0001
IC50 (µg/mL)
0
50
100
150
200
Viruses ordered by 10E8 potency
Tri-MAb
VRC01
PGT121
10E8
Viruses ordered by VRC01 potency
Viruses ordered by PGT121 potency

FIG. 17D

		Potency (µg/mL)			
Sensitivity	N	**VRC01**	**PGT121**	**Bi-ScFv***	**Bi-NAb**
VRC01(S) × PGT121(S)	125	0.27	0.019	0.144 (2.1x, 0.1x)	0.131(1.9x, 0.1x)
VRC01(R) × PGT121(S)	8	>50	0.11	0.174 (0.7x)	0.164 (0.7x)
VRC01(S) × PGT121(R)	63	0.30	>50	1.46 (0.1x)	1.28 (0.2x)
VRC01(R) × PGT121(R)	12	>50	>50	21.7 (NA, NA)	4.9 (NA, NA)
All Viruses	208	0.29	0.013	0.210 (1.4x, 0.1x)	0.198 (1.3x, 0.1x)

Sensitivity	N	Potency (µg/mL)			
		VRC01	**PGT121**	**10E8**	**Tri-NAb**
VRC01(S) × PGT121(S) × 10E8(S)	122	0.274	0.013	0.392	0.037 (7.5x, 0.3x, 10.7x)
VRC01(R) × PGT121(S) × 10E8(S)	7	>50	0.03	0.224	0.98 (0.3x, 2.3x)
VRC01(S) × PGT121(R) × 10E8(S)	62	0.299	>50	0.394	0.170 (1.8x, 2.3x)
VRC01(S) × PGT121(S) × 10E8(R)	3	0.487	0.118	>50	0.131 (3.7x, 0.9x)
VRC01(R) × PGT121(R) × 10E8(S)	12	>50	>50	0.746	2.36 (0.4x)
VRC01(R) × PGT121(S) × 10E8(R)	1	>50	0.001	>50	0.017 (0.0x)
VRC01(S) × PGT121(R) × 10E8(R)	1	0.512	>50	>50	0.772 (0.7x)
VRC01(R) × PGT121(R) × 10E8(R)	0	NA	NA	NA	NA
All Viruses	208	0.2865	0.013	0.392	0.063 (4.5x, 0.2x, 6.2x)

FIG. 17E

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
0260.v5.c36	A	0.124	0.416	0.170	0.131	0.062	0.523	0.039	9.87	0.070
0330.v4.c3	A	0.082	0.241	0.092	0.085	0.054	0.066	0.041	1.12	>50
0439.v5.c1	A	1.57	2.93	0.471	1.02	0.216	0.228	>50	1.23	0.010
3365.v2.c20	A	0.070	0.232	0.078	0.077	0.068	0.060	0.059	1.60	>50
3415.v1.c1	A	0.354	0.847	0.312	0.425	0.300	0.087	>50	4.69	>50
3718.v3.c11	A	0.116	0.295	0.147	0.128	0.141	0.411	1.40	0.838	>50
398-F1_F6_20	A	0.015	0.147	0.005	0.017	0.019	0.102	0.002	0.704	>50
BB201.B42	A	0.015	0.063	0.049	0.054	0.051	0.262	0.003	0.613	>50
BB539.2B13	A	1.82	1.29	0.296	0.835	0.415	0.105	>50	0.591	>50
BG505.W6M.C2	A	0.048	0.134	0.089	0.131	0.077	0.037	0.032	0.689	>50
BI369.9A	A	0.087	0.103	0.072	0.237	0.081	0.047	0.008	0.356	0.007
BS208.B1	A	0.061	0.096	0.026	0.034	0.033	0.027	>50	0.319	>50
KER2008.12	A	0.067	0.233	0.131	0.148	0.033	0.487	2.22	>50	0.00003
KER2018.11	A	0.064	0.271	0.211	0.279	0.131	0.348	>50	1.89	0.005
KNH1209.18	A	0.014	0.049	0.034	0.051	0.045	0.119	0.002	0.406	0.040
MB201.A1	A	0.008	0.035	0.030	0.023	0.023	0.241	0.005	0.411	>50
MB539.2B7	A	1.94	4.16	0.772	2.33	1.14	0.512	>50	>50	1.49
MI369.A5	A	0.056	0.155	0.107	0.150	0.075	0.236	0.022	0.671	0.004
MS208.A1	A	0.504	0.994	0.405	0.755	1.17	0.174	>50	0.187	>50
Q23.17	A	0.060	0.131	0.038	0.041	0.036	0.099	0.004	0.461	0.003
Q259.17	A	0.013	0.043	0.042	0.046	0.041	0.085	>50	4.76	>50
Q769.d22	A	0.175	0.489	0.315	0.254	0.149	0.036	>50	1.91	0.053
Q769.h5	A	0.094	0.325	0.229	0.168	0.126	0.072	>50	2.89	0.635
Q842.d12	A	0.030	0.139	0.048	0.039	0.027	0.034	0.016	2.82	0.006
QH209.14M.A2	A	0.175	0.456	0.168	0.161	0.070	0.026	>50	1.30	>50
RW020.2	A	0.006	0.032	0.021	0.033	0.022	0.217	0.002	0.902	0.009
UG037.8	A	0.104	0.333	0.092	0.138	0.084	0.073	0.065	0.048	0.063
246-F3.C10.2	AC	0.028	0.092	0.037	0.039	0.017	0.254	>50	0.210	0.021
3301.V1.C24	AC	0.029	0.085	0.070	0.075	0.066	0.095	0.009	2.97	0.057
3589.V1.C4	AC	0.127	0.475	0.208	0.230	0.112	0.081	>50	5.77	0.004
6540.v4.c1	AC	>50	>50	28.7	>50	>50	>50	>50	2.24	>50

IC50 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (1/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
6545.V4.C1	AC	>50	>50	5.56	9.91	1.51	>50	>50	2.54	>50
0815.V3.C3	ACD	0.031	0.109	0.058	0.071	0.033	0.029	0.020	0.491	0.002
6095.V1.C10	ACD	0.146	0.331	0.012	0.047	0.063	0.631	37.3	0.0005	>50
3468.V1.C12	AD	0.015	0.045	0.025	0.021	0.019	0.058	0.042	0.381	0.005
Q168.a2	AD	0.067	0.141	0.136	0.184	0.095	0.101	>50	0.463	0.051
Q461.e2	AD	3.28	6.73	4.41	5.04	0.208	0.420	>50	2.29	0.0010
620345.c1	AE	>50	>50	26.3	>50	>50	>50	>50	0.989	>50
BJOX009000.02.4	AE	0.228	0.654	0.043	0.115	0.180	1.74	14.7	0.251	>50
BJOX010000.06.2	AE	4.85	18.8	0.177	0.409	0.326	8.40	>50	0.060	3.00
BJOX025000.01.1	AE	>50	>50	0.357	0.113	0.100	20.2	>50	0.228	3.00
BJOX028000.10.3	AE	0.152	0.285	0.076	0.071	0.036	0.188	>50	0.167	0.004
C1080.c3	AE	6.87	5.02	0.077	0.089	0.071	2.63	>50	0.108	0.005
C2101.c1	AE	1.47	1.86	0.138	0.093	0.051	0.269	>50	1.20	0.007
C3347.c11	AE	1.77	1.73	0.005	0.031	0.057	0.213	>50	0.019	0.004
C4118.09	AE	2.00	2.26	0.113	0.077	0.020	0.285	>50	0.421	0.0003
CM244.ec1	AE	0.237	0.867	0.117	0.142	0.139	0.116	>50	0.365	0.023
CNE3	AE	>50	>50	3.85	3.12	0.479	1.79	>50	1.37	0.020
CNE5	AE	1.01	4.59	0.183	0.140	0.192	0.398	>50	1.17	>50
CNE55	AE	1.78	5.74	0.233	0.340	0.229	0.358	>50	0.038	>50
CNE56	AE	2.74	6.54	0.476	1.02	0.754	0.525	>50	0.060	>50
CNE59	AE	2.83	2.26	0.065	0.331	0.603	0.368	>50	0.0010	>50
CNE8	AE	0.356	1.14	0.005	0.035	0.095	0.299	>50	0.140	>50
M02138	AE	4.92	7.18	0.031	0.138	0.221	0.898	>50	0.014	>50
R1166.c1	AE	4.58	10.6	0.035	0.124	0.275	2.09	>50	0.488	>50
R2184.c4	AE	0.791	2.33	0.557	0.859	0.525	0.106	>50	0.576	0.052
R3265.c6	AE	2.87	6.81	0.894	1.16	0.773	0.382	>50	1.58	>50
TH023.6	AE	5.07	1.05	0.015	0.042	0.056	0.546	>50	0.0003	0.086
TH966.8	AE	2.29	3.47	0.027	0.135	0.227	0.390	>50	0.039	>50
TH976.17	AE	1.05	2.96	0.256	0.370	0.282	0.299	>50	0.392	1.00
235-47	AG	0.071	0.246	0.067	0.094	0.096	0.043	0.110	0.244	>50
242-14	AG	33.2	>50	1.23	19.3	3.01	>50	>50	0.568	>50

