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(54) Title: BROADLY NEUTRALIZING ANTIBODIES TARGETING THE CD4 BINDING SITE ON HIV ENV

(57) Abstract: The present disclosure relates to monoclonal human antibodies or binding fragments thereof which are directed against the CD4 binding site of the human immunodeficiency virus HIV-1, a pharmaceutical composition comprising such monoclonal human antibodies or binding fragments thereof, a kit comprising such antibodies or binding fragments thereof, and the monoclonal antibodies or binding fragments thereof and the pharmaceutical composition and the kit for use as a medicament, and in the treatment or prevention of a disease caused by the human immunodeficiency virus HIV-1.



Broadly neutralizing antibodies targeting the CD4 binding site on HIV Env**Technical field**

The present invention relates to monoclonal human antibodies or binding fragments thereof which are directed against the CD4 binding site of the human immunodeficiency virus HIV-1, a pharmaceutical composition comprising such monoclonal human antibodies or binding fragments thereof, a kit comprising such antibodies or binding fragments thereof, and the monoclonal antibodies or binding fragments thereof and the pharmaceutical composition and the kit for use as a medicament, and in the treatment, cure or prevention of a disease caused by the human immunodeficiency virus HIV-1.

Background of the Invention

Despite the widespread use of effective antiretroviral therapy (ART), the combat against the global HIV-1 epidemic still remains a public health priority. Highly potent and broadly neutralizing anti-HIV-1 antibodies (bNAbs) represent promising and powerful tools that open unprecedented opportunities for alternative treatment and prevention strategies. In recent years, potent bNAbs have been isolated from HIV-1-infected donors that bind to distinct vulnerable epitopes on the HIV-1 envelope (Env) trimer. These epitopes encompass the CD4 binding site (CD4bs), the V1/V2 loop, the V3 loop glycan patch, the membrane-proximal external region, the fusion peptide, the silent face and the interface between the gp120 and gp41 Env subunits.

Among these sites, the CD4bs is of particular interest because CD4 serves as the primary receptor for viral entry. BNAbs that are encoded by the immunoglobulin heavy chain gene segment IGHV1-2 often mimic the CD4 contact residues for the viral envelope protein, display high levels of somatic hypermutation, and rely on a five amino acid light chain complementarity determining region (CDRL3). These antibodies are also referred to as VRC01-class antibodies and comprise the majority of the most potent and broadest CD4bs bNAbs identified to date. Due to their remarkable antiviral capacities, several CD4bs bNAbs have entered advanced clinical. Representative members of the VRC01-class include VRC01, VRC07, VRC07-523-LS, 3BNC117, NIH45-46, N6, and N49-P7.

Other CD4bs bNAbs are encoded by the immunoglobulin heavy chain gene segment IGHV1-46 and do not require a five amino acid CDRL3. Representative members of this antibody class are 8ANC131, CH235, and 1B2530. These antibodies mostly display lower potencies and breadth when compared to VRC01-class antibodies. One exception is the recently identified bNAb 1-18 that exhibits high levels of potency and breadth against large multiclade HIV-1 pseudovirus reference panels.

In pre-clinical animal models bNAbs have demonstrated robust protection from infection even at low levels of serum concentrations and have shown favorable safety and pharmacokinetic profiles. Moreover, administration of bNAbs in HIV-1-infected individuals has led to prolonged suppression of viremia and

delayed viral rebound following analytical treatment interruption (ATI). While the recent Antibody Mediated Prevention (AMP) efficacy trials using the CD4bs bNAb VRC01 did not demonstrate overall efficacy in preventing HIV-1 infection, subgroup analyses showed efficacy of VRC01 on prevention of infection with VRC01-sensitive strains ($IC_{80} < 1 \mu g/ml$). Therefore, the identification and development of highly potent CD4bs bNAbs is of utmost relevance for HIV-1 prevention. These bNAbs need to have enhanced neutralization potency, breadth and restricted viral escape pathways. Therefore, there remains a strong demand for the isolation of novel CD4bs bNAbs that exceed the antiviral activity of known anti-HIV-1 bNAbs.

10 The present invention aims to provide novel human monoclonal antibodies targeting HIV-1 that exhibit remarkable levels of breadth and potency against various virus strains and subtypes.

Moreover, it is a further objective of this invention to provide novel human monoclonal antibodies directed against HIV-1 that limit the emergence of viral escape variants and remain effective against such variants.

Summary of the Invention

These objects have been solved by the aspects of the present invention as specified hereinafter.

20 According to the first aspect of the present invention, an antibody or antigen-binding fragment thereof directed against the CD4 binding site of the human immunodeficiency virus HIV-1 is provided, wherein the antibody or antigen-binding fragment thereof comprises a combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody from the group comprising **04_A06** (having a CDR-H1 amino acid sequence of SEQ ID No. 1, a CDR-H2 amino acid sequence of SEQ ID No. 2, a CDR-H3 amino acid sequence of SEQ ID No. 3, a CDR-L1 amino acid sequence of SEQ ID No. 4, a CDR-L2 amino acid sequence of SEQ ID No. 5, and a CDR-L3 amino acid sequence of SEQ ID No. 6), **01_D03** (having a CDR-H1 amino acid sequence of SEQ ID No. 7, a CDR-H2 amino acid sequence of SEQ ID No. 8, a CDR-H3 amino acid sequence of SEQ ID No. 9, a CDR-L1 amino acid sequence of SEQ ID No. 10, a CDR-L2 amino acid sequence of SEQ ID No. 11, and a CDR-L3 amino acid sequence of SEQ ID No. 12), **04_B09** (having a CDR-H1 amino acid sequence of SEQ ID No. 13, a CDR-H2 amino acid sequence of SEQ ID No. 14, a CDR-H3 amino acid sequence of SEQ ID No. 15, a CDR-L1 amino acid sequence of SEQ ID No. 16, a CDR-L2 amino acid sequence of SEQ ID No. 17, and a CDR-L3 amino acid sequence of SEQ ID No. 18), **05_C07** (having a CDR-H1 amino acid sequence of SEQ ID No. 19, a CDR-H2 amino acid sequence of SEQ ID No. 20, a CDR-H3 amino acid sequence of SEQ ID No. 21, a CDR-L1 amino acid sequence of SEQ ID No. 22, a CDR-L2 amino acid sequence of SEQ ID No. 23, and a CDR-L3 amino acid sequence of SEQ ID No. 24), and **01_B01** (having a CDR-H1 amino acid sequence of SEQ ID No. 25, a CDR-H2 amino acid sequence of SEQ ID No. 26, a CDR-H3 amino acid sequence of SEQ ID No. 27, a CDR-L1 amino acid sequence of SEQ ID No. 28, a CDR-L2 amino acid sequence of SEQ ID No. 29, and a CDR-L3 amino acid sequence of SEQ ID No. 30).

According to a preferred embodiment of the first aspect of the invention, the antibody or antigen-binding fragment thereof comprises a combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of one antibody selected from the group comprising **04_A06** (consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence of SEQ ID No. 41), **01_D03** (consisting of the heavy chain amino acid sequence of SEQ ID No. 33 and the light chain amino acid sequence of SEQ ID No. 34), **04_B09** (consisting of the heavy chain amino acid sequence of SEQ ID No. 35 and the light chain amino acid sequence of SEQ ID No. 36), **05_C07** (consisting of the heavy chain amino acid sequence of SEQ ID No. 37 and the light chain amino acid sequence of SEQ ID No. 38), and **01_B01** (consisting of the heavy chain amino acid sequence of SEQ ID No. 39 and the light chain amino acid sequence of SEQ ID No. 40).

According to a preferred embodiment of the first aspect of the invention or of the preceding embodiment of the first aspect of the invention, the amino acid sequences comprised in the antibody or the antigen-binding fragment thereof are of one antibody selected from the group comprising 04_A06, 01_D03, 04_B09 and 05_C07, preferably of one antibody from the group comprising 04_A06, 01_D03, and 04_B09, more preferably of one antibody from the group comprising 04_A06 and 01_D03, particularly preferably of the antibody 04_A06.

According to the second aspect of the present invention, a pharmaceutical composition is provided comprising an antibody or antigen-binding fragment thereof according to the first aspect of the present invention, and at least one pharmaceutically acceptable excipient.

According to a preferred embodiment of the second aspect of the invention, the pharmaceutical composition is a vaccination composition for a human subject.

According to the third aspect of the present invention, a kit is provided comprising an antibody or antigen-binding fragment thereof according to the first aspect of the present invention and a container.

According to the fourth aspect of the present invention, an antibody or antigen-binding fragment thereof according to the first aspect of the present invention, a pharmaceutical composition according to the second aspect of the present invention, or a kit according to the third aspect of the present invention is provided for use as a medicament.

According to the fifth aspect of the present invention, an antibody or antigen-binding fragment thereof according to the first aspect of the present invention, a pharmaceutical composition according to the second aspect of the present invention, or a kit according to the third aspect of the present invention is provided for use in the treatment or prevention of an infection with the human immunodeficiency virus HIV-1 in mammalian subjects.

According to the sixth aspect of the present invention, an antibody or antigen-binding fragment thereof according to the first aspect of the present invention, a pharmaceutical composition according to the second aspect of the present invention, or a kit according to the third aspect of the present invention is provided for use in the treatment or prevention of a disease caused by the human immunodeficiency virus HIV-1 in mammalian subjects.

In a preferred embodiment of the fifth or sixth aspect of the present invention, the use is in human subjects.

10 In one preferred embodiment of the fifth or sixth aspect of the present invention, the use is in the treatment or prevention of acquired immune deficiency syndrome (AIDS).

According to the seventh aspect of the present invention, a nucleic acid is provided encoding an antibody or antigen-binding fragment thereof according to the first aspect of the present invention.

According to the eighth aspect of the present invention, an expression vector comprising the nucleic acid of the seventh aspect of the present invention is provided in functional association with an expression control sequence.

20 According to the ninth aspect of the present invention, a host cell is provided comprising a nucleic acid according to the seventh aspect of the present invention or the expression vector according to the eighth aspect of the present invention.

According to the tenth aspect of the present invention, a method of production of an antibody or antigen-binding fragment thereof according to the first aspect of the present invention is provided, comprising cultivating the host cell of the ninth aspect of the present invention under conditions allowing expression of the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof.

30

Description of Figures

Figure 1 shows the neutralizing activity of antibodies of the present invention against a panel of 12 global reference pseudovirus strains of HIV-1 with different envelope amino acid sequences (as described in de Camp *et al.*, *J Virol.* 2014 Mar; 88(5): 2489–2507) when tested in the TZM-bl cell pseudovirus neutralization assay (see below in the Examples section); antibodies 04_A06 and 01_D03 as described herein neutralize 100% (12/12) strains of the “Global Panel” and display high levels of potency.

40 Figure 2 shows the neutralizing activity (IC_{50}) of antibody 04_A06 of the present invention against a multiclade panel of 119 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay (as described in Schoofs *et al.*, *Immunity*,

2019 Jun 18;50(6):1513-1529.e9); data for antibody 04_A06 compared to the most potent and broadest CD4bs bNAbs of the prior art (3BNC117, 1-18, N49P7, N6, VRC07, and VRC07-523-LS) are shown; for presentation in this figure, 99 pseudovirus strains from the 119 multiclade panel for which neutralization data of reference antibodies was published were selected; neutralization data of the reference bNAbs were retrieved from the antibody neutralization database CATNAP (Yoon et al., Nucleic Acids Research, Volume 43, Issue W1, 1 July 2015, Pages W213–W219, <https://doi.org/10.1093/nar/gkv404>).

Figure 3 shows the neutralizing activity (IC_{50} and breadth) of the antibody 04_A06 of the present invention compared to anti-HIV-1 antibodies of the prior art against a multiclade panel of 119 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay (as in Figure 2, and described in Schoofs et al., Immunity, 2019 Jun 18;50(6):1513-1529.e9); for presentation in this figure, 76 pseudovirus strains (A) or 99 pseudovirus strains (B) from the 119 multiclade panel for which neutralization data of reference antibodies was published were selected; (A) shows the neutralizing activity of 04_A06 compared to other anti-HIV-1 bNAbs that target a large variety of different known epitopes, (B) presents the neutralizing activity of antibody 04_A06 compared to the most potent and broadest anti-HIV-1 bNAbs of the prior art; neutralization data of the reference bNAbs were retrieved from the antibody neutralization database CATNAP (Yoon et al., 2015, *supra*).