IC50 (ug/mL)

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (2/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
263-8	AG	0.250	0.683	0.227	0.133	0.112	0.176	1.23	0.229	>50
269-12	AG	0.056	0.239	0.083	0.069	0.055	0.313	0.164	0.124	>50
271-11	AG	0.008	0.032	0.020	0.024	0.012	0.059	11.7	0.891	0.080
928-28	AG	1.16	2.63	0.088	0.206	0.349	0.394	31.0	0.079	>50
DJ263.8	AG	0.016	0.100	0.006	0.038	0.055	0.047	0.064	0.009	0.030
T250-4	AG	0.007	0.028	0.025	0.015	0.016	>50	0.0010	1.07	>50
T251-18	AG	2.86	8.32	0.052	0.064	0.048	4.21	10.8	0.666	>50
T253-11	AG	0.217	2.29	0.239	0.243	0.128	0.397	>50	1.21	2.89
T255-34	AG	0.034	0.089	0.013	0.016	0.016	0.500	>50	0.228	0.005
T257-31	AG	0.480	1.71	0.247	0.260	0.194	1.72	>50	0.336	>50
T266-60	AG	0.534	1.40	0.684	0.418	0.282	1.81	0.160	>50	>50
T278-50	AG	>50	>50	2.36	1.51	0.165	>50	>50	0.357	2.27
T280-5	AG	0.014	0.050	0.024	0.047	0.018	0.032	0.002	0.715	>50
T33-7	AG	0.088	0.180	0.105	0.079	0.044	0.018	>50	0.818	1.00
3988.25	B	0.021	0.058	0.033	0.045	0.055	0.494	0.002	0.070	0.609
5768.04	B	0.042	0.108	0.124	0.108	0.074	0.365	0.039	1.63	0.074
6101.10	B	0.018	0.036	0.010	0.016	0.013	0.035	0.002	0.0010	>50
6535.3	B	0.006	0.031	0.007	0.017	0.023	1.93	0.003	0.190	>50
7165.18	B	0.085	0.196	0.055	0.100	0.116	28.2	0.019	0.659	>50
45_01dG5	B	0.008	0.051	0.015	0.035	0.018	0.018	0.002	0.106	>50
89.6.DG	B	0.124	0.160	0.007	0.020	0.023	0.762	0.016	0.318	>50
AC10.29	B	0.179	0.534	0.128	0.289	0.308	1.81	0.028	0.102	>50
ADA.DG	B	0.075	0.193	0.022	0.084	0.082	0.470	0.002	0.055	>50
Bal.01	B	0.007	0.028	0.031	0.032	0.014	0.095	0.011	0.421	0.002
BaL.26	B	0.023	0.060	0.036	0.062	0.031	0.042	0.010	0.518	0.0010
BG1168.01	B	2.03	2.27	0.580	1.54	1.46	0.869	>50	0.396	>50
BL01.DG	B	>50	>50	4.01	10.5	1.70	>50	>50	0.362	0.009
BR07.DG	B	0.162	0.338	0.031	0.070	0.049	1.57	0.064	0.118	0.002
BX08.16	B	0.059	0.052	0.020	0.077	0.082	0.274	0.002	0.213	0.040
CAAN.A2	B	0.052	0.104	0.058	0.040	0.026	1.03	0.005	1.45	0.0010
CNE10	B	0.024	0.090	0.018	0.029	0.027	0.565	0.005	0.014	>50

IC50 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (3/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CNE12	B	0.023	0.073	0.021	0.031	0.025	0.866	0.002	0.301	0.010
CNE14	B	0.012	0.048	0.018	0.021	0.018	0.275	0.002	0.151	0.004
CNE4	B	0.353	0.966	0.063	0.187	0.100	0.910	11.5	0.059	0.010
CNE57	B	0.050	0.155	0.029	0.075	0.066	0.563	0.006	0.059	>50
HO86.8	B	>50	>50	5.94	4.32	0.527	>50	>50	0.326	>50
HT593.1	B	0.445	1.10	0.094	0.370	0.416	0.476	>50	0.049	>50
HXB2.DG	B	0.003	0.065	0.008	0.019	0.017	0.034	>50	0.003	17.3
JRCSF.JB	B	0.036	0.278	0.158	0.167	0.146	0.362	0.061	0.429	0.070
JRFL.JB	B	0.021	0.087	0.053	0.081	0.055	0.028	0.017	0.174	0.020
MN.3	B	0.009	0.057	0.006	0.027	0.036	0.020	>50	0.0003	0.009
PVO.04	B	0.497	1.28	0.732	0.858	0.302	0.511	0.132	1.60	45.0
QH0515.01	B	0.462	1.29	0.535	0.445	0.364	1.01	8.70	2.25	>50
QH0692.42	B	0.966	1.70	0.331	0.514	0.323	1.54	0.940	0.531	0.100
REJO.67	B	0.046	0.178	0.072	0.091	0.049	0.075	8.87	0.302	0.003
RHPA.7	B	0.035	0.110	0.065	0.088	0.088	0.034	0.014	1.01	>50
SC422.8	B	0.167	0.488	0.166	0.220	0.198	0.127	0.098	0.343	>50
SF162.LS	B	0.0006	0.017	0.010	0.007	0.005	0.207	0.004	0.245	>50
SS1196.01	B	0.017	0.063	0.002	0.011	0.018	0.304	0.002	0.244	0.068
THRO.18	B	1.70	2.60	0.316	0.664	0.458	3.16	>50	0.092	>50
TRJO.58	B	0.321	0.820	0.377	0.411	0.088	0.101	4.31	1.13	0.0010
TRO.11	B	0.033	0.138	0.033	0.058	0.066	0.469	0.006	0.028	>50
WITO.33	B	0.298	0.893	0.086	0.160	0.123	0.102	0.787	0.031	7.24
X2278.C2.B6	B	0.064	0.188	0.051	0.082	0.020	0.151	0.007	0.442	0.0001
YU2.DG	B	0.047	0.200	0.049	0.074	0.070	0.076	0.068	1.17	>50
BJOX002000.03.2	BC	0.048	0.132	0.066	0.081	0.081	>50	0.018	0.384	45.0
CH038.12	BC	0.043	0.104	0.024	0.033	0.043	0.447	0.004	0.271	>50
CH070.1	BC	0.039	0.096	0.002	0.011	0.027	14.0	0.003	6.65	>50
CH117.4	BC	0.008	0.059	0.018	0.021	0.013	0.105	>50	0.270	>50
CH119.10	BC	0.070	0.173	0.023	0.041	0.033	0.833	0.029	0.591	38.2
CH181.12	BC	0.048	0.124	0.063	0.061	0.053	0.487	0.007	0.754	10.6
CNE15	BC	0.105	0.359	0.009	0.018	0.024	0.141	19.0	0.844	>50

IC50 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (4/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CNE19	BC	0.002	0.020	0.007	0.009	0.004	0.247	0.007	0.251	0.0010
CNE20	BC	0.002	0.017	0.018	0.028	0.023	7.39	0.002	0.131	>50
CNE21	BC	0.026	0.098	0.031	0.041	0.042	0.274	0.004	0.979	0.062
CNE40	BC	0.234	0.440	0.005	0.025	0.063	0.433	0.224	0.0010	0.006
CNE7	BC	0.055	0.175	0.033	0.098	0.091	0.187	0.032	0.130	0.003
286.36	C	0.005	0.037	0.009	0.015	0.012	0.223	0.002	1.19	0.009
288.38	C	0.030	0.085	0.007	0.023	0.027	1.38	0.006	0.435	>50
0013095-2.11	C	0.306	0.864	0.004	0.011	0.014	0.086	>50	0.009	>50
001428-2.42	C	0.018	0.085	0.038	0.035	0.033	0.014	0.023	1.71	>50
0077_V1.C16	C	0.224	0.482	0.171	0.185	0.147	1.13	>50	1.86	0.005
00836-2.5	C	0.005	0.047	0.024	0.014	0.013	0.122	31.8	0.666	>50
0921.V2.C14	C	0.050	0.135	0.093	0.092	0.064	0.230	>50	0.908	>50
16055-2.3	C	0.016	0.070	0.047	0.048	0.041	0.100	1.02	1.10	>50
16845-2.22	C	2.99	5.21	0.068	0.189	0.510	2.95	9.41	0.020	>50
16936-2.21	C	0.011	0.038	0.003	0.010	0.009	0.154	0.003	0.264	0.0010
25710-2.43	C	0.034	0.154	0.037	0.055	0.049	0.487	0.014	0.064	>50
25711-2.4	C	0.028	0.103	0.063	0.038	0.049	0.559	0.010	0.516	>50
25925-2.22	C	0.060	0.188	0.072	0.076	0.061	0.550	0.024	0.402	>50
26191-2.48	C	0.107	0.406	0.136	0.142	0.115	0.183	0.150	1.83	>50
3168.V4.C10	C	0.638	1.68	0.544	0.583	0.232	0.129	0.485	2.83	0.004
3637.V5.C3	C	11.4	18.9	2.07	8.72	6.20	1.97	>50	2.12	>50
3873.V1.C24	C	0.322	1.48	0.007	0.031	0.068	2.81	0.015	5.51	>50
426c	C	0.419	0.953	0.429	0.466	0.298	1.93	>50	0.445	>50
6322.V4.C1	C	15.2	5.00	1.67	0.058	0.029	>50	>50	0.923	>50
6471.V1.C16	C	>50	>50	>50	>50	>50	>50	>50	4.98	>50
6631.V3.C10	C	1.91	2.63	1.39	1.82	0.976	>50	>50	0.934	>50
6644.V2.C33	C	0.021	0.143	0.027	0.044	0.025	0.153	0.018	0.013	0.037
6785.V5.C14	C	0.059	0.149	0.134	0.125	0.066	0.253	0.019	0.701	>50
6838.V1.C35	C	0.002	0.015	0.012	0.014	0.009	0.288	0.119	0.292	>50
96ZM651.02	C	0.026	0.070	0.011	0.027	0.035	0.807	0.009	0.033	>50
BR025.9	C	0.019	0.033	0.014	0.015	0.013	0.528	0.002	0.307	0.005

IC50 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (5/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CAP210.E8	C	2.55	4.88	0.526	0.303	0.231	>50	>50	0.474	0.019
CAP244.D3	C	0.455	1.28	0.015	0.034	0.057	1.34	>50	0.369	>50
CAP256.206.C9	C	0.054	0.102	0.049	0.045	0.021	1.07	0.010	0.713	0.033
CAP45.G3	C	0.166	0.373	0.168	0.122	0.083	6.75	2.08	0.722	0.016
Ce1176.A3	C	0.051	0.120	0.071	0.055	0.049	1.85	0.016	0.252	0.005
CE703010217.B6	C	0.009	0.038	0.018	0.023	0.019	0.195	0.002	0.096	>50
CNE30	C	0.224	0.609	0.094	0.152	0.172	0.693	0.061	0.456	>50
CNE31	C	0.713	2.30	1.01	0.628	0.382	0.772	0.789	1.32	>50
CNE53	C	0.050	0.106	0.010	0.028	0.038	0.112	0.022	0.213	>50
CNE58	C	0.970	2.32	0.366	0.735	0.651	0.252	>50	0.229	0.070
DU123.06	C	0.053	0.199	0.002	0.021	0.044	5.70	0.033	0.132	>50
DU151.02	C	0.011	0.044	0.059	0.085	0.067	10.5	0.005	0.461	8.00
DU156.12	C	0.005	0.036	0.015	0.036	0.020	0.077	0.005	0.023	>50
DU172.17	C	0.059	0.140	0.039	0.061	0.112	>50	0.104	0.057	>50
DU422.01	C	0.057	0.187	0.182	0.290	0.267	>50	0.164	0.224	>50
MW965.26	C	0.012	0.051	0.005	0.011	0.022	0.043	0.011	0.0010	5.60
SO18.18	C	0.004	0.023	0.0010	0.005	0.007	0.052	0.002	1.60	>50
TV1.29	C	0.319	0.473	1.17	1.57	0.774	>50	0.118	0.248	>50
TZA125.17	C	2.11	5.14	0.428	0.557	0.746	>50	9.96	0.217	>50
TZBD.02	C	0.007	0.035	0.017	0.023	0.022	0.043	0.005	1.41	>50
ZA012.29	C	0.023	0.062	0.060	0.064	0.059	0.327	0.005	1.47	>50
ZM106.9	C	0.023	0.089	0.049	0.070	0.045	0.264	0.005	>50	>50
ZM109.4	C	0.091	0.351	0.060	0.094	0.077	0.142	13.7	0.161	>50
ZM135.10a	C	1.20	2.12	0.004	0.019	0.076	1.40	1.50	0.033	>50
ZM176.66	C	0.071	0.221	0.030	0.071	0.060	0.045	13.8	0.267	>50
ZM197.7	C	1.14	4.15	0.161	0.410	0.484	0.532	>50	0.055	>50
ZM214.15	C	0.857	2.30	0.533	0.509	0.417	0.957	0.682	2.22	>50
ZM215.8	C	0.021	0.100	0.023	0.031	0.021	0.362	0.014	0.044	0.015
ZM233.6	C	0.070	0.268	0.090	0.112	0.054	1.98	4.14	0.270	0.005
ZM249.1	C	0.486	0.810	0.279	0.211	0.056	0.107	>50	0.830	0.0010
ZM53.12	C	0.0008	0.010	0.011	0.014	0.010	0.702	0.002	2.62	>50

IC50 (ug/mL)

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (6/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/ dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
ZM55.28a	C	0.137	0.407	0.212	0.240	0.155	0.241	0.070	2.34	>50
3326.V4.C3	CD	0.008	0.017	0.018	0.020	0.017	0.107	>50	1.40	>50
3337.V2.C6	CD	0.051	0.110	0.039	0.054	0.037	0.105	21.1	1.09	0.0009
3817.v2.c59	CD	7.23	11.6	0.230	0.446	0.407	>50	>50	0.229	>50
191821.E6.1	D	0.487	1.57	0.921	0.724	0.342	0.438	>50	1.91	>50
231965.c1	D	1.31	1.41	0.261	0.269	0.074	0.392	>50	11.0	>50
247-23	D	19.5	18.3	0.639	0.695	0.113	1.63	>50	0.344	0.0010
3016.v5.c45	D	0.043	0.078	0.037	0.052	0.041	0.117	>50	0.710	>50
57128.vrc15	D	0.438	0.875	0.098	0.136	0.129	>50	2.16	0.212	0.043
6405.v4.c34	D	0.092	0.259	0.103	0.104	0.056	1.69	0.019	0.461	0.004
A03349M1.vrc4a	D	0.071	0.194	0.189	0.136	0.048	4.42	0.013	0.270	0.0009
A07412M1.vrc12	D	0.011	0.057	0.041	0.037	0.017	0.101	0.012	0.140	>50
NKU3006.ec1	D	3.28	8.54	1.91	3.65	0.904	0.460	>50	0.673	0.004
UG021.16	D	0.275	0.340	0.016	0.055	0.083	0.451	2.41	0.046	>50
UG024.2	D	2.28	0.830	0.035	0.119	0.126	0.219	>50	0.053	>50
P0402.c2.11	G	0.024	0.081	0.0010	0.020	0.025	0.207	0.004	0.057	0.006
P1981.C5.3	G	0.006	0.024	0.025	0.052	0.032	0.336	0.004	0.024	0.0003
X1193.c1	G	0.128	0.239	0.009	0.032	0.051	0.124	0.028	0.341	0.020
X1254.c3	G	0.072	0.188	0.010	0.054	0.062	0.055	0.024	3.67	>50
X1632.S2.B10	G	0.088	0.178	0.065	0.069	0.056	0.131	>50	0.387	0.564
X2088.c9	G	0.016	0.029	0.017	0.033	0.032	>50	0.003	>50	>50
X2131.C1.B5	G	0.023	0.079	0.016	0.031	0.026	0.467	0.010	0.039	>50
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50