Figure 4 shows the neutralizing activity (IC_{50}) of the antibody 04_A06 of the present invention against a multiclade panel of 100 clade C HIV-1 pseudoviruses (Subtype C panel) with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay (as described in Hraber et al., J Virol., 2017 Sep 12;91(19):e00991-17); data for antibody 04_A06 compared to the most potent and broadest CD4bs bNAbs of the prior art (3BNC117, 1-18, N6, VRC01, and VRC07-523-LS) are shown; neutralization data of the reference bNAbs were retrieved from the antibody neutralization database CATNAP (Yoon et al., 2015, *supra*).

Figure 5 shows the neutralizing activity (IC_{50}) of the antibody 04_A06 of the present invention against a panel of 9 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay; the pseudoviruses of this panel are known to display extraordinary resistance against CD4bs bNAbs; viral strains of this panel are constituents of the 119 multiclade and Subtype C pseudovirus panel (as described in Schoofs et al., Immunity, 2019 Jun 18;50(6):1513-1529.e9; and Hraber et al., J Virol., 2017 Sep 12;91(19):e00991-17); data for antibody 04_A06 compared to the most potent and broadest CD4bs bNAbs described to date (VRC07-523-LS, N6, 3BNC117, 1-18, VRC01) are shown; neutralization data of the reference bNAbs were retrieved from the antibody neutralization database CATNAP (Yoon et al., 2015, *supra*).

Figure 6 shows the neutralizing activity (IC_{50}) of the antibody 04_A06 of the present invention against a panel of 7 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay; tested virus strains were selected from a reference pseudovirus panel that is known as "Resistant panel to CD4bs bNAbs" and is described in Zhou et al., PLoS Pathog. 2019

Jun 13;15(6):e1007819. doi: 10.1371/journal.ppat.1007819. PMID: 31194843; PMCID: PMC6592578 2017 Sep 12;91(19):e00991-17; the pseudoviruses of this published reference panel display extraordinary resistance against CD4bs bNAbs; data for antibody 04_A06 compared to the most potent and broadest CD4bs bNAbs of the prior art (VRC07, N6, NIH45-46, 1-18, VRC01, N49P7, 3BNC117) are shown; all antibodies were tested in duplicates in independent assays.

Figure 7 shows the neutralizing activity (IC_{50}) of the antibody 01_D03 of the present invention against a panel of 8 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay; viral strains of this panel are constituents of the 119 multiclade (as described in Schoofs et al., Immunity, 2019 Jun 18;50(6):1513-1529.e9); selected pseudoviruses of this panel are insensitive against neutralization by CD4bs bNAbs; insensitivity against antibody neutralization was defined by an $IC_{50} > 1 \mu g/ml$; data for antibody 01_D03 compared to the most potent and broadest CD4bs bNAbs of the prior art (VRC07-523-LS, N6, VRC07, N49P7, 1-18) are shown; all antibodies were tested in duplicates in independent assays. neutralization data of the reference bNAbs were retrieved from the antibody neutralization database CATNAP (Yoon et al., 2015, *supra*).

Figure 8 shows the neutralizing activity (IC_{50} and IC_{80}) of the antibody 04_A06 of the present invention against a panel of 185 pseudoviruses with different HIV-1 envelope amino acid sequences when tested in the TZM-bl cell pseudovirus neutralization assay; viral strains of this pseudovirus panel were generated from virus sequences obtained from the Antibody Mediated Prevention Trials (as described in Corey L, Two Randomized Trials of Neutralizing Antibodies to Prevent HIV-1 Acquisition. N Engl J Med. 2021 Mar 18;384(11):1003-1014. doi: 10.1056/NEJMoa2031738. PMID: 33730454; PMCID: PMC8189692); data for antibody 04_A06 compared to reference bNAbs VRC01 is shown; neutralization data for other reference bNAbs was not publicly available; neutralization data of the reference bNAbs VRC01 was retrieved from Corey L, Two Randomized Trials of Neutralizing Antibodies to Prevent HIV-1 Acquisition. N Engl J Med. 2021 Mar 18;384(11):1003-1014. Doi: 10.1056/NEJMoa2031738. PMID: 33730454; PMCID: PMC8189692.

Figure 9 shows HIV-1 RNA plasma copy numbers (top) and their $\log_{10}$ change (bottom, compared to baseline (HIV-1 RNA plasma copy number of day -1)) in humanized mice infected with HIV-1_{YU2} (NL4-3 virus with envelope gene substituted for that of YU2); mice were treated subcutaneously with a 1 mg loading dose per antibody, followed by subcutaneous injections of 0.5 mg per antibody every 3-4 days; mice treated with the known CD4bs antibodies VRC01 and VRC07 show a transient reduction of viremia, followed by rapid viral rebound; in contrast, mice treated with antibody 04_A06 of the invention alone show a persistent reduction of viremia throughout the treatment period of 84 days; black lines indicate mean $\log_{10}$ change.

Figure 10 shows HIV-1 RNA plasma copy numbers (top) and their $\log_{10}$ change (bottom, compared to baseline (HIV-1 RNA plasma copy number at day 28)) in humanized mice infected with HIV-1_{YU2} (NL4-3/YU2) that were pre-treated with the CD4 binding site antibody VRC01 (as shown in Figure 8); after four weeks of treatment, antibody 04_A06 was added to the previous treatment regimen that was continued

throughout; 04_A06 was administered subcutaneously with a 1 mg loading dose, followed by 0.5 mg every 3 days to 4 days; despite viral rebound after pre-treatment with the CD4 binding site antibody VRC01, treatment with antibody 04_A06 resulted in maintained viral suppression in all treated animals.

Detailed Description of the Invention

The present inventors have dedicated themselves to solving the problem of the present invention and were successful to find novel and useful human monoclonal antibodies against HIV-1 which overcome the disadvantages and shortcomings of known antibodies.

10

Herein, the inventors describe novel CD4 binding site antibodies that exceed the potency and breadth of known CD4bs bNAbs such as antibody 1-18 as disclosed in WO 2021/110764 A1 or others. These properties are unprecedented and open new possibilities for antibody-based therapies and prevention of HIV-1 associated disease.

20

Thus, the present invention provides in a first aspect an antibody or antigen-binding fragment thereof directed against the CD4 binding site of the human immunodeficiency virus HIV-1, wherein the antibody or antigen-binding fragment thereof comprises a combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody from the group comprising **04_A06** (having a CDR-H1 amino acid sequence of SEQ ID No. 1, a CDR-H2 amino acid sequence of SEQ ID No. 2, a CDR-H3 amino acid sequence of SEQ ID No. 3, a CDR-L1 amino acid sequence of SEQ ID No. 4, a CDR-L2 amino acid sequence of SEQ ID No. 5, and a CDR-L3 amino acid sequence of SEQ ID No. 6), **01_D03** (having a CDR-H1 amino acid sequence of SEQ ID No. 7, a CDR-H2 amino acid sequence of SEQ ID No. 8, a CDR-H3 amino acid sequence of SEQ ID No. 9, a CDR-L1 amino acid sequence of SEQ ID No. 10, a CDR-L2 amino acid sequence of SEQ ID No. 11, and a CDR-L3 amino acid sequence of SEQ ID No. 12), **04_B09** (having a CDR-H1 amino acid sequence of SEQ ID No. 13, a CDR-H2 amino acid sequence of SEQ ID No. 14, a CDR-H3 amino acid sequence of SEQ ID No. 15, a CDR-L1 amino acid sequence of SEQ ID No. 16, a CDR-L2 amino acid sequence of SEQ ID No. 17, and a CDR-L3 amino acid sequence of SEQ ID No. 18), **05_C07** (having a CDR-H1 amino acid sequence of SEQ ID No. 19, a CDR-H2 amino acid sequence of SEQ ID No. 20, a CDR-H3 amino acid sequence of SEQ ID No. 21, a CDR-L1 amino acid sequence of SEQ ID No. 22, a CDR-L2 amino acid sequence of SEQ ID No. 23, and a CDR-L3 amino acid sequence of SEQ ID No. 24), and **01_B01** (having a CDR-H1 amino acid sequence of SEQ ID No. 25, a CDR-H2 amino acid sequence of SEQ ID No. 26, a CDR-H3 amino acid sequence of SEQ ID No. 27, a CDR-L1 amino acid sequence of SEQ ID No. 28, a CDR-L2 amino acid sequence of SEQ ID No. 29, and a CDR-L3 amino acid sequence of SEQ ID No. 30).

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Within the context of the present invention, the antibodies, which have been generated and described herein, may be used and claimed as the complete monoclonal human antibody or as any functional or binding fragment thereof. Preferably, the monoclonal human antibody or any kind of functional or antigen-

binding fragment thereof should at least comprise the complementarity determining regions (CDR) 1 to 3 of the heavy chain and CDR 1 to 3 of the light chain of the human monoclonal antibody.

The CDR regions of the antibody sequences described herein are preferably defined according to the numbering scheme of IMGT which is an adaptation of the numbering scheme of Chothia (ImMunoGeneTics information system®; Lefranc et al., NAR 27: 209-212 (1999); <http://www.imgt.org>).

According to the present invention, the CDR sequences of the light and heavy chain variable region sequences of the antibodies and antigen-binding fragments thereof described herein are as follows:

Source	CDR1	SEQ ID CDR1	CDR2	SEQ ID CDR2	CDR3	SEQ ID CDR3
Heavy chain 04_A06	DSFTVYRPRLS AYFIGEFN	1	VNIFRG AV	2	AWDKNVDDN PWRLDS	3
Light chain 04_A06	QDVGWA	4	TAH	5	QVFDS*	6
Heavy chain 01_D03	GYSFRDYR	7	MQPRY GAV	8	VTHKLYDGE DASDLTWRL DP	9
Light chain 01_D03	QDITNA	10	DGS	11	QHYEF**	12
Heavy chain 04_B09	AYFIGEFN	13	VNIFRG AV	14	AWDKNVDDN PWRLDS	15
Light chain 04_B09	QDVGWA	16	TTS	17	QVFES*	18
Heavy chain 05_C07	GYTFRDYR	19	LHPAY GAV	20	VTHKLYDGE DASDLTWRL DP	21
Light chain 05_C07	QDITNA	22	DGS	23	QHYEF**	24
Heavy chain 01_B01	GYSFRDYR	25	IQPRY GAV	26	VTHKLYDGE DASDLAWRL DP	27
Light chain 01_B01	QDITNA	28	DGS	29	QHYEF**	30

- 10 * The CDRL3 of antibodies 04_A06 and 04_B09 could not precisely be determined using the IMGT software. The reason is assumed to lie in the fact that the specific sequences did not fulfil the criteria of the numbering scheme and CDR3 sequence definition of IMGT. CDRL3 sequences of 04_A06 and 04_B09 as stated are defined herein on the assumption of an amino acid substitution in the J-region motif (F/W-G-X-G) from F-G-P-G to F-A-P-G and in comparison to gene sequences from other IGHV1-2 and IGKV1-33 encoded CD4bs bNAbs.

** The CDRL3 of antibody 01_D03, 05_C07 and 01_B01 could not precisely be determined using the IMGT software. The reason is assumed to lie in the fact that the specific sequence did not fulfil the criteria of the numbering scheme and CDR3 sequence definition of IMGT. CDRL3 sequence of 01_D03 as stated is defined herein on the assumption of an amino acid substitution in the J-region motif (F/W-G-X-G) from **F-G-P-G** to **F-P-P-V** and in comparison to gene sequences from other IGHV1-2 and IGKV1-33 encoded CD4bs bNAbs. CDRL3 sequences of 05_C07 and 01_B01 as stated are defined herein on the assumption of an amino acid substitution in the J-region motif (F/W-G-X-G) from **F-G-P-G** to **F-P-P-A** and in comparison to gene sequences from other IGHV1-2 and IGKV1-33 encoded CD4bs bNAbs.

- 10 Also, one preferred embodiment of the first aspect of the present invention provides antibodies or antigen-binding fragments directed against the CD4 binding site of HIV-1, wherein the antibody or antigen-binding fragment thereof comprises the combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of one antibody selected from the group comprising **04_A06** (consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence of SEQ ID No. 32, or preferably consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence as defined by SEQ ID No. 41), **01_D03** (consisting of the heavy chain amino acid sequence of SEQ ID No. 33 and the light chain amino acid sequence of SEQ ID No. 34), **04_B09** (consisting of the heavy chain amino acid sequence of SEQ ID No. 35 and the light chain amino acid sequence of SEQ ID No. 36), **05_C07** (consisting of the heavy chain amino acid sequence of SEQ ID No. 37 and the light chain amino acid sequence of SEQ ID No. 38), and **01_B01** (consisting of the heavy chain amino acid sequence of SEQ ID No. 39 and the light chain amino acid sequence of SEQ ID No. 40).

According to the present invention, the light and heavy chain variable region sequences of the preferred antibodies and antigen-binding fragments thereof described herein with the internal designations 04_A06, 01_D03, 04_B09, 05_C07 and 01_B01 are as follows:

SEQ ID NO	Source	Sequence
31	Heavy chain variable domain 04_A06	SVRLDQSGTAVKKPGASVRVSCRAPDSFTVYRPLSAYFIGEFNIHW LRQAPGQGLEWLGFVNIFRGAVKYSSRFQGRITITRDTSSSETSYLDL GALKADDTATYYCAWDKNVDDNPWRLDSWGQGTIVIVSS
32	Light chain variable domain 04_A06	YIQVTQSPSSLSASIGDTITVACEVSQDVGWAVNWWYHQRPGRPYPYN LIYTAHNLAPGVASRFRGSRVGTYFTLTINNLLPEDVGTYCQVFDSF APGGTRVDL
33	Heavy chain variable domain 01_D03	HVQLWQSGAEVKKPGASVKISCSAWGFGTTSYGYSFRDYRVHWWVRH IAGQGFQWMGHMQPRYGAVNYARQFQGRITMTREVSFDASGGTAY MELRSLRSDDTAVYYCVTHKLYDGEDASDLTWRLDPWGQGTRVIVS S

WO 2024/252014		PCT/EP2024/065842
34	Light chain variable domain 01_D03	GVHLTQSPASLSASVGDRVFTFCQASQDITNAINWYQLRPGKTPKL MIHDGSTLRRGVPSRFAGSGFGTKFTFTIDNLQPEDLATYFCQHYEF FPPVGTQVELN
35	Heavy chain variable domain 04_B09	SVRLDQSGTAVKKPGASVRVSCRAPDSFTVYRPRLSAYFIGEFNIHW LRQAPGQGLEWLGFVNIFRGAVKYSSAFRGRITITRDTSLTSYLDLG ALKPDDTATYFCAWDKNVDDNPWRLDSWGQGTLVVSS
36	Light chain variable domain 04_B09	YIQVTQSPSSLSASVGDTITVACQVSQDVGWAVNWWYHQRPGRPYPY NLIYTTSNLAPGVASRFRGTRVATYFTLTINLLPEDVGTYTCQVFES FAPGGTRVDLR
37	Heavy chain variable domain 05_C07	HVQLWQSGAEVKKPGASVKISCSAWGFGTRSGYTFRDYRIHWVRQ VPRQGFEWMGHLHPAYGAVNYARQFQGRIIMTREVSTFDGSGGTVY MELRSLRSDDTATYYCVTHKLYDGEDASDLTWRLDPWGQGTRVTV SS
38	Light chain variable domain 05_C07	GVSLTQSPASLCASVGDRVFTFCQASQDITNAINWYQLRPGKTPKL MIHDGSTLRRGVPSRFAGSGFGTRFTFTIDNLQPEDLATYFCQHYEF FPPAGTQVDFN
39	Heavy chain variable domain 01_B01	HVQLWQSGAEVKKPGASVKISCAAWGFGTTSGYSTFRDYRIHWVRQ VPGQGFQWMGHIQPRYGAVNYAHQFQGRITMTREVSTFDASGGTAY MELRSLRSDDTATYYCVTHKLYDGEDASDLAWRLDPWGQGTRVTV SS
40	Light chain variable domain 01_B01	GVHLTQSPASLSASVGDRVFTFCQASQDITNAINWYQLRPGKTPKL MIHDGSTLRRGVPSRFAGGGFGTKFTFTIDNLQPEDLATYFCQHYEF FPPAGTQVELN
41	Light chain variable domain 04_A06	YIQVTQSPSSLSASIGDTITVACEVSQDVGWAVNWWYHQRPGRPYPY LIYTAHNLAPGVASRFRGSRVGTYFTLTINLLPEDVGTYTCQVFDSF APGGTRVDLR

According to one embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 04_A06 (SEQ ID No. 31). According to one embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 01_D03 (SEQ ID No. 33). According to one embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 04_B09 (SEQ ID No. 35). According to one embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 05_C07 (SEQ ID No. 37).

10 According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence of antibody 01_B01 (SEQ ID No. 39).

According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 04_A06 as defined by SEQ ID

No. 32. According to an alternative and preferred embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 04_A06 as defined by SEQ ID No. 41. In accordance with this embodiment, the light chain variable region amino acid sequence of antibody 04_A06 may preferably be defined herein as SEQ ID NO: 41. According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 01_D03 (SEQ ID No. 34). According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 04_B09 (SEQ ID No. 36). According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 05_C07 (SEQ ID No. 38). According to an embodiment of the present invention, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence of antibody 01_B01 (SEQ ID No. 40).

According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 31 and a light chain variable region amino acid sequence of SEQ ID No. 32. According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 31 and a light chain variable region amino acid sequence of SEQ ID No. 41. According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 33 and a light chain variable region amino acid sequence of SEQ ID No. 34. According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 35 and a light chain variable region amino acid sequence of SEQ ID No. 36. According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 37 and a light chain variable region amino acid sequence of SEQ ID No. 38. According to a preferred embodiment of the present invention, the antibody or antigen-binding fragment comprises a heavy chain variable region amino acid sequence of SEQ ID No. 39 and a light chain variable region amino acid sequence of SEQ ID No. 40.

According to a specific embodiment of the present invention, the antibody consists of two heavy chains of sequence SEQ ID No. 31 and two light chains of sequence SEQ ID No. 32, or the antibody consists of two heavy chains of sequence SEQ ID No. 31 and two light chains of sequence SEQ ID No. 41, or the antibody consists of two heavy chains of sequence SEQ ID No. 33 and two light chains of sequence SEQ ID No. 34, or the antibody consists of two heavy chains of sequence SEQ ID No. 35 and two light chains of sequence SEQ ID No. 36, or the antibody consists of two heavy chains of sequence SEQ ID No. 37 and two light chains of sequence SEQ ID No. 38, or the antibody consists of two heavy chains of sequence SEQ ID No. 39 and two light chains of sequence SEQ ID No. 40.

According to a preferred embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody or antigen-binding fragment thereof according to the present invention is selected

from the group comprising 04_A06, 01_D03, 04_B09 and 05_C07, preferably from the group comprising 04_A06, 01_D03, and 04_B09, more preferably from the group comprising 04_A06 and 01_D03, particularly preferably is the antibody 04_A06.

In one embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 04_A06. In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 01_D03. In one embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 04_B09. In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 05_C07. In another embodiment of the present invention, the antibody used as a source for sequences comprised in the antibody of the invention is 01_B01.

In one preferred embodiment, the antibody is a monoclonal antibody or a fragment thereof that retains binding specificity and ability to neutralize infectious pathogen. In one preferred embodiment, the antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. For example, the antibody may be an antibody comprising an Fc domain of any human IgG isotype (e.g. IgG1, IgG2, IgG3, or IgG4).

Optionally, the antigen-binding compound consists of or comprises a Fab, Fab', Fab'-SH, F(ab)₂, Fv, a diabody, single-chain antibody fragment, or a multispecific antibody comprising multiple different antibody fragments.

Within the present invention, an antibody or binding fragment directed against the CD4 binding site of HIV-1 means an antibody binding to the CD4 binding site region within the gp120 envelope glycoprotein of HIV-1 with an at least 10-fold, more preferably at least 50-fold, particularly preferably at least 100-fold increased affinity compared to unrelated epitopes, proteins or protein regions. In general, the term CD4 binding site herein designates the CD4 binding site region within the gp120 envelope glycoprotein of HIV-1.

The present invention further provides and encompasses monoclonal human antibodies or binding fragments which comprise antibody amino acid sequences being at least 80% identical to the sequences as defined and disclosed herein as long as they are still directed against the CD4 binding site of the human immunodeficiency virus HIV-1. This is meant to include sequences having trivial mutations of the antibody amino acid sequence which do not interfere with structural folds and the affinity of the antibody to the CD4 binding site.

Commonly and preferably, such mutations which do not substantially interfere with the structure and function of the antibody lie outside of the CDRs of the antibody, more preferably such mutations lie outside the CDRs and outside the framework regions of the antibody. Accordingly, it is preferred that the antibody amino acid sequences which are at least 80% identical to the sequences as defined herein do not carry mutations in the CDRs, more preferably not in the CDRs or in the framework regions of the antibody, if any mutations are present at all.

It is a trivial task for a skilled person to determine if an antibody which exhibits a certain degree of identity is directed against the CD4 binding site of the human immunodeficiency virus HIV-1 based on the above or the common general knowledge.

The determination of percent identity between two sequences is accomplished according to the present invention by using the mathematical algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA (1993) 90: 5873-5877). Such an algorithm is the basis of the BLASTN and BLASTP programs of Altschul et al. (J. Mol. Biol. (1990) 215: 403-410). BLAST nucleotide searches are performed with the BLASTN program.

10 To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described by Altschul et al. (Nucleic Acids Res. (1997) 25: 3389-3402). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used.

According to a preferred embodiment of the present invention, antibody amino acid sequences form part of the invention which consist of or comprise a nucleic acid sequence being at least 85% identical to the sequences defined above and disclosed herein, more preferably at least 90% identical, even more preferred at least 95% identical. According to one preferred embodiment, the mutations in the amino acid sequence which may lead to a sequence identity of less than 100% lie outside of the CDRs disclosed herein, more preferably such mutations lie outside the CDR and the framework regions of the antibody sequences disclosed herein.

20 Within the present invention, the antibodies disclosed are tested for their neutralizing activity against a wide selection of pseudoviruses. The TZM-bl cell pseudovirus neutralization assay used to study the breadth and strength of neutralization is a highly standardized assay which is commonly used in the art and in the technical field of the invention for an analysis of neutralization potency of antibodies against various HIV-1 strains. In brief, antibodies and virus strains are incubated together before TZM-bl target cells are added. These cells exhibit luciferase activity in case of a successful infection, resulting in a detectable luminescence signal in the presence of luciferin after lysis of cells. Neutralizing antibodies are able to prevent infection and therefore the generation of luminescence. The potency of a neutralizing antibody is

30 determined by the concentration of the antibody required to reduce virus infectivity by a particular amount.

An exemplary and standardized way of setting up and employing this assay is disclosed and described in detail in Sarzotti-Kelsoe *et al.*; *J Immunol Methods*. 2014 July ; 0: 131–146. doi:10.1016/j.jim.2013.11.022. Preferably, the method described in the “Examples” section below is used to determine the neutralizing activity. This description alone and in combination with the common prior art enables the skilled person to establish and determine a conclusive readout of the TZM-bl cell pseudovirus neutralization assay as used herein.

Neutralization potency as defined herein preferably takes only those virus variants into account that could positively be determined as being neutralized by the respective antibodies. Based on the selection of positively neutralized variants, a geometric mean IC_{50} is then determined across the neutralized strains.

According to a preferred embodiment of the first aspect of the present invention, the antibody or antigen-binding fragment thereof comprises a combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of the antibody 04_A06 (consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence as defined by SEQ ID No. 32, or preferably consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence as defined by SEQ ID No. 41).

Preferably, the antibodies or antigen-binding fragments as described herein are mammalian, more preferably human. Further preferably, the antibodies or antigen-binding fragments as described herein are monoclonal, and may be used and administered in a monoclonal fashion, i.e. without a concurrent or overlapping administration of another monoclonal antibody to a patient. Alternatively, preferably, the antibodies or antigen-binding fragments of the present invention may be administered in combination with one or more different monoclonal antibodies, e.g. in a concurrent or overlapping administration, so that all of said antibodies deliver their effects concomitantly.

In the description of the present application, antibody designations may be used. It is pointed out that the antibodies consist of heavy and light chains which also form part of the present description. If reference is made to an antibody by its designation or to a SEQ ID No., it should be understood that these ways of reference are interchangeable.