IC50 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 18 (7/8)

	Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/dVRC01-5X-PGT121	35O22-5X-10E8/dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
# Viruses	208	208	208	208	208	208	208	208	208
Total VS Neutralized									
IC50 <50ug/ml	199	198	207	205	205	188	133	203	94
IC50 <10ug/ml	195	193	205	203	205	184	122	202	89
IC50 <1.0ug/ml	162	147	192	189	198	152	108	152	79
IC50 <0.1ug/ml	112	58	131	117	133	40	93	42	75
IC50 <0.01ug/ml	23	0	24	3	5	0	47	10	45
% VS Neutralized									
IC50 <50ug/ml	95.7	95.2	99.5	98.6	98.6	90	64	97.6	45
IC50 <10ug/ml	94	93	99	98	99	88	59	97	43
IC50 <1.0ug/ml	78	71	92	91	95	73	52	73	38
IC50 <0.1ug/ml	54	28	63	56	64	19	45	20	36
IC50 <0.01ug/ml	11	0	12	1	2	0	23	5	22
Median IC50	0.071	0.198	0.063	0.079	0.063	0.287	0.019	0.392	0.010
Geometric Mean	0.108	0.297	0.069	0.101	0.077	0.301	0.045	0.299	0.025
Note: Median and Geometric Mean titers are calculated only for samples with IC50 <50ug/ml									

FIG. 18 (8/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/dVRC01-5X-PGT121	35O22-5X-10E8/dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
0260.v5.c36	A	0.378	0.958	0.716	0.498	0.224	1.35	0.143	21.7	>50
0330.v4.c3	A	0.216	0.619	0.403	0.391	0.216	0.202	0.194	3.64	>50
0439.v5.c1	A	2.60	7.07	2.27	3.39	0.829	0.438	>50	3.95	>50
3365.v2.c20	A	0.150	0.534	0.301	0.273	0.210	0.125	1.34	4.56	>50
3415.v1.c1	A	0.752	2.18	1.40	1.33	0.850	0.177	>50	11.5	>50
3718.v3.c11	A	0.337	0.685	0.573	0.470	0.448	5.58	8.64	4.42	>50
398-F1_F6_20	A	0.049	0.322	0.027	0.066	0.062	0.479	0.011	6.17	>50
BB201.B42	A	0.053	0.137	0.161	0.182	0.148	0.614	0.011	1.96	>50
BB539.2B13	A	3.17	3.46	1.97	2.73	2.02	0.407	>50	13.0	>50
BG505.W6M.C2	A	0.130	0.338	0.293	0.457	0.276	0.125	0.256	2.14	>50
BI369.9A	A	0.418	0.563	0.340	1.19	0.477	0.532	0.043	1.29	>50
BS208.B1	A	0.139	0.245	0.114	0.124	0.125	0.080	>50	3.27	>50
KER2008.12	A	0.221	0.673	0.421	0.451	0.115	1.46	>50	>50	0.002
KER2018.11	A	0.213	0.800	0.979	1.05	0.442	0.976	>50	7.16	0.281
KNH1209.18	A	0.037	0.110	0.101	0.159	0.121	0.298	0.007	2.39	>50
MB201.A1	A	0.025	0.089	0.077	0.078	0.074	0.452	0.026	1.36	>50
MB539.2B7	A	4.72	11.0	2.45	6.80	4.08	1.24	>50	>50	>50
MI369.A5	A	0.204	0.509	0.642	0.490	0.342	0.843	0.087	1.77	0.819
MS208.A1	A	1.94	3.39	1.79	3.16	4.51	0.593	>50	1.14	>50
Q23.17	A	0.117	0.352	0.149	0.167	0.115	0.212	0.019	1.60	0.020
Q259.17	A	0.032	0.080	0.147	0.174	0.107	0.242	>50	12.0	>50
Q769.d22	A	0.435	1.14	1.11	1.08	0.530	0.098	>50	4.47	>50
Q769.h5	A	0.223	0.703	0.650	0.788	0.464	0.145	>50	7.44	>50
Q842.d12	A	0.096	0.351	0.169	0.156	0.083	0.075	0.047	7.58	>50
QH209.14M.A2	A	0.577	1.48	0.705	0.613	0.281	0.094	>50	4.09	>50
RW020.2	A	0.021	0.076	0.073	0.096	0.069	0.647	0.009	2.92	>50
UG037.8	A	0.339	0.990	0.483	0.741	0.286	0.186	0.237	0.353	>50
246-F3.C10.2	AC	0.108	0.301	0.181	0.179	0.062	0.650	>50	1.49	>50
3301.V1.C24	AC	0.070	0.170	0.207	0.222	0.165	0.223	0.030	9.50	>50
3589.V1.C4	AC	0.315	1.10	0.709	0.774	0.369	0.199	>50	11.7	0.080
6540.v4.c1	AC	>50	>50	>50	>50	>50	>50	>50	7.01	>50

IC80 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (1/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
6545.V4.C1	AC	>50	>50	31.4	>50	30.1	>50	>50	7.50	>50
0815.V3.C3	ACD	0.081	0.356	0.186	0.244	0.115	0.085	0.072	1.81	0.018
6095.V1.C10	ACD	0.557	0.938	0.048	0.153	0.230	2.04	>50	0.004	>50
3468.V1.C12	AD	0.040	0.101	0.072	0.074	0.054	0.117	1.05	2.04	6.66
Q168.a2	AD	0.127	0.377	0.382	0.510	0.329	0.230	>50	2.88	>50
Q461.e2	AD	7.51	17.0	11.5	16.4	1.18	1.02	>50	4.68	0.009
620345.c1	AE	>50	>50	>50	>50	>50	>50	>50	3.73	>50
BJOX009000.02.4	AE	0.762	1.92	0.279	0.504	0.675	4.80	>50	1.47	>50
BJOX010000.06.2	AE	15.5	>50	1.70	4.19	2.52	20.9	>50	0.476	>50
BJOX025000.01.1	AE	>50	>50	2.98	8.47	8.29	>50	>50	1.54	>50
BJOX028000.10.3	AE	0.804	1.01	0.432	0.414	0.206	1.03	>50	0.876	>50
C1080.c3	AE	18.1	22.4	0.973	0.896	0.415	9.98	>50	0.613	>50
C2101.c1	AE	3.46	4.64	0.816	0.521	0.271	0.581	>50	4.12	0.090
C3347.c11	AE	3.82	3.88	0.060	0.183	0.302	0.452	>50	0.089	>50
C4118.09	AE	5.11	7.21	1.04	0.648	0.109	0.720	>50	2.30	0.003
CM244.ec1	AE	0.741	2.48	0.913	1.20	1.05	0.452	>50	1.46	>50
CNE3	AE	>50	>50	20.9	17.2	3.12	11.0	>50	4.01	>50
CNE5	AE	3.00	11.0	1.67	1.57	1.07	0.914	>50	2.52	>50
CNE55	AE	4.29	17.1	1.44	2.77	1.28	0.933	>50	0.605	>50
CNE56	AE	7.51	28.5	2.06	5.04	3.27	1.30	>50	0.314	>50
CNE59	AE	11.8	9.43	0.339	1.47	2.62	1.70	>50	0.010	>50
CNE8	AE	1.46	4.45	0.025	0.123	0.441	0.965	>50	1.42	>50
M02138	AE	21.4	25.8	0.135	0.718	1.18	3.02	>50	0.126	>50
R1166.c1	AE	13.7	44.6	0.463	1.11	1.90	5.39	>50	2.02	>50
R2184.c4	AE	2.26	6.10	1.94	3.18	1.87	0.288	>50	2.20	>50
R3265.c6	AE	9.74	19.7	4.97	7.77	4.54	1.41	>50	9.28	>50
TH023.6	AE	21.1	4.46	0.115	0.301	0.411	5.75	>50	0.034	>50
TH966.8	AE	5.60	12.7	0.180	0.558	1.35	0.983	>50	0.291	>50
TH976.17	AE	2.93	7.63	1.18	1.84	1.22	0.726	>50	1.75	>50
235-47	AG	0.265	0.857	0.309	0.320	0.310	0.154	0.842	0.786	>50
242-14	AG	>50	>50	8.76	>50	34.7	>50	>50	3.17	>50

IC80 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (2/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
263-8	AG	0.674	2.34	1.00	0.889	0.525	0.536	7.73	0.991	>50
269-12	AG	0.155	0.632	0.308	0.223	0.191	0.679	1.26	0.475	>50
271-11	AG	0.028	0.096	0.078	0.079	0.045	0.200	>50	4.34	>50
928-28	AG	2.92	7.95	0.677	1.86	1.35	0.968	>50	0.365	>50
DJ263.8	AG	0.090	0.328	0.061	0.180	0.158	0.490	0.202	0.100	12.0
T250-4	AG	0.024	0.078	0.092	0.058	0.044	>50	0.012	3.45	>50
T251-18	AG	8.52	26.9	0.951	0.736	0.235	12.0	>50	2.55	>50
T253-11	AG	0.603	5.03	1.16	1.14	0.516	1.23	>50	4.05	>50
T255-34	AG	0.141	0.312	0.160	0.120	0.077	1.31	>50	1.14	>50
T257-31	AG	1.21	4.87	1.16	1.43	1.12	4.55	>50	1.58	>50
T266-60	AG	1.64	5.19	3.57	2.43	1.51	5.78	0.620	>50	>50
T278-50	AG	>50	>50	>50	>50	7.64	>50	>50	2.10	>50
T280-5	AG	0.043	0.129	0.103	0.216	0.065	0.094	0.010	4.77	>50
T33-7	AG	0.236	0.545	0.367	0.311	0.166	0.047	>50	2.83	>50
3988.25	B	0.058	0.118	0.084	0.132	0.085	1.12	0.008	0.293	>50
5768.04	B	0.108	0.245	0.318	0.291	0.188	0.956	0.897	5.26	>50
6101.10	B	0.057	0.132	0.038	0.068	0.052	0.112	0.018	0.005	>50
6535.3	B	0.029	0.122	0.031	0.074	0.083	5.77	0.011	1.28	>50
7165.18	B	0.218	0.489	0.355	0.402	0.386	>50	0.074	2.71	>50
45_01dG5	B	0.028	0.131	0.058	0.086	0.060	0.047	0.009	0.703	>50
89.6.DG	B	0.410	0.444	0.052	0.099	0.099	2.03	0.077	1.48	>50
AC10.29	B	0.475	1.21	0.549	0.898	0.952	2.76	0.118	0.512	>50
ADA.DG	B	0.225	0.474	0.097	0.274	0.281	1.32	0.015	0.358	>50
Bal.01	B	0.023	0.073	0.079	0.099	0.046	0.307	0.044	1.91	0.040
BaL.26	B	0.063	0.100	0.132	0.185	0.094	0.138	0.050	2.39	0.005
BG1168.01	B	5.61	5.88	1.68	3.76	3.82	3.57	>50	1.48	>50
BL01.DG	B	>50	>50	22.8	>50	10.1	>50	>50	1.57	0.049
BR07.DG	B	0.492	0.998	0.171	0.308	0.176	4.69	0.338	0.445	0.006
BX08.16	B	0.198	0.223	0.102	0.313	0.387	1.09	0.007	1.30	>50
CAAN.A2	B	0.116	0.300	0.170	0.155	0.077	3.71	0.027	5.70	0.027
CNE10	B	0.065	0.207	0.094	0.108	0.092	1.59	0.027	0.169	>50

IC80 (ug/mL)
<0.001
.001-.01
.01-.100
100-1.00
1.00-10.0
>10.0

FIG. 19 (3/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CNE12	B	0.061	0.169	0.102	0.096	0.085	2.14	0.014	1.09	10.0
CNE14	B	0.035	0.109	0.071	0.069	0.052	0.813	0.007	0.649	>50
CNE4	B	1.19	2.69	0.579	0.914	0.358	2.96	>50	0.437	>50
CNE57	B	0.117	0.427	0.165	0.271	0.228	1.26	0.035	0.317	>50
HO86.8	B	>50	>50	45.9	>50	4.72	>50	>50	1.52	>50
HT593.1	B	1.47	3.63	0.441	1.41	1.53	1.62	>50	0.285	>50
HXB2.DG	B	0.019	0.166	0.028	0.056	0.041	0.093	>50	0.015	>50
JRCSF.JB	B	0.126	0.747	0.452	0.573	0.389	0.925	0.219	1.89	>50
JRFL.JB	B	0.062	0.223	0.156	0.250	0.166	0.087	0.071	0.768	49.0
MN.3	B	0.035	0.138	0.026	0.098	0.119	0.063	>50	0.0010	0.039
PVO.04	B	1.21	3.10	2.37	2.97	1.06	1.20	0.436	6.43	>50
QH0515.01	B	1.08	2.99	1.91	1.56	1.18	2.85	>50	5.54	>50
QH0692.42	B	2.41	4.40	1.59	1.86	1.06	3.98	9.70	2.35	>50
REJO.67	B	0.105	0.415	0.254	0.353	0.138	0.180	>50	1.18	0.040
RHPA.7	B	0.102	0.300	0.246	0.347	0.267	0.112	0.046	5.10	>50
SC422.8	B	0.474	1.10	0.934	1.25	0.662	0.321	0.362	1.15	>50
SF162.LS	B	0.004	0.041	0.032	0.035	0.018	0.580	0.017	1.06	>50
SS1196.01	B	0.051	0.144	0.014	0.040	0.070	0.679	0.011	1.25	4.18
THRO.18	B	6.69	12.5	1.13	3.22	2.11	9.91	>50	0.587	>50
TRJO.58	B	0.777	2.25	1.02	1.14	0.231	0.231	>50	4.18	25.0
TRO.11	B	0.098	0.326	0.150	0.160	0.169	1.19	0.032	0.286	>50
WITO.33	B	0.800	2.98	0.365	0.699	0.553	0.295	3.25	0.305	>50
X2278.C2.B6	B	0.140	0.410	0.196	0.273	0.064	0.356	0.034	2.24	0.0010
YU2.DG	B	0.111	0.500	0.181	0.324	0.191	0.188	0.178	5.46	>50
BJOX002000.03.2	BC	0.129	0.337	0.273	0.304	0.280	>50	0.068	1.56	>50
CH038.12	BC	0.109	0.241	0.094	0.122	0.119	1.04	0.020	1.41	>50
CH070.1	BC	0.116	0.272	0.014	0.039	0.084	>50	0.015	13.5	>50
CH117.4	BC	0.026	0.125	0.060	0.065	0.036	0.241	>50	0.859	>50
CH119.10	BC	0.176	0.514	0.153	0.150	0.113	2.15	0.116	2.36	>50
CH181.12	BC	0.134	0.334	0.253	0.263	0.195	1.22	0.039	2.79	>50
CNE15	BC	0.235	0.709	0.062	0.119	0.152	0.375	>50	2.97	>50

IC80 (ug/mL)

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (4/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CNE19	BC	0.010	0.044	0.024	0.023	0.018	0.659	0.063	1.11	>50
CNE20	BC	0.009	0.036	0.059	0.092	0.064	>50	0.008	0.732	>50
CNE21	BC	0.081	0.236	0.129	0.159	0.146	0.903	0.018	3.25	>50
CNE40	BC	0.855	1.29	0.048	0.114	0.192	4.55	1.85	0.009	0.070
CNE7	BC	0.146	0.558	0.206	0.391	0.307	0.605	0.096	0.603	0.015
286.36	C	0.023	0.085	0.054	0.046	0.043	0.715	0.009	5.00	>50
288.38	C	0.106	0.176	0.022	0.061	0.081	3.46	0.035	3.08	>50
0013095-2.11	C	0.639	2.57	0.025	0.046	0.099	0.287	>50	0.077	>50
001428-2.42	C	0.062	0.217	0.144	0.135	0.102	0.035	0.076	6.28	>50
0077_V1.C16	C	0.697	1.82	1.12	0.841	0.453	3.10	>50	7.11	0.030
00836-2.5	C	0.021	0.107	0.078	0.054	0.035	0.462	>50	1.77	>50
0921.V2.C14	C	0.090	0.325	0.280	0.289	0.182	0.519	>50	3.03	>50
16055-2.3	C	0.046	0.159	0.125	0.127	0.101	0.224	12.2	3.31	>50
16845-2.22	C	11.2	22.6	0.389	1.60	2.90	12.3	>50	0.172	>50
16936-2.21	C	0.033	0.111	0.030	0.031	0.031	0.435	0.013	1.31	0.010
25710-2.43	C	0.108	0.396	0.130	0.181	0.164	1.40	0.055	0.304	>50
25711-2.4	C	0.081	0.242	0.160	0.113	0.124	1.44	0.041	1.69	>50
25925-2.22	C	0.142	0.442	0.224	0.206	0.159	1.34	0.072	1.53	>50
26191-2.48	C	0.331	0.953	0.543	0.498	0.378	0.679	0.393	4.90	>50
3168.V4.C10	C	1.50	2.64	2.05	1.92	0.617	0.325	1.94	8.18	>50
3637.V5.C3	C	37.1	>50	9.98	46.9	28.0	6.17	>50	6.68	>50
3873.V1.C24	C	0.664	2.03	0.069	0.151	0.230	6.97	0.106	15.7	>50
426c	C	0.967	2.48	1.20	1.39	0.831	4.41	>50	1.60	>50
6322.V4.C1	C	>50	29.0	12.0	2.11	0.281	>50	>50	3.68	>50
6471.V1.C16	C	>50	>50	>50	>50	>50	>50	>50	14.9	>50
6631.V3.C10	C	6.28	9.28	8.27	6.72	4.44	>50	>50	3.36	>50
6644.V2.C33	C	0.075	0.448	0.100	0.156	0.090	0.421	0.171	0.124	>50
6785.V5.C14	C	0.138	0.390	0.383	0.431	0.216	0.686	0.072	2.42	>50
6838.V1.C35	C	0.007	0.031	0.036	0.048	0.024	0.616	0.840	1.01	>50
96ZM651.02	C	0.099	0.200	0.087	0.118	0.123	2.54	0.044	0.177	>50
BR025.9	C	0.047	0.091	0.061	0.054	0.050	2.37	0.008	1.11	0.020