The present invention further relates in a second aspect to a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to the first aspect of the present invention, and at least one pharmaceutically acceptable excipient. Preferably, the pharmaceutical composition according to the second aspect of the present invention is a vaccination composition for a human subject.

Preferably, the pharmaceutical composition according to the second aspect of the present invention may further comprise one or more antibodies directed against a different epitope within HIV-1, preferably directed against the HIV-1 envelope (Env) trimer, more preferably directed against the V3 glycan site.

The present invention also encompasses in a third aspect a kit comprising an antibody or antigen-binding fragment thereof according to the first aspect of the present invention and a container.

In a fourth aspect of the present invention, the antibody or antigen-binding fragment thereof according to the first aspect of the present invention, the pharmaceutical composition according to the second aspect, and the kit according to the third aspect of the present invention are for use as a medicament.

In a fifth aspect of the present invention, the antibody or antigen-binding fragment thereof according to the first aspect of the present invention, the pharmaceutical composition according to the second aspect, and the kit according to the third aspect of the present invention are for use in the treatment or prevention of an infection with the human immunodeficiency virus HIV-1 in mammalian subjects.

According to a sixth aspect of the present invention, the antibody or antigen-binding fragment thereof according to the first aspect of the present invention, the pharmaceutical composition according to the second aspect, and the kit according to the third aspect of the present invention are for use in the treatment or prevention of a disease caused by the human immunodeficiency virus HIV-1 in mammalian subjects.

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In a preferred embodiment of the fifth or sixth aspect of the present invention, the use is in human subjects. In another preferred embodiment of the fifth or sixth aspect of the present invention, the use is for vaccination and/or in the form of a vaccination composition. In yet another preferred embodiment of the fifth or sixth aspect of the present invention, the use is in the treatment or prevention of acquired immune deficiency syndrome (AIDS).

20

In one other aspect, the present invention is also directed to a method of treatment of a patient suffering from a disease caused by the human immunodeficiency virus HIV-1 in human subjects, preferably for use in the treatment or prevention of acquired immune deficiency syndrome (AIDS) in human subjects, wherein the patient is administered an effective amount of the antibody or antigen-binding fragment thereof according to the invention or a pharmaceutical composition of the invention.

In another aspect, the present invention is also directed to the use of the antibody or antigen-binding fragment thereof according to the invention or a pharmaceutical composition of the invention in the manufacture of a medicament for treatment of a disease caused by the human immunodeficiency virus HIV-1 in human subjects, preferably for treatment or prevention of acquired immune deficiency syndrome (AIDS) in human subjects.

30

According to the seventh aspect of the present invention, a nucleic acid is provided encoding an antibody or antigen-binding fragment thereof according to the first aspect of the present invention.

According to the eighth aspect of the present invention, an expression vector comprising the nucleic acid of the seventh aspect of the present invention is provided in functional association with an expression control sequence.

According to the ninth aspect of the present invention, a host cell is provided comprising a nucleic acid according to the seventh aspect of the present invention or the expression vector according to the eighth aspect of the present invention.

According to the tenth aspect of the present invention, a method of production of an antibody or antigen-binding fragment thereof according to the first aspect of the present invention is provided, comprising cultivating the host cell of the ninth aspect of the present invention under conditions allowing expression of the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof.

All embodiments of the present invention as described herein are deemed to be combinable in any combination, unless the skilled person considers such a combination to not make any technical sense.

10

Examples

A) Experimental Methods

Isolation of Monoclonal Antibody Sequences

Blood and leukapheresis samples were obtained under protocols approved by the Institutional Review Board of the University of Cologne (protocols 13-364 and 16-054) and the participant provided written informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation and stored at -150°C in 90% FBS and 10% DMSO. B cells were isolated from PBMCs by magnetic cell separation and labeled with anti-human CD19-AF700, anti-human IgG-APC, DAPI (BD), and an HIV-1 Env bait protein for 30 minutes on ice.

20

The HIV-1 Env bait protein was either BG505^{SOSIP.664}-GFP (Sliepen K et al., *Biomolecules*, 2015 Oct 23;5(4):2919-34) or biotinylated (EZ-Link Sulfo NHS Biotinylation and Labeling Kit, Thermo Fisher) YU2_{gp140} (Yang et al., *J Virol.* 2000 Jun;74(12):5716-25. doi: 10.1128/jvi.74.12.5716-5725.2000) that was labeled with Streptavidin-PE. Env-reactive CD19⁺IgG⁺DAPI⁻ single cells were sorted as described before (Gieselmann et al., *Nat Protoc.* 2021 Jul;16(7):3639-3671. doi: 10.1038/s41596-021-00554-w.). Sorted cells were incubated with random hexamer primer, NP-40, and RNase-free H₂O for 1 min at 65°C. Subsequently, cDNA was generated using SuperScript IV in the presence of RT Buffer, dNTPs, DTT, H₂O, RNasin, and RNaseOUT. Antibody sequences for single cell analysis were amplified by semi-nested PCRs using Taq polymerase and the previously described primers CG_RT (Ozawa et al., *Biotechniques*. 2006 Apr; 40(4):469-70, 472, 474 passim. doi: 10.2144/000112123, first PCR), IgG_Internal RT (Tiller et al., *J Immunol Methods*. 2008 Jan 1;329(1-2):112-24. doi: 10.1016/j.jim.2007.09.017. Epub 2007 Oct 31. , second PCR), and the OPT5/oPR-primer mix (Kreer et al., *J Immunol Methods*. 2020 May;480:112752. doi: 10.1016/j.jim.2020.112752, both PCRs)

30

Antibody Sequence Analysis

Sequences of 2nd PCR products with a mean Phred score ≥ 28 and a minimal length of 240 nucleotides were annotated with IgBLAST (Ye et al., 2013) and trimmed from framework region (FWR) 1 of the variable region to the end of the J gene. Base calls with a Phred score < 16 were masked and sequences with > 15 masked nucleotides, frameshifts, or stop codons were excluded from further analyses. To analyze the sequences for potential clonalities, all productive heavy chain sequences were grouped by identical V genes and the pairwise Levenshtein distance of their CDRH3s was determined.

10 Individual sequences were grouped into clones when they shared the same V gene and had a minimal CDRH3 identity of 75%. After 10 rounds with a randomized input of sequences the result that yielded the lowest number of unassigned (non-clonal) sequences was selected for further analyses. All clones were re-validated manually by the investigators in order to identify shared mutations. Sequences that were initially assigned to different clones but shared the same VDJ genes and amino acid and/or silent nucleotide mutations were subsequently grouped into subclones. Nucleotide sequence identity to germline was calculated using IgBLAST.

Monoclonal Antibody Production

20 For cloning of single cell-derived antibodies, the 1st PCR product of single cell-PCR was used as template and amplified using Q5 High Fidelity Polymerase and specific forward- and reverse primers that resembled the respective nucleotide sequence of the V- and J-regions (Giesemann et al., 2021, *supra*) with expression vector overhangs for subsequent sequence and ligation independent cloning (SLIC). PCR products were cloned into human antibody expression vectors (IgG1, kappa, or lambda chain) by SLIC assembly as previously described (von Boehmer et al., Nat Protoc. 2016 Oct;11(10):1908-1923. doi: 10.1038/nprot.2016.102). Antibodies were produced in HEK293-6E cells by transfection using polyethylenimine. After 5-7 days, antibodies were purified from supernatants after protein G incubation and subsequent elution from chromatography columns using 0.1 M glycine (pH=3.0). After buffer neutralization, buffer exchange to PBS, and filter-sterilization, antibodies were stored at 4°C.

Pseudovirus Production

30 Pseudoviruses were produced in HEK293T cells by co-transfection with pSG3ΔEnv plasmid as described previously (Doria-Rose et al., PLoS Pathog. 2017 Jan 4;13(1):e1006148. doi: 10.1371/journal.ppat; Sarzotti-Kelsoe et al., J Immunol Methods. 2014 Jul;409:131-46. doi: 10.1016/j.jim.2013.11.022; Hraber et al., J Virol. 2017 Sep 12;91(19):e00991-17. doi: 10.1128/JVI.00991-17.; Seaman et al., J Virol. 2010 Feb;84(3):1439-52. doi: 10.1128/JVI.02108-09).

TZM-bl Cell Neutralization Assay

Neutralization assays were performed as previously described (Sarzotti-Kelsoe et al., 2014, *supra*; Seaman et al., 2010, *supra*). Murine leukemia virus (MuLV)-pseudotyped virus was used to determine unspecific activity. Antibodies were tested in duplicates. For the global reference panel, bioluminescence was

determined after adding a luciferin/lysis-buffer (10 mM MgCl₂, 0.3 mM ATP, 0.5mM Coenzyme A, 17 mM IGEPAL (all Sigma-Aldrich), and 1mM D-Luciferin (GoldBio) in Tris-HCL).

HIV-1-infected Humanized Mice

Humanized mice were generated as previously described (Klein et al., 2012) with modifications. NOD.Cg-*Rag1^{tm1mom}Il2rg^{tm1Wjl}/SzJ* (NRG) mice were humanized within five days of birth and 3-6 hours after sublethal irradiation by intrahepatic injection of human CD34⁺ hematopoietic cord blood and/or placental tissue stem cells. Humanized mice were infected by intraperitoneal challenge using replication-competent recombinant HIV-1_{YU2} (YU2 *env* in NL4-3 backbone (Zhang et al., J Virol. 2002 Jun;76(12):6332-43. doi: 10.1128/jvi.76.12.6332-6343.2002) that was harvested from supernatants of transfected HEK293T cells.

HIV-1 Viral Load Measurements

Plasma RNA was extracted from EDTA plasma samples using the MinElute Virus Mini Spin Kit including an on-column DNase I digestion step. Viral loads were determined by quantitative real-time PCR using *pol*-specific primers described previously (Horwitz et al., Proc Natl Acad Sci U S A. 2013 Oct 8;110(41):16538-43. doi: 10.1073/pnas.1315295110). qPCR was performed on a QuantStudio 5 using the Taqman RNA-to-Ct 1-Step-Kit. Viral loads were quantified by including a standard curve derived from a sample of known copy number with every qPCR run. The limit of accuracy of the qPCR was determined as 784 copies/ml.

Antibody Treatment of HIV-1-infected Humanized Mice

Antibodies diluted in PBS were injected subcutaneously. Following a 1 mg loading dose, doses of 0.5 mg were injected every 3-4 days.

B) Specific Examples of Antibodies of the Invention

Example I – Isolation of Broad and Potent VH1-2 Derived HIV-1-Neutralizing Antibodies from an HIV-1-Infected Elite Neutralizer

Human B cells reactive to the HIV-1 envelope protein were isolated from an HIV-1-infected individual who was previously identified as having exceptional serum neutralizing activity against HIV-1 in *in vitro* assays.

To this end, isolated B cells were incubated with fluorochrome-labeled soluble HIV-1 Env protein (YU2_{gp140} or BG505_{SOSIP.664}) and single-cell sorted (Giesemann et al., 2021, *supra*). This approach enabled the subsequent amplification of heavy and light antibody gene segments from an individual HIV-1 Env-reactive B cell by PCR. This allowed for the cloning of the PCR products into expression vectors for the recombinant production of the corresponding antibody encoded by an individual B cell, enabling functional testing of the antibody.

From the analysis of the sequences of HIV-1-Env reactive B cells, expanded VH1-2-derived B cell clones could be identified. One of these clones was characterized by a twelve amino acid (aa) insertion and a deletion of one amino acid in heavy chain CDR1/FWR1 region. Antibody 04_A06 is representative of this

B cell clone. Another of these clones, encompasses a 6 aa CDRH1/FWRH1 and a 4 aa FWRH3 insertion. Antibody 01_D03 is a representative of this B cell clone.

To determine the overall neutralizing potency and breadth, isolated antibody 04_A06 as well as other antibodies were tested for neutralizing activity in the TZM-bl cell neutralization assay against a reference panel of 12 HIV-1 pseudovirus strains that is referred to as “global panel” (**Figure 1**). This panel of pseudoviruses was previously designed to be a representation of the diversity of the global HIV-1 epidemic and enable a standardized assessment of the activity of neutralizing antibodies.