IC80 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (5/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/ dVRC01-5X-PGT121	35O22-5X-10E8/ dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
CAP210.E8	C	11.0	20.1	2.68	2.67	1.16	>50	>50	2.01	>50
CAP244.D3	C	1.14	3.48	0.117	0.241	0.297	3.53	>50	1.48	>50
CAP256.206.C9	C	0.138	0.308	0.141	0.116	0.089	2.72	0.045	2.97	>50
CAP45.G3	C	0.398	0.963	0.483	0.377	0.251	>50	>50	3.41	>50
Ce1176.A3	C	0.120	0.300	0.212	0.191	0.136	4.92	0.070	1.15	2.00
CE703010217.B6	C	0.022	0.087	0.053	0.066	0.063	0.584	0.011	0.679	>50
CNE30	C	0.688	1.44	0.466	0.668	0.584	2.15	0.249	2.29	>50
CNE31	C	2.23	7.20	2.93	3.11	1.74	2.10	2.68	3.57	>50
CNE53	C	0.091	0.254	0.061	0.144	0.126	0.302	0.054	1.01	>50
CNE58	C	3.09	5.78	2.46	3.36	1.74	0.582	>50	1.09	>50
DU123.06	C	0.216	0.501	0.027	0.095	0.223	46.1	0.101	0.423	>50
DU151.02	C	0.036	0.116	0.204	0.294	0.188	>50	0.021	1.71	>50
DU156.12	C	0.030	0.117	0.045	0.103	0.052	0.188	0.023	0.120	>50
DU172.17	C	0.244	0.480	0.197	0.272	0.428	>50	0.846	0.238	>50
DU422.01	C	0.231	0.757	0.719	1.36	0.843	>50	0.365	0.812	>50
MW965.26	C	0.044	0.127	0.024	0.101	0.076	0.128	0.051	0.007	>50
SO18.18	C	0.013	0.061	0.008	0.019	0.034	0.085	0.006	4.48	>50
TV1.29	C	0.897	1.80	3.39	6.06	3.88	>50	0.318	0.719	>50
TZA125.17	C	7.29	22.1	3.14	3.21	2.81	>50	>50	1.19	>50
TZBD.02	C	0.026	0.090	0.060	0.084	0.075	0.147	0.060	4.31	>50
ZA012.29	C	0.055	0.129	0.165	0.195	0.152	0.602	0.021	4.12	>50
ZM106.9	C	0.059	0.161	0.155	0.233	0.143	0.513	0.018	>50	>50
ZM109.4	C	0.312	0.926	0.368	0.329	0.281	0.414	>50	1.07	>50
ZM135.10a	C	3.95	7.79	0.093	0.258	0.568	6.10	9.25	0.408	>50
ZM176.66	C	0.232	0.727	0.171	0.394	0.239	0.207	>50	1.73	>50
ZM197.7	C	3.92	15.6	0.706	2.57	2.42	1.64	>50	0.369	>50
ZM214.15	C	3.40	8.45	2.68	2.94	1.30	3.36	2.37	5.98	>50
ZM215.8	C	0.066	0.281	0.083	0.100	0.082	0.937	0.057	0.230	>50
ZM233.6	C	0.199	0.628	0.322	0.335	0.161	6.95	>50	0.737	>50
ZM249.1	C	1.58	3.30	0.817	0.868	0.226	0.442	>50	2.27	0.006
ZM53.12	C	0.004	0.021	0.031	0.037	0.028	1.88	0.016	6.72	>50

IC80 (ug/mL)
<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (6/8)

		Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
Virus ID	Clade	dVRC01-5X-PGT121 ScFv	5X-PGT121 Fc	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/dVRC01-5X-PGT121	35O22-5X-10E8/dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
ZM55.28a	C	0.358	1.06	0.726	0.741	0.531	0.629	0.233	6.78	>50
3326.V4.C3	CD	0.035	0.042	0.075	0.067	0.057	2.98	>50	4.29	>50
3337.V2.C6	CD	0.119	0.241	0.178	0.159	0.103	0.211	>50	4.87	0.070
3817.v2.c59	CD	35.4	>50	4.56	11.2	6.02	>50	>50	1.43	>50
191821.E6.1	D	1.15	3.89	3.01	2.41	1.19	1.34	>50	5.89	>50
231965.c1	D	3.30	3.35	1.15	0.882	0.434	1.01	>50	20.4	>50
247-23	D	>50	>50	2.06	2.59	0.443	12.3	>50	1.29	>50
3016.v5.c45	D	0.086	0.186	0.118	0.118	0.096	0.252	>50	2.17	>50
57128.vrc15	D	1.37	2.73	1.03	0.897	0.473	>50	>50	1.50	>50
6405.v4.c34	D	0.255	0.645	0.357	0.383	0.151	4.24	0.080	1.80	>50
A03349M1.vrc4a	D	0.184	0.433	0.462	0.407	0.191	13.9	0.155	0.663	>50
A07412M1.vrc12	D	0.034	0.134	0.106	0.097	0.062	0.339	0.104	0.873	>50
NKU3006.ec1	D	10.1	26.7	6.89	11.3	2.70	1.29	>50	2.46	0.022
UG021.16	D	0.929	1.18	0.103	0.333	0.482	1.59	>50	0.362	>50
UG024.2	D	7.55	2.13	0.204	0.528	0.509	0.667	>50	0.241	>50
P0402.c2.11	G	0.064	0.188	0.019	0.075	0.088	0.488	0.026	0.460	1.20
P1981.C5.3	G	0.018	0.054	0.072	0.093	0.092	0.691	0.013	0.124	0.004
X1193.c1	G	0.332	0.709	0.071	0.159	0.154	0.350	0.091	1.15	0.400
X1254.c3	G	0.159	0.535	0.040	0.176	0.182	0.132	0.069	15.7	>50
X1632.S2.B10	G	0.322	0.582	0.251	0.359	0.189	0.526	>50	1.76	>50
X2088.c9	G	0.046	0.097	0.126	0.157	0.090	>50	0.009	>50	>50
X2131.C1.B5	G	0.080	0.197	0.076	0.137	0.106	1.41	0.044	0.175	>50
SIVmac251.30.SG3	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50	>50	>50	>50	>50	>50

IC80 (ug/mL)

<0.001
.001-.01
.01-.100
.100-1.00
1.00-10.0
>10.0

FIG. 19 (7/8)

	Bi-ScFv	Bi-NAb	Tri-NAb	Tetra-NAb1	Tetra-NAb2	Parental mAbs			
	dVRC01-5X-PGT121 ScFv	dVRC01-5X-PGT121 IgG	10E8/dVRC01-5X-PGT121	10E8-5X-35O22/dVRC01-5X-PGT121	35O22-5X-10E8/dVRC01-5X-PGT121	VRC01	PGT121	10E8	35022
# Viruses	208	208	208	208	208	208	208	208	208
Total VS Neutralized									
IC80 <50ug/ml	196	194	204	200	205	182	112	203	35
IC80 <10ug/ml	185	176	198	195	201	175	111	193	31
IC80 <1.0ug/ml	144	122	155	149	160	106	99	61	27
IC80 <0.1ug/ml	62	19	55	37	51	13	72	10	24
IC80 <0.01ug/ml	4	0	1	0	0	0	11	5	8
% VS Neutralized									
IC80 <50ug/ml	94	93	98.1	96	98.6	88	54	97.6	17
IC80 <10ug/ml	89	85	95	94	97	84	53	93	15
IC80 <1.0ug/ml	69	59	75	72	77	51	48	29	13
IC80 <0.1ug/ml	30	9	26	18	25	6	35	5	12
IC80 <0.01ug/ml	2	0	0	0	0	0	5	2	4
Median IC80	0.216	0.540	0.235	0.310	0.226	0.703	0.059	1.69	0.040
Geometric Mean	0.298	0.749	0.298	0.384	0.296	0.793	0.081	1.34	0.087
Note: Median and Geometric Mean titers are calculated only for samples with IC80 <50ug/ml									

FIG. 19 (8/8)

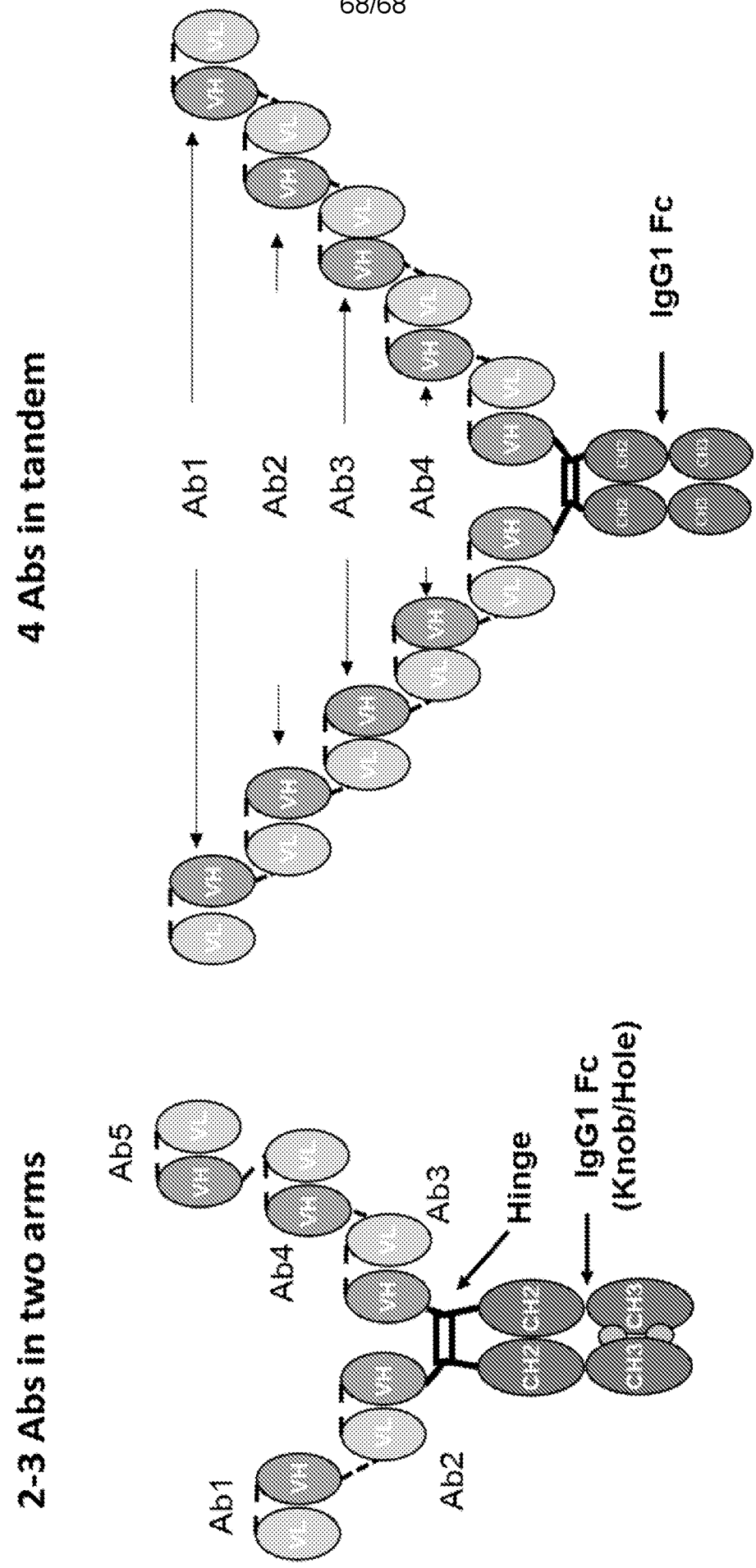

4 Abs in tandem
Ab1
Ab2
Ab3
Ab4
IgG1 Fc
2-3 Abs in two arms
Ab5
Ab4
Ab3
Hinge
IgG1 Fc
(Knob/Hole)
Ab1
Ab2

FIG. 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/57053

A CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395, C07K 16/46, C07K 16/10, A61P 31/18 (2018.01)
CPC - A61K 39/42, C07K 2317/31, C07K 16/1063, A61K 2039/505, C07K 2317/76

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	WO 2013/163427 A1 (THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 31 October 2013 (31.10.2013) p 1, ln 8-9; p 8, ln 38 to p 39, ln 1; p 9, ln 9-15; p 21, ln 34 to p 22, ln 2; p 30, ln 33-37; p 31, ln 1-2; p 44, ln 7-10	1-4, 6 ---------- 11, 13/11, 14/11, 19-20, 23-25, 47-52, ********** 31
Y	WO 2016/037154 A1 (THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 10 March 2016 (10.03.2016) p 1, ln 8-9; p 28, ln 31-39; p 46, ln 5-13	11, 13/11, 14/11, 20, 24, 47-52
Y	US 2012/0244166 A1 (MASCOLA et al.) 27 September 2012 (27.09.2012) para [0020], [0558]; SEQ ID NOs: 1 and 2	19, 23, 25, 51
Y	US 2016/0213779 A1 (BETH ISRAEL DEACONESS MEDICAL CENTER, INC.) 28 July 2016 (28.07.2016) para [0127], [0128]; SEQ ID NOs: 7 and 8	20, 24, 25
A	US 2015/0037334 A1 (AMGEN RESEARCH (MUNICH) GMBH) 5 February 2015 (05.02.2015) SEQ ID NO: 90	31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2018	01 MAR 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/57053

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.: 33-46
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:

------- please see extra sheet -------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 6, 11, (13-14)/11, 19-20, 23-25, 31, 47-52, limited to the VRC01/PGT121 multispecific antibody (SEQ ID NOs: 1-17)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/57053