10 Notably, all antibodies of the present invention (i.e. 04_A06, 01_D03, 04_B09, 05_C07 and 01_B01) demonstrated high neutralizing activity against 100% (12/12) of the strains included in the panel (**Figure 1**). The neutralizing potency is routinely represented by the 50% inhibitory concentration (IC₅₀). When tested against the “global panel”, all of the antibodies of the present invention exhibited a geometric mean IC₅₀ of less than 0.05 µg/ml, wherein 04_A06 and 01_D03 were highly active with a geometric mean IC₅₀ of 0.038 µg/ml or 0.021 µg/ml against the neutralized virus strains (**Figure 1**).

Example II – High Potency and Breadth of Antibody 04_A06

20 To confirm the neutralizing potency and breadth of 04_A06, the antibody was tested against an extended panel of 119 HIV-1 pseudoviruses as previously tested in Schoofs et al., 2019. This panel of pseudoviruses represents a development of a panel of pseudoviruses described in detail in Seaman et al., 2010. It provides a representation of the genetic and global diversity of HIV-1 Env variants. It includes HIV-1 variants of different clades or subtypes, including variants isolated from transmitted/founder viruses and difficult-to-neutralize viruses.

Notably, 04_A06 demonstrated high neutralizing potency and breadth when tested on the 119-pseudovirus multiclade panel (**Figure 2**). In particular, when tested at antibody concentrations up to 10 µg/ml, antibody 04_A06 neutralized 99% of the tested pseudoviruses and showed a geometric mean IC₅₀ of 0.036 µg/ml against the neutralized pseudoviruses (**Figure 2**). This breadth and potency are higher than seen for other HIV-1 neutralizing antibodies that also target the CD4 binding site and are in advanced stages of clinical testing (e.g. 3BNC117, VRC01, and VRC07-523-LS) (**Figure 2**). Moreover, the potency of antibody 04_A06 in the 119-multiclade panel was higher than that of N6, another VH1-2-derived CD4 binding site antibody that is currently investigated in clinical testing (**Figure 2**).

In addition, when compared to other HIV-1 neutralizing antibodies and other CD4bs bNAbs, for which results against a total of 76 (**Figure 3 A**) and 99 (**Figure 3 B**) identical HIV-1 pseudovirus were available (Yoon et al., 2015, *supra*), 04_A06 demonstrated the highest levels of potency and/or breadth.

Example III – High Potency and Breadth of Antibody 04_A06 against Clade C HIV-1 Pseudoviruses

40 HIV-1 viruses of the group M can be divided into nine clades and different recombinants. HIV-1 viruses from clade C are responsible for approximately half of current HIV-1 infections and represent the most

common lineage worldwide. Therefore, clade C viruses are investigated in many different antibody and vaccine studies. In order to confirm the antiviral activity of antibody 04_A06 against clinically relevant HIV-1 clades, we determined the neutralization profile of the antibody against a published reference panel, that consists of 100 HIV-1 pseudoviruses isolated from different stages of infection and difficult-to-neutralize viruses. The so-called Subtype C panel is described in detail in Hraber et al., J Virol., 2017 Sep 12;91(19):e00991-17.

Notably, 04_A06 demonstrated high neutralizing potency and breadth when tested against the 100 strain Subtype C pseudovirus panel (**Figure 4**). In particular, when tested at antibody concentrations up to 10 µg/ml, antibody 04_A06 neutralized 99% of the tested pseudoviruses and showed a geometric mean IC₅₀ of 0.057 µg/ml against the neutralized pseudoviruses (**Figure 4**). The breadth and potency of 04_A06 is higher than observed for the best CD4bs bNAbs investigated against the Subtype C pseudovirus panel so far (3BNC117, 1-18, N6, VRC01, VRC07-523-LS) (**Figure 4**). Neutralization data for the CD4bs reference bNAbs was obtained from the CATNAP database (Yoon et al., 2015, *supra*).

Example IV – Neutralizing Activity against Pseudoviruses that are Poorly Neutralized by other CD4 binding site antibodies

Although CD4 binding site antibodies can achieve high levels of neutralizing breadth (i.e., have activity against a large number of different HIV-1 Env variants), antibody-resistant HIV-1 variants exist. Thus, it is important to identify novel CD4 binding site antibodies that are highly active against such HIV-1 variants. Therefore, we determined the neutralization profile of antibody 04_A06 described in this invention against a panel of nine (**Figure 5**) and seven (**Figure 6**) HIV-1 variants that are resistant against neutralization by CD4bs bNAbs.

Tested virus strains illustrated in **Figure 5** are constituents of the 119 multiclade and Subtype C pseudovirus panel (as described in as described in Schoofs et al., Immunity, 2019 Jun 18;50(6):1513-1529.e9, Hraber et al., J Virol., 2017 Sep 12;91(19):e00991-17). Tested virus strains illustrated in **Figure 6** were selected from a reference pseudovirus panel that is known as “Resistant panel to CD4bs bNAbs” and is described in Zhou et al., PLoS Pathog. 2019 Jun 13;15(6):e1007819. Doi: 10.1371/journal.ppat.1007819. PMID: 31194843; PMCID: PMC6592578 2017 Sep 12;91(19):e00991-17.

Compared to CD4 binding site antibodies in advanced stages of clinical testing and to previously published best in class CD4bs bNAbs (VRC07-523-LS, N6, 3BNC117, 1-18, VRC01), antibody 04_A06 neutralizes resistant pseudovirus variants with much higher breadth and/or potency (**Figure 5** and **Figure 6**).

For example, 100% of the nine pseudovirus panel and 86% of the seven pseudovirus panel are neutralized by 04_A06 when tested at concentrations up to 10 µg/ml in the TZM-bl pseudovirus neutralization assay. In contrast, the broadest and most potent CD4bs reference bNAbs described so far only neutralize between 0% - 67% of the nine pseudovirus panel (**Figure 5**) and 0% - 57% of the seven pseudovirus

panel (**Figure 6**). In addition, 04_A06 displays the highest potency (IC_{50} of 0.153 $\mu\text{g/ml}$) against strains of the seven pseudovirus panel compared to reference CD4bs bNAbs (**Figure 6**).

Thus, antibodies of this invention provide a solution to the poor neutralization of viruses that are resistant against neutralization by CD4bs bNAbs.

Example V – Neutralizing Activity of Antibody 01_D03 Against Pseudoviruses that are Insensitive to Neutralization by CD4bs bNAbs

In addition to virus variants that are resistant, there are also variants that are insensitive against neutralization by most CD4bs bNAbs. Clinical trials have shown that the sensitivity of virus variants critically determine the success of bNAb-mediated treatment or prevention strategies. The inventors investigated the neutralization profile of antibody 01_D03 against a virus panel that consists of eight pseudovirus strains (**Figure 7**) which are known to be insensitive against neutralization by most CD4bs bNAbs.

Viral strains of this panel are constituents of the 119 multiclade pseudovirus panel (as described in Schoofs et al., Immunity, 2019 Jun 18;50(6):1513-1529.e9). Insensitivity against neutralization by a given antibody was defined by an $IC_{50} > 1 \mu\text{g/ml}$. Antibody 01_D03 neutralizes 88% (7/8) of the viral strains of the eight pseudovirus panel whereas the broadest and most potent reference bNAbs against CD4bs described so far only neutralize 25% – 75% (**Figure 7**). Thus, antibodies of this invention provide a solution to the poor neutralization of viruses that are insensitive against neutralization by most other CD4bs bNAbs.

Example VI – Neutralizing Activity against Pseudoviruses generated from the Antibody Mediated Prevention Trials

The recent Antibody Mediated Prevention (AMP) Trials have provided proof of concept that bNAb administration can in principle be effective to prevent HIV-1 infections in humans. In this trial, the prevention efficacy of CD4bs bNAb VRC01 strongly depended on the sensitivity of circulating viral strains against the antibody. VRC01 displayed a prevention efficacy of 75% against virus strains that were neutralized with an IC_{80} below 1 $\mu\text{g/ml}$ (as described in Corey L, Two Randomized Trials of Neutralizing Antibodies to Prevent HIV-1 Acquisition. N Engl J Med. 2021 Mar 18;384(11):1003-1014. Doi: 10.1056/NEJMoa2031738. PMID: 33730454; PMCID: PMC8189692).

Antibody 04_A06 neutralized up to 98% of 185 pseudovirus strains generated from the virus sequences obtained from the AMP trials (**Figure 8**). In contrast, VRC01 only displayed a breadth of up to 83% against the tested pseudovirus panel. In addition, the GeoMean IC_{50} (0.084 $\mu\text{g/ml}$) of 04_A06 against the virus panel was approximately 10-fold higher than for VRC01 (0.839 $\mu\text{g/ml}$). Thus, 04_A06 can be regarded as a promising bNAb candidate for future antibody-based prevention strategies.

Example VII – Maintaining Viral Suppression by Antibody Monotherapy in an *In Vivo* Model of HIV-1-Infection

Humanized mice infected with HIV-1_{YU2} (Zhang et al., 2002, *supra*) provide a model to study the antiviral activity of neutralizing HIV-1 antibodies *in vivo*. To generate humanized mice, immunodeficient NRG mice are irradiated within the first days after birth and injected intrahepatically with human hematopoietic CD34⁺ stem cells. This results in the development of human lymphocytes that can be infected with replication-competent HIV-1. These mice can maintain stable levels of viremia (i.e., HIV-1 RNA copy numbers in plasma) and show a rate of HIV-1 sequence diversification that is similar to what is observed in humans (Klein et al., Nature. 2012 Dec 6;492(7427):118-22. doi: 10.1038/nature11604). This mouse model has previously been used to determine the antiviral activity of several CD4 binding site antibodies given as monotherapy *in vivo* (179NC75: Freund et al., PLoS Pathog. 2015 Oct 30;11(10):e1005238. doi: 10.1371/journal.ppat.1005238; 3BNC117: Horwitz et al, Proc Natl Acad Sci U S A. 2013 Oct 8;110(41):16538-43. doi: 10.1073/pnas.1315295110; 45-46^{G54W}: Klein et al, 2012, *supra*; NC37: Freund et al., Sci Transl Med. 2017 Jan 18;9(373):eaal2144. doi: 10.1126/scitranslmed.aal2144).

In all of these studies, only transient effects on the viral load were observed and viral rebound (i.e., return of viremia towards baseline levels) rapidly developed. Moreover, this viral rebound was associated with the development of mutations in the antibody target sites. Thus, novel CD4 binding site antibodies are required that are capable of maintaining viral suppression when given as monotherapy.

When HIV-1_{YU2}-infected humanized mice were treated with the CD4 binding site antibody VRC01 or VRC07 (1 mg loading dose per antibody subcutaneously (s.c.), followed by 0.5 mg given s.c. every 3-4 days), only a transient reduction of the HIV-1 RNA copy number was observed, and viremia returned to baseline levels in most mice within 2-4 weeks (**Figure 9**). In contrast, when mice were treated according to the same dosing scheme with the representative antibody 04_A06 of the invention, viral suppression compared to the viral copy number at the start of treatment was observed in all treated mice for a period of 84 days of treatment (**Figure 9**). In all mice (100%), the HIV-1 RNA plasma copy number was reduced to levels below the limit of accuracy of the used assay (784 copies/ml).

Thus, 04_A06 is capable to effectively induce and maintain viral suppression in HIV-1_{YU2}-infected humanized mice even when given as monotherapy.

Example VIII - Maintaining Viral Suppression by Antibody Monotherapy in an *In Vivo* Model of HIV-1-Infection After Viral Rebound from VRC01-Class Therapy

Failure of antibody monotherapy using CD4 binding site antibodies in HIV-1-infected humanized mice results in viral rebound and is associated with viral resistance against the administered antibody (Freund et al., 2015, *supra*; Horwitz et al, 2013, *supra*; Klein et al, 2012, *supra*; Freund et al., 2017, *supra*). Thus, novel CD4 binding site antibodies are required that provide an *in vivo* treatment option after previous failure of CD4 binding site therapy.

Following the viral rebound observed during four weeks of treatment with VRC01 (as described in **Example VII**), the addition of antibody 04_A06 of the invention to treatment regimen (1 mg loading dose s.c. followed by 0.5 mg given s.c. every 3 days to 4 days) resulted in a sustained reduction of viremia in all (100%) of treated mice (**Figure 10**). Thus, antibody 04_A06 provides an option for the *in vivo* control of HIV-1 even after the failure of pretreatment with other CD4 binding site antibodies.

Claims

1. Antibody or antigen-binding fragment thereof directed against the CD4 binding site of the human immunodeficiency virus HIV-1, wherein the antibody or antigen-binding fragment thereof comprises a combination of the heavy chain CDR1 to CDR3 and the light chain CDR1 to CDR3 amino acid sequence of one antibody selected from the group comprising:

04_A06 (having a CDR-H1 amino acid sequence of SEQ ID No. 1, a CDR-H2 amino acid sequence of SEQ ID No. 2, a CDR-H3 amino acid sequence of SEQ ID No. 3, a CDR-L1 amino acid sequence of SEQ ID No. 4, a CDR-L2 amino acid sequence of SEQ ID No. 5, and a CDR-L3 amino acid sequence of SEQ ID No. 6),

01_D03 (having a CDR-H1 amino acid sequence of SEQ ID No. 7, a CDR-H2 amino acid sequence of SEQ ID No. 8, a CDR-H3 amino acid sequence of SEQ ID No. 9, a CDR-L1 amino acid sequence of SEQ ID No. 10, a CDR-L2 amino acid sequence of SEQ ID No. 11, and a CDR-L3 amino acid sequence of SEQ ID No. 12),

04_B09 (having a CDR-H1 amino acid sequence of SEQ ID No. 13, a CDR-H2 amino acid sequence of SEQ ID No. 14, a CDR-H3 amino acid sequence of SEQ ID No. 15, a CDR-L1 amino acid sequence of SEQ ID No. 16, a CDR-L2 amino acid sequence of SEQ ID No. 17, and a CDR-L3 amino acid sequence of SEQ ID No. 18),

05_C07 (having a CDR-H1 amino acid sequence of SEQ ID No. 19, a CDR-H2 amino acid sequence of SEQ ID No. 20, a CDR-H3 amino acid sequence of SEQ ID No. 21, a CDR-L1 amino acid sequence of SEQ ID No. 22, a CDR-L2 amino acid sequence of SEQ ID No. 23, and a CDR-L3 amino acid sequence of SEQ ID No. 24), and

01_B01 (having a CDR-H1 amino acid sequence of SEQ ID No. 25, a CDR-H2 amino acid sequence of SEQ ID No. 26, a CDR-H3 amino acid sequence of SEQ ID No. 27, a CDR-L1 amino acid sequence of SEQ ID No. 28, a CDR-L2 amino acid sequence of SEQ ID No. 29, and a CDR-L3 amino acid sequence of SEQ ID No. 30).

2. Antibody or antigen-binding fragment according to claim 1, wherein the antibody or antigen-binding fragment thereof comprises a combination of the variable region heavy chain amino acid sequence and of the variable region light chain amino acid sequence of one antibody selected from the group comprising:

04_A06 (consisting of the heavy chain amino acid sequence of SEQ ID No. 31 and the light chain amino acid sequence of SEQ ID No. 41),

01_D03 (consisting of the heavy chain amino acid sequence of SEQ ID No. 33 and the light chain amino acid sequence of SEQ ID No. 34),

04_B09 (consisting of the heavy chain amino acid sequence of SEQ ID No. 35 and the light chain amino acid sequence of SEQ ID No. 36),

05_C07 (consisting of the heavy chain amino acid sequence of SEQ ID No. 37 and the light chain amino acid sequence of SEQ ID No. 38), and

10 **01_B01** (consisting of the heavy chain amino acid sequence of SEQ ID No. 39 and the light chain amino acid sequence of SEQ ID No. 40).

3. Antibody or antigen-binding fragment thereof according to claim 1 or 2, wherein the amino acid sequences comprised are of one antibody selected from the group comprising 04_A06, 01_D03, 04_B09 and 05_C07, preferably of one antibody from the group comprising 04_A06, 01_D03, and 04_B09, more preferably of one antibody from the group comprising 04_A06 and 01_D03, particularly preferably of the antibody 04_A06.

20 4. Pharmaceutical composition comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 to 3 and at least one pharmaceutically acceptable excipient.

5. Pharmaceutical composition according to claim 4, wherein the pharmaceutical composition is a vaccination composition for a human subject.

6. Kit comprising an antibody or antigen-binding fragment thereof according to any one of claims 1 to 3 and a container.

7. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, pharmaceutical composition according to any one of claims 4 or 5, or kit according to claim 6 for use as a medicament.

30 8. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, pharmaceutical composition according to any one of claims 4 or 5, or kit according to claim 6 for use in the treatment or prevention of an infection with the human immunodeficiency virus HIV-1 in mammalian subjects.

9. Antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, pharmaceutical composition according to any one of claims 4 or 5, or kit according to claim 6 for use in the treatment or prevention of a disease caused by the human immunodeficiency virus HIV-1 in mammalian subjects.

40 10. Antibody or antigen-binding fragment thereof, pharmaceutical composition, or kit for use according to any one of claims 8 or 9, wherein the use is in human subjects.

11. Antibody or antigen-binding fragment thereof, pharmaceutical composition, or kit for use according to any one of claims 8 to 10, wherein the use is in the treatment or prevention of acquired immune deficiency syndrome (AIDS).
12. Nucleic acid encoding an antibody or antigen-binding fragment thereof according to any one of claims 1 to 3.
13. An expression vector comprising the nucleic acid of claim 12 in functional association with an expression control sequence.
14. Host cell comprising a nucleic acid according to claim 12 or the expression vector according to claim 13.
15. Method of production of an antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, comprising
- (a) cultivating the host cell of claim 14 under conditions allowing expression of the antibody or antigen-binding fragment thereof, and
 - (b) recovering the antibody or antigen-binding fragment thereof.

Figures

Antibody	Global virus panel												GeoMean	Breadth	
	Tro11	25710	398F1	CNE8	X2278	BuOX20 00	X1632	CE1176	246F3	CH119	CEO217	CNE55	IC ₅₀ (µg/ml)	(%)	
	IC ₅₀ (µg/ml)														
01_D03	0.015	0.017	0.010	0.028	0.010	0.072	0.031	0.040	0.016	0.023	0.011	0.028	0.021	100	
	0.047	0.031	0.040	0.028	0.047	0.024	0.060	0.084	0.063	0.032	0.018	0.024	0.038	100	
	0.038	0.037	0.079	0.081	0.017	0.018	0.039	0.114	0.108	0.040	0.019	0.027	0.042	100	
	0.041	0.045	0.041	0.063	0.031	0.096	0.032	0.231	0.031	0.054	0.018	0.027	0.046	100	
05_C07	0.034	0.035	0.025	0.037	0.023	0.085	0.230	0.142	0.022	0.111	0.031	0.037	0.049	100	
01_B01															
1-18	0.017	0.030	0.070	0.010	0.020	0.040	0.023	0.214	0.030	0.050	0.040	0.020	0.033	100	
	0.102	0.079	0.033	0.095	0.032	0.044	0.061	0.208	0.044	0.052	0.034	0.081	0.061	100	
	0.096	0.127	0.037	0.096	0.024	0.385	0.006	0.271	0.125	0.065	0.052	0.055	0.071	100	
VRC07-523-LS															
VRC07	0.196	0.180	0.104	0.241	0.064	>20	0.038	0.901	0.319	0.111	0.091	0.144	0.151	92	
3BNC117	0.046	0.136	0.073	0.082	0.021	>20	3.260	0.259	0.102	4.734	0.042	0.119	0.158	92	
N49P7	0.073	0.190	0.101	0.079	0.046	2.860	12.046	0.310	0.172	0.108	0.131	0.050	0.208	92	
NIH45-46	1.482	0.239	0.178	0.330	0.060	>20	0.074	1.311	0.761	0.206	0.135	0.259	0.274	92	
VRC01	0.366	0.533	0.151	0.541	0.137	>20	0.119	2.261	0.275	0.715	0.261	0.360	0.354	92	
8ANC195	0.174	2.419	0.786	0.407	0.066	0.510	0.347	5.408	>20	2.113	>20	1.583	0.700	83	

Figure 1

119 multiclade panel (99 common virus strains)									
Virus		Clade							
		04_A06	3BNC117	1-18	N49P7	N6	VR07	VR07 _{-s23-LS}	
		IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	
6405_V4_C34	D	0.110	0.167	>10	0.304	0.112	0.567	0.239	
CAP45_G3	C	0.063	1.366	>10	0.131	0.042	0.378	0.033	
DU172_17	C	0.028	0.416	>10	0.235	0.038	0.279	0.102	
P1981_C5_3	G	0.039	0.505	>10	0.178	0.125	0.157	0.046	
T278_50	AG	0.203	>10	>10	>10	>10	>10	>10	
ZM109_4	C	0.024	0.083	9.916	0.050	0.039	0.072	0.030	
ZM135_10A	C	0.088	0.059	1.729	0.301	0.153	0.105	0.073	
THRO4156	B	0.554	2.491	1.584	3.846	1.000	2.663	0.960	
3016_V5_C45	D	0.015	0.808	1.227	0.595	0.019	0.179	0.020	
16845_2_22	C	0.081	>10	0.862	1.553	0.398	1.430	0.450	
CAP210_E8	C	2.375	9.699	0.818	3.971	5.358	>10	1.708	
BJOX028000_10_3	AE	0.007	0.012	0.806	0.070	0.006	0.039	0.002	
CAAN5342	B	0.155	0.600	0.804	0.634	0.138	0.418	0.153	
BJOX010000_06_2	AE	0.236	1.681	0.526	0.186	0.593	2.629	1.206	
BJOX009000_02_4	AE	0.194	0.352	0.386	0.195	0.219	0.958	0.356	
AC10_29	B	0.812	6.917	0.369	9.466	0.319	0.950	0.500	
CE1172_H1	C	0.039	>10	0.295	8.499	0.530	>10	>10	
1394_C9G1	C	0.054	>10	0.287	3.841	0.067	0.085	0.012	
ZM53_12	C	0.122	0.227	0.256	0.823	0.223	0.355	0.166	
CE1176_A3	C	0.061	0.259	0.214	0.310	0.208	0.901	0.271	
928_28	AG	0.044	0.157	0.189	0.240	0.067	0.097	0.097	
CNE5	AE	0.057	0.304	0.184	0.248	0.082	0.159	0.065	
0013095_2_11	C	0.003	0.279	0.151	0.088	0.041	0.014	0.012	
620345_C1	AE	>10	>10	0.145	>10	1.336	>10	>10	
CE2010_F5	C	0.036	0.053	0.139	0.130	0.102	0.213	0.088	
3817_V2_C59	CD	0.251	0.187	0.131	1.272	0.272	2.580	0.155	
703010200_1E5	C	0.104	0.307	0.121	0.184	0.093	0.292	0.088	
BJOX025000_01_1	AE	0.040	0.067	0.120	0.128	0.043	1.310	0.124	
231965_C1	D	0.036	0.047	0.115	0.151	0.044	0.068	0.018	
231966_C2	D	0.030	0.244	0.096	0.102	0.052	0.067	0.028	
CE1086_B2	C	0.021	0.077	0.092	0.089	0.027	0.170	0.088	
ZM197_7	C	0.123	0.337	0.091	0.203	0.061	0.277	0.158	
CNE53	BC	0.039	0.070	0.089	0.056	0.036	0.012	0.012	
QH0692_42	B	0.200	0.237	0.089	1.087	0.595	1.151	0.571	