Continuation of: Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-32, 47-58, drawn to a multispecific antibody. The multispecific antibody will be searched to the extent that the multispecific antibody is the VRC01/PGT121 multispecific antibody. Note that the VRC01/PGT121 multispecific antibody has the sequence of SEQ ID NO: 1 (claim 31), and is further characterized by the following:
- the VRC01 antibody VL and VH sequences of SEQ ID NOs: 2 and 6 respectively, the VL CDR 1-3 sequences of SEQ ID NOs: 3-5, the VH CDR 1-3 sequences of SEQ ID NOs: 7-9 (claims 19, 23, 25);
- the PGT121 antibody VL and VH sequences of SEQ ID NOs: 10 and 14 respectively, the VL CDR 1-3 sequences of SEQ ID NOs: 11-13, the VH CDR 1-3 sequences of SEQ ID NOs: 15-17 (claims 20, 24, 25);
- wherein the first antibody binds to an epitope in the CD4-binding site of the of the envelope protein of HIV-1 (the VRC01 antibody) and the second antibody binds to an epitope in the V3- glycan region of the of the envelope protein of HIV-1 (the PGT121 antibody) (claims 11, 13, 14);
- wherein the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers (claim 6, 52); and
- only comprises variable domain and does not comprise a constant domain.
It is believed that claims 1-4, 6, 11, 13-14, 19-20, 23-25, 31, 47-52, limited to the VRC01/PGT121 multispecific antibody (SEQ ID NOs: 1-17), encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass the VRC01/PGT121 multispecific antibody. [Note: Claims 8-10, 32, 54-56 require a third light chain, not required by the VRC01/PGT121 multispecific antibody (SEQ ID NO: 1) of the first named invention. Claims 57-58 require the multispecific antibody to comprise a constant domain comprising an IgG-like amino acid sequence, not required by the VRC01/PGT121 multispecific antibody (SEQ ID NO: 1) of the first named invention]. Additional multispecific antibodies will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected multispecific antibodies. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein the multispecific antibody is the 35022/10E8 multispecific antibody. Note that the 35022/10E8 multispecific antibody has the sequence of SEQ ID NO: 30 (claim 31), and is further characterized by the following:
- the 35022 antibody VL and VH sequences of SEQ ID NOs: 31 and 35 respectively, the VL CDR 1-3 sequences of SEQ ID NOs: 32-34, the VH CDR 1-3 sequences of SEQ ID NOs: 36-38 (claims 21, 23, 29);
- the 10E8 antibody VL and VH sequences of SEQ ID NOs: 39 and 43 respectively, the VL CDR 1-3 sequences of SEQ ID NOs: 40-42, the VH CDR 1-3 sequences of SEQ ID NOs: 44-46 (claims 22, 24, 29);
- wherein the first antibody binds to an epitope in the gp120/gp41 interface of the of the envelope protein of HIV-1 (the 35022 antibody) and the second antibody binds to an epitope in the MPER of the of the envelope protein of HIV-1 (the 10E8 antibody) (claims 15, 17, 18);
- wherein the VH from the first light chain and the VL from the second light chain are connected by five tetra-glycine serine (G4S) protein linkers (claim 6, 52); and
- only comprises variable domain and does not comprise a constant domain,
i.e. claims 1-7, 15, 17, 18, 21-24, 29, 31, 57-58, limited to the 35022/10E8 multispecific antibody (SEQ ID NOs: 30-46). [Note: Claims 8-10, 32, 54-56 require a third light chain, not required by the 35022/10E8 multispecific antibody (SEQ ID NO: 30) of the exemplary invention. Claims 47-56 requires binding to an epitope on CD4s and an epitope on V1, V2, or V3 glycan of HIV-1, not required by the 35022/10E8 multispecific antibody. Claims 57-58 require the multispecific antibody to comprise a constant domain comprising an IgG-like amino acid sequence, not required by the 35022/10E8 multispecific antibody (SEQ ID NO: 30) of the first named invention].

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

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

Additionally, even if the inventions listed as Group I+ were considered to share the technical features, these shared technical features are previously disclosed by prior art, as further discussed below.

Common Technical Features

The feature shared by the inventions listed as Group I+ is a multispecific antibody, or an antigen-binding fragment thereof, comprising:
a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof; and
b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof,
wherein the first light chain and the second light chain bind non-overlapping epitopes of the envelope protein of human immunodeficiency virus-1 (HIV-1), and
wherein the VH from the first light chain and the VL from the second light chain are connected by one or more linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by one or more linkers.

------- please see continuation on next extra sheet -------

Continuation of: Box No. III Observations where unity of invention is lacking

Another feature shared by the inventions listed as Group I+ is wherein the linker comprises two or more tetra-glycine serine (G4S) protein linkers.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by WO 2013/163427 A1 to THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES (hereinafter 'HHS').

HHS discloses a multispecific antibody, or an antigen-binding fragment thereof (p 1, ln 8-9 - "This application relates to multispecific antibodies that specifically bind an human immunodeficiency virus (HIV)-l envelope protein"), comprising:
a) a first light chain comprising a first light chain variable region (VL) and a first heavy chain comprising a first heavy chain variable region (VH), wherein the first light chain and the first heavy chain are derived from a first antibody or an antigen-binding fragment thereof (p 8, ln 38 to p 39, ln 1 - "these multispecific antibodies include a first antigen binding domain that specifically binds to an HIV-1 antigen and is neutralizing"; p 30, ln 33-37 - "In several embodiments, the isolated multispecific antibody includes a first antigen binding domain that specifically binds to gpl20, such as a VRC01-like monoclonal antibody, or a functional fragment thereof (e.g., an scFv or a Fab), for example, as described herein. In some embodiments, the first antigen binding domain specifically binds to the CD4 binding site on gpl20. The HIV-1 specific antigen binding domain is neutralizing"; p 9, ln 9-15 - "In some embodiments, an isolated bispecific monoclonal antibody is disclosed that includes a first antigen binding domain and a second antigen binding domain. The first antigen binding domain can be a Fab or a scFv, specifically binds to gpl20 or gp41, and is neutralizing. The Fab and/or the scFv of the first antigen binding domain includes a heavy chain variable region [VH] including a heavy chain complementarity determining region (H-CDR)l, an H-CDR2, an H-CDR3, and a light chain variable region [VL] including a light chain complementarity determining region (L-CDR)l, an L-CDR2, and an L- CDR3"); and
b) a second light chain comprising a second light chain variable region (VL) and a second heavy chain comprising a second heavy chain variable region (VH), wherein the second light chain and the second heavy chain are derived from a second antibody or an antigen-binding fragment thereof (p 8, ln 38 to p 39, ln 2 - "these multispecific antibodies include a first antigen binding domain . . . and a second antigen binding domain that specifically binds to CD3, activates T cells, and activates HIV-1 in T cells"; p 9, ln 9-15 - "In some embodiments, an isolated bispecific monoclonal antibody is disclosed that includes a first antigen binding domain and a second antigen binding domain. The first antigen binding domain can be a Fab or a scFv, specifically binds to gpl20 or gp41, and is neutralizing. The Fab and/or the scFv of the first antigen binding domain includes a heavy chain variable region [VH] including a heavy chain complementarity determining region (H-CDR)l, an H-CDR2, an H-CDR3, and a light chain variable region [VL] including a light chain complementarity determining region (L-CDR)l, an L-CDR2, and an L- CDR3");
wherein the first light chain binds an epitope of the envelope protein of human immunodeficiency virus-1 (HIV-1) (p 8, ln 38 to p 39, ln 1 - "these multispecific antibodies include a first antigen binding domain that specifically binds to an HIV-1 antigen and is neutralizing"), wherein the VH from the first light chain and the VL from the second light chain are connected by one or more linkers or wherein the VL from the first light chain and the VH from the second light chain are connected by one or more linkers (p 44, ln 7-10 - "In an additional example, the multispecific antibody includes a scFv including VH and VL chains joined by a peptide linker . . . In some embodiments, the peptide linker includes an amino acid sequence having four glycine residues followed by a serine, or polymers thereof").
HHS does not specifically teach wherein the second light chain binds a non-overlapping epitope of the envelope protein of human immunodeficiency virus-1 (HIV-1). However, HHS does teach that the antibody is a multispecific antibody that specifically bind to HIV-1 envelope protein epitopes (p 1, ln 8-9 - "This application relates to multispecific antibodies that specifically bind an human immunodeficiency virus (HIV)-l envelope protein") and further teaches that several antibodies that bind to HIV-1 envelope proteins are known in the art (p 31, ln 1-2 - In some embodiments, the first antigen binding domain includes the heavy and light chain CDRs of a VRC01-like monoclonal antibody, such as VRC01, VRC07, or a variant thereof. VRC01-like monoclonal antibodies include, but are not limited to VRC01, VRC07, and variants thereof"). Given that that the multispecific antibody of HHS targets HIV-1 envelope protein epitopes, one of ordinary skill in the art would have found it obvious that the multispecifica antbody can have more than one antibody or antigen binding domain thereof that binds to non-overlapping HIV-1 envelope protein epitopes.

HHS further teaches wherein the linker comprises two or more tetra-glycine serine (G4S) protein linkers (p 21, ln 34 to p 22, ln 2 - "Linker: A bi-functional molecule that can be used to link two molecules into one contiguous molecule, for example, to link a first antigen binding domain to a second antigen binding domain in a multispecific antibody. . . .In some examples, the linker is a peptide linker, such as a (GGGGS)1 (SEQ ID NO: 1763), (GGGGS)2 (SEQ ID NO: 1764), or a (GGGGS)3 (SEQ ID NO: 1765) linker"; p 44, ln 7-10 - "In an additional example, the multispecific antibody includes a scFv including VH and VL chains joined by a peptide linker . . . In some embodiments, the peptide linker includes an amino acid sequence having four glycine residues followed by a serine, or polymers thereof").

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

The inventions listed as Group I+ therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.