Figure 2

119 multiclade panel (99 common virus strains)								
Virus	Clade	04_A06 IC ₅₀ (µg/ml)	3BNC117 IC ₅₀ (µg/ml)	1-18 IC ₅₀ (µg/ml)	N49P7 IC ₅₀ (µg/ml)	N6 IC ₅₀ (µg/ml)	VRC07 IC ₅₀ (µg/ml)	VRC07_523-LS IC ₅₀ (µg/ml)
X2088_C9	G	0.044	>10	0.089	0.185	0.041	>10	>10
CE2060_G9	C	0.087	0.222	0.086	0.661	0.055	0.213	0.045
704809221_1B3	C	0.027	0.076	0.078	0.141	0.217	0.298	0.154
3415_V1_C1	A	0.024	0.099	0.076	0.213	0.032	0.072	0.020
CNE30	C	0.081	0.321	0.076	0.271	0.171	0.392	0.166
6535_3	B	0.070	0.380	0.068	0.230	0.104	0.271	0.125
DU422_1	C	0.053	>10	0.063	4.496	0.034	>10	2.033
TRJ04551_58	B	0.060	0.070	0.060	0.165	0.095	0.073	0.044
Q461_E2	A	0.052	0.063	0.053	0.533	0.130	0.245	0.110
X2131_C1_B5	G	0.092	0.346	0.053	0.110	0.086	0.163	0.102
T257_31	AG	0.069	0.127	0.050	0.250	0.182	0.591	0.300
X1193_C1	G	0.007	0.067	0.050	0.038	0.072	0.087	0.037
ZM214_15	C	0.073	0.099	0.048	0.560	0.071	0.254	0.158
T251_18	AG	0.106	0.217	0.044	0.198	0.389	1.148	0.266
A07412M1_VRC12	D	0.016	0.031	0.043	0.249	0.043	0.100	0.031
P0402_C2_11	G	0.027	0.057	0.043	0.037	0.049	0.071	0.045
T255_34	AG	0.035	0.042	0.042	0.069	0.052	0.116	0.040
ZM233_6	C	0.048	0.202	0.042	0.327	0.066	0.268	0.071
C4118_9	AE	0.050	0.035	0.040	0.058	0.052	0.078	0.017
CNE21	BC	0.026	>10	0.039	1.587	0.040	0.100	0.045
C2101_C1	AE	0.071	0.056	0.038	0.115	0.056	0.266	0.061
CE0393_C3	C	0.007	0.201	0.036	0.195	0.038	0.276	0.065
CNE20	BC	0.018	>10	0.033	0.064	0.014	0.056	0.009
703010054_2A2	C	0.042	0.331	0.032	0.132	0.108	0.256	0.091
9004SS_A3_4	A	0.048	0.066	0.031	0.125	0.081	0.218	0.051
MS208_A1	A	0.040	0.022	0.031	0.196	0.058	0.133	0.036
BF1266_431A	C	0.013	0.028	0.030	0.057	0.007	0.032	0.009
C3347_C11	AE	0.030	0.031	0.027	0.034	0.008	0.075	0.029
X1254_C3	G	0.042	0.073	0.027	5.111	0.033	0.051	0.035
3365_V2_C2	A	0.016	0.017	0.025	0.063	0.016	0.026	0.007
191084_B7_19	A	0.043	0.068	0.023	0.047	0.020	0.039	0.010
ZM247_V1	C	0.006	>10	0.023	0.062	0.025	0.099	0.025
X1632_S2_B10	G	0.034	3.260	0.023	>10	0.061	0.038	0.006
DU156_12	C	0.020	0.032	0.022	0.047	0.011	0.047	0.009

Figure 2 (continued)

119 multiclade panel (99 common virus strains)

3BNC117	1-18	N49P7	N6	VRC07	VRC07 _{523-LS}
IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
0.134	0.020	0.254	0.187	0.725	0.170
0.010	0.020	0.042	0.020	0.025	0.010
0.043	0.020	0.060	0.019	0.055	0.021
3.951	0.019	0.102	0.012	0.043	0.005
>10	0.019	2.070	0.012	>10	>10
0.017	0.018	1.044	0.155	0.035	0.008
0.046	0.017	0.073	0.102	0.196	0.096
>10	0.017	1.811	0.025	0.791	1.081
0.387	0.017	0.045	0.021	0.087	0.025
0.069	0.017	0.153	0.114	0.130	0.088
0.014	0.017	0.118	0.046	0.039	0.031
0.028	0.016	0.073	0.032	0.065	0.029
0.030	0.016	0.034	0.023	0.018	0.005
0.032	0.015	0.041	0.030	0.077	0.039
0.024	0.015	0.108	0.017	0.031	0.009
0.181	0.010	0.441	0.090	0.324	0.173
0.082	0.010	0.079	0.095	0.241	0.096
0.081	0.010	0.142	0.059	0.243	0.113
0.015	0.009	0.028	0.010	0.022	0.002
0.045	0.009	0.155	0.065	0.072	0.031
0.011	0.007	0.010	0.004	0.002	0.001
0.028	0.007	0.064	0.012	0.018	0.005
0.025	0.006	0.023	0.021	0.111	0.045
0.004	0.006	0.030	0.012	0.018	0.009
0.197	0.006	0.149	0.313	1.540	0.390
0.026	0.006	0.102	0.047	0.074	0.052
>10	0.006	0.094	0.018	0.422	0.102
0.031	0.005	0.036	0.012	0.028	0.008
>10	0.005	>10	0.026	>10	>10
>10	0.004	8.558	4.252	>10	>10
0.034	0.004	0.020	0.039	0.070	0.018
0.108	0.049	0.194	0.062	0.140	0.051
85	95	96	99	91	93

Figure 2 (continued)

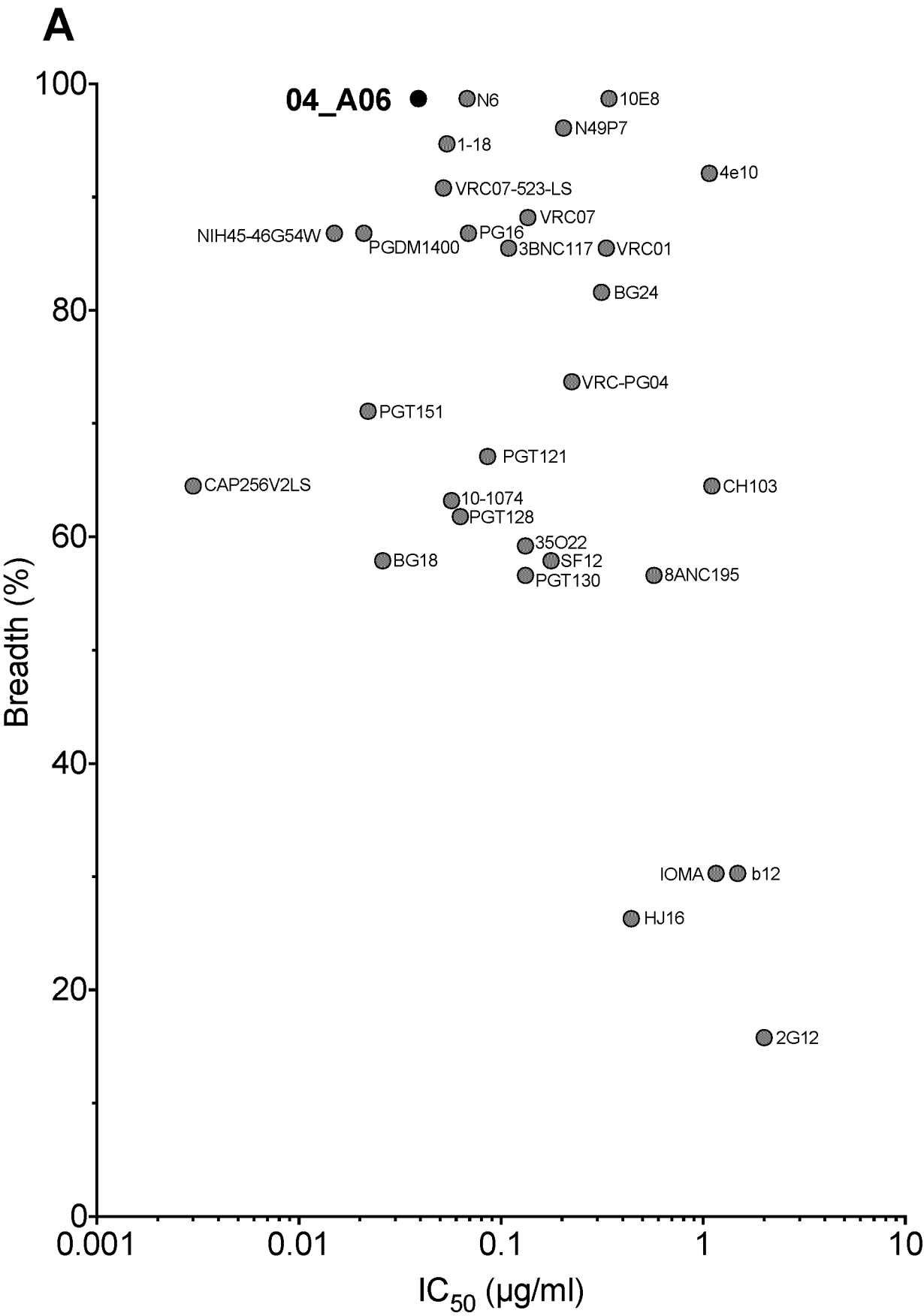


Figure 3

B

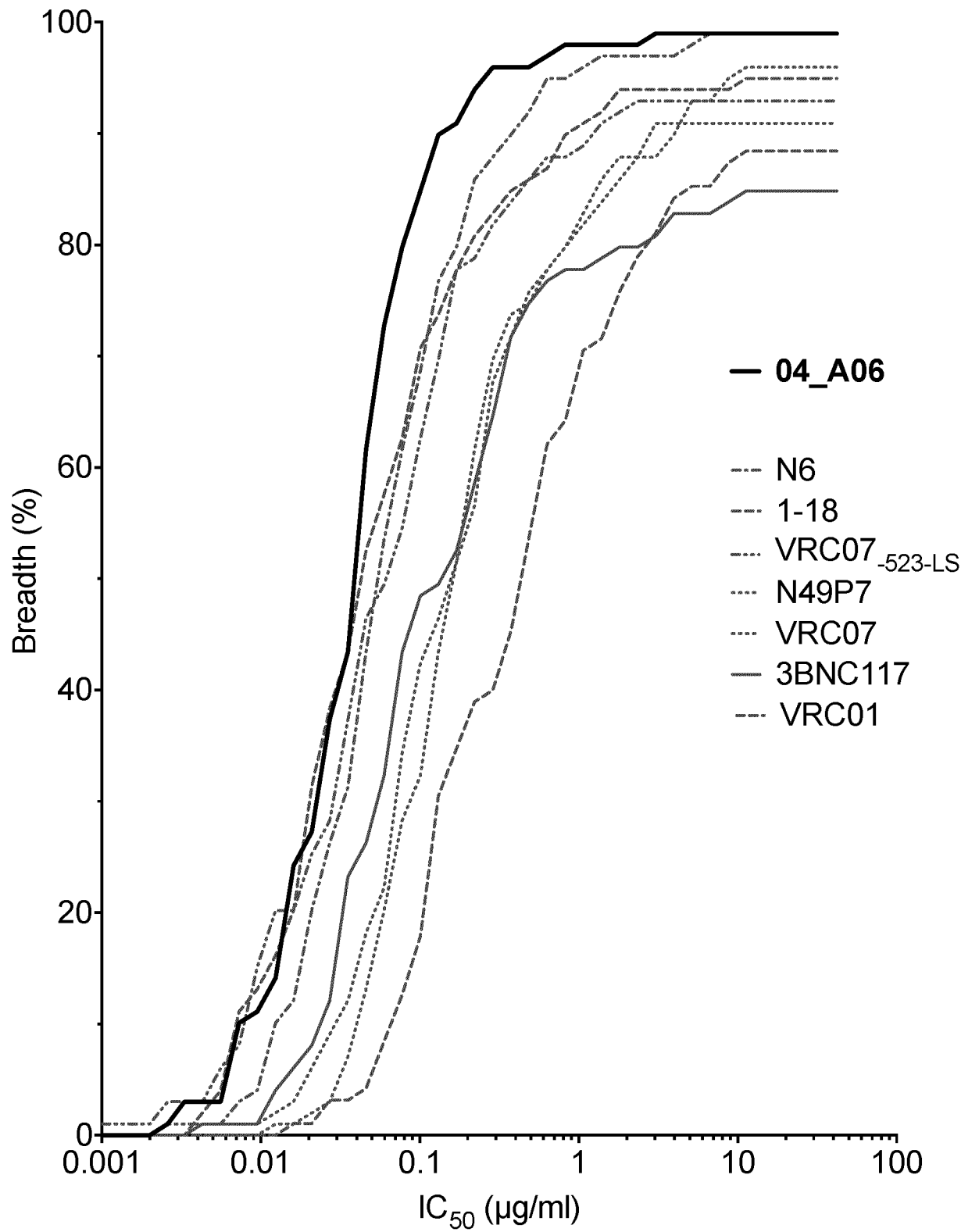


Figure 3 (continued)

Subtype C panel

Virus	04_A06	3BNC117	1-18	N6	VRC01	VRC07 ^{-523-LS}
	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
CAP308_2_00_E11_35	>10	>10	>10	>10	>10	>10
20417927_07	0.009	>10	0.396	>10	>10	>10
CAP225_1_06_A2_18	0.004	6.174	0.176	>10	1.170	5.152
CAP210_TA5	2.116	5.720	0.904	>10	>10	0.921
CT565_C7_48	1.089	>10	2.263	5.233	>10	0.513
21203310_G7_C3	0.764	5.812	0.374	2.063	>10	1.055
1245045	0.115	3.298	0.253	0.904	3.440	0.312
6022_V7_C24	0.770	0.021	4.221	0.842	1.980	0.101
20927783	0.087	>10	>10	0.702	>10	>10
CE1172_H1	0.044	>10	0.295	0.530	>10	>10
705010185_TF	0.080	0.050	0.062	0.444	>10	0.262
CA146_H3_3	0.124	0.578	>10	0.388	>10	0.091
21502011_F12_E2	1.015	1.582	0.309	0.372	9.770	0.605
21197826_V1	0.300	>10	0.825	0.313	>10	0.366
2969249	0.456	0.640	>10	0.280	1.370	0.222
SO405_T24_5	0.196	0.330	0.073	0.279	3.410	0.459
0984_V2_C2	0.055	>10	0.094	0.272	1.490	0.282
2768732_C5_16	0.299	0.304	0.246	0.271	1.600	0.198
CA392_H2_6	0.046	0.036	0.037	0.186	0.180	0.088
KO459_T68_4	0.084	0.173	0.159	0.156	1.270	0.120
ZM135_10A	0.098	0.059	1.729	0.153	0.855	0.073
556_F2_3_25	0.344	4.418	0.253	0.151	3.320	0.173
CAP363_2_00_10_3	0.102	0.286	0.704	0.148	2.120	0.160
SO032_A2_8_1	0.364	4.873	0.035	0.147	1.980	0.101
CAP323_2_00_B6_45	0.125	0.141	0.600	0.145	>10	0.053
20286961_C1_H8	0.061	0.130	0.058	0.144	0.400	0.113
KO426_T78_10	0.063	0.169	0.056	0.136	1.339	0.276
2865_A11_12	0.161	0.268	0.411	0.135	0.880	0.113
SO607_B6_9	0.084	0.124	0.071	0.131	0.490	0.059
CAP291_2_00_H2_15	0.099	0.095	0.060	0.130	0.580	0.023
ME178_G6_16	0.109	0.425	0.176	0.119	0.400	0.047
703010054_2A2	0.062	0.331	0.032	0.108	0.535	0.091
0041_V3_C18	0.089	0.100	0.061	0.103	0.360	0.058
CAP244_D3	0.076	0.096	0.082	0.102	0.688	0.164
CT810_G4_7	0.117	1.191	0.071	0.100	0.510	0.093

Figure 4

Subtype C panel

Virus	04_A06	3BNC117	1-18	N6	VRC01	VRC07 _{-523-LS}
	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
CE0665_F2	0.047	0.125	0.048	0.092	0.480	0.148
722_G4_16	0.149	4.086	0.193	0.091	>10	0.054
CAP136_1_16_E6_1	1.502	0.253	>10	0.087	>10	0.170
6146_V7_C23	0.047	>10	0.072	0.083	0.900	0.132
CA240_A5_5	0.047	0.631	0.562	0.082	1.330	0.143
6631_V3_C10	0.037	>10	0.053	0.081	>10	0.153
CH0694	0.052	0.647	0.124	0.076	>10	0.111
20358510_01	0.046	0.192	0.014	0.074	0.390	0.012
704810053_2B7	0.052	1.582	0.076	0.072	>10	0.069
ZM214_15	0.113	0.099	0.048	0.071	0.827	0.158
20258279_V2	0.036	0.303	0.078	0.069	0.540	0.104
20965238	0.107	0.157	0.135	0.069	1.100	0.083
CAP200_B8A	0.076	0.142	>10	0.066	0.480	0.085
21492713_B11_E3	0.049	0.089	0.031	0.066	0.140	0.021
ZM233_6	0.032	0.202	0.042	0.066	2.455	0.071
3312_D6_2	0.018	0.828	0.013	0.065	0.180	0.035
ZM197_7	0.176	0.337	0.091	0.061	0.507	0.158
CT140_140_B6	0.103	0.126	0.142	0.060	>10	0.103
19252094_A5_G2	0.042	5.147	0.067	0.059	0.470	0.109
20198102_E9_G1	0.020	0.366	0.028	0.053	0.160	0.035
19314479_A2_5	0.114	0.046	0.113	0.053	0.840	0.062
B005018_8_F6_3	0.038	0.093	0.020	0.051	0.280	0.118
6040_V4_C15	0.017	0.032	0.022	0.049	0.150	0.062
704010042_2E5	0.031	0.040	0.012	0.046	0.160	0.009
CAP307_2_00_24_1	0.123	0.125	2.387	0.045	0.290	0.050
3603_C11_13	0.022	0.015	0.040	0.045	0.310	0.048
CE2052_G10	0.020	0.048	0.003	0.043	0.180	0.016
ZM215_8	0.084	0.014	0.039	0.043	0.176	0.050
CAP45_G3	0.074	1.366	>10	0.042	5.849	0.033
21283649	0.023	0.165	0.074	0.042	0.310	0.027
KO870_C2_10	0.058	0.131	0.157	0.042	0.230	0.030
569_F1_37_10	0.031	0.226	0.260	0.041	0.300	0.016
19707346_E8_C6	0.049	>10	0.177	0.040	1.730	0.074
234_F1_16_57	0.057	>10	0.028	0.039	>10	1.352
CT966_E1_7	0.120	0.200	0.057	0.038	0.560	0.072

Figure 4 (continued)

Subtype C panel

Virus	04_A06	3BNC117	1-18	N6	VRC01	VRC07 _{-523-LS}
	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
706010164_1A7	0.031	>10	0.035	0.036	0.240	0.024
3426_V5_C17	0.025	0.049	0.037	0.034	0.260	0.067
DU422_1	0.061	>10	0.063	0.034	>10	2.033
CT184_D3_15	0.008	0.133	1.076	0.032	0.100	0.012
541_F1_A7_2	0.120	0.070	0.099	0.032	0.170	0.023
235080_3G7_REV	0.012	>10	0.322	0.028	8.410	0.004
ZM106_9	0.028	0.081	0.011	0.026	0.255	0.030
CAP69_1_12_TA7_1	6.778	>10	>10	0.025	>10	0.047
0933_V4_C4	0.068	>10	>10	0.023	0.910	0.554
CAP290_2_00_23_6	0.053	>10	0.071	0.023	1.980	0.095
3514597	0.029	0.038	0.103	0.022	0.500	0.054
703010131_1E2	0.019	0.287	0.027	0.020	>10	0.028
ZM249_1	0.008	0.043	0.020	0.019	0.077	0.021
CT072_56_7	0.040	0.014	0.011	0.019	0.060	0.029
20104663_E11_D2	0.035	>10	>10	0.015	>10	0.628
20883229_C9_H6	0.045	>10	0.056	0.015	0.400	0.024
19157834_V1	0.016	0.041	0.012	0.015	0.350	0.032
6980_V0_C31	0.018	0.072	0.022	0.012	0.100	0.036
CAP177_1A3	0.033	0.527	0.018	0.011	0.150	0.017
CAP188_1_D1_14_REV	0.031	1.364	0.012	0.010	0.180	0.014
CH505_W4_3	0.004	0.011	0.007	0.010	0.027	0.013
CAP266_2_00_E9_H6	0.006	>10	0.010	0.009	>10	>10
CAP304_2_00_F6_6	0.033	1.282	0.026	0.009	0.040	0.008
CAP174_1_06_F3_1B	0.001	>10	0.084	0.007	0.050	0.002
20915593	0.035	>10	0.046	0.006	>10	0.075
21561324_D3_B5	0.009	6.983	0.006	0.005	0.440	0.019
18814602_H8_F3	0.012	0.007	0.015	0.004	0.020	0.005
CAP330_2_00_F2_41	0.005	>10	0.017	0.003	0.560	0.002
SO431_C1_1	0.012	0.198	0.018	0.003	0.560	0.012
1170887_08	0.007	0.004	0.003	0.001	0.110	0.004
GeoMean IC ₅₀ of neutralized (µg/ml)	0.057	0.209	0.074	0.058	0.470	0.068
Breadth (%)	99	77	90	96	76	95

Figure 4 (continued)

CD4bs bNAb resistant virus strains						
Virus	04_A06	VRC07 _{-s23-LS}	N6	3BNC117	1-18	VRC01
	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)
20927783	0.087	>10	0.702	>10	>10	>10
20417927_07	0.009	>10	>10	>10	0.396	>10
T278_50	0.203	>10	>10	>10	>10	>10
TV1.29	0.284	2.080	>10	>10	>10	>10
CAP210_TA5	2.116	0.921	>10	5.720	0.904	>10
20104663_E11_D2	0.035	0.628	0.015	>10	>10	>10
CAP136_1_16_E6_1	1.502	0.170	0.087	0.253	>10	>10
CA146_H3_3	0.124	0.091	0.388	0.578	>10	>10
CAP69_1_12_TA7_1	6.778	0.047	0.025	>10	>10	>10
GeoMean IC ₅₀ of neutralized (µg/ml)	0.253	0.309	0.098	0.942	0.598	>10
Breadth (%)	100	67	56	33	22	0

Figure 5

Resistant panel to CD4bs bNAbs									
Virus	04 A06	VRC07	N6	NIH45-46	1-18	VRC01	N49P7	3BNC117	
	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₅₀ (µg/ml)	
BL01DG	>10	>10	>10	>10	>10	>10	>10	>10	
TV1.29	0.284	>10	>10	>10	>10	>10	>10	>10	
CN23	1.072	1.576	2.382	2.365	>10	>10	1.060	>10	
TZA125.17	0.102	>10	0.526	>10	4.113	>10	0.121	>10	
CNE6	0.014	>10	>10	>10	0.857	>10	>10	>10	
CNE64	2.934	2.975	0.502	>10	0.411	9.393	5.854	>10	
BjOX2000	0.010	>10	0.027	>10	0.016	>10	0.415	>10	
GeoMean IC ₅₀ of neutralized (µg/ml)									
Breadth (%)									
	0.153	2.165	0.360	2.365	0.391	>10	>10	>10	
	86	29	57	14	57	14	57	0	

Figure 6

Insensitive virus panel to CD4bs bNAbs															
Virus		Clade		01_D03		VRC07 _{-523-LS}		N6		VRC07		N49P7		1-18	
				IC ₅₀ (µg/ml)		IC ₅₀ (µg/ml)		IC ₅₀ (µg/ml)		IC ₅₀ (µg/ml)		IC ₅₀ (µg/ml)		IC ₅₀ (µg/ml)	
CAP210_E8	C	0.499		>1		>1		>1		>1		>1		0.818	
6545_V4_C1	AC	>1		>1		>1		>1		>1		>1		0.004	
THRO4156	B	0.196		0.960		1.000		>1		>1		>1		>1	
6405_V4_C34	D	0.113		0.239		0.112		0.567		0.304		>1		>1	
DU172_17	C	0.061		0.102		0.038		0.279		0.235		>1		>1	
P1981_C5_3	G	0.082		0.046		0.125		0.157		0.178		>1		>1	
ZM109_4	C	0.227		0.030		0.039		0.072		0.050		>1		>1	
ZM135_10A	C	0.021		0.073		0.153		0.105		0.301		>1		>1	
GeoMean IC ₅₀ of neutralized (µg/ml)				0.115		0.115		0.121		0.180		0.180		0.057	
Breadth (%)				88		75		75		63		63		25	

Figure 7

Antibody Mediated Prevention Trial Panel

Virus	04_A06		VRC01	
	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)
H703_0015_110s	>10	>10	>10	>10
H703_0132_200s_10H3	0.190	0.460	>10	>10
H703_0157_090s	0.005	0.020	>10	>10
H703_0455_160_RE_cs	0.040	0.140	>10	>10
H703_0482_250Es	0.030	0.080	>10	>10
H703_0514_150s_6C1	1.820	4.970	>10	>10
H703_0902_140_RE_p01s	0.010	0.030	>10	>10
H703_1446_040s	0.170	0.690	>10	>10
H703_1515_120Es	0.040	0.110	>10	>10
H703_1670_220_RE_cs	0.260	1.010	>10	>10
H703_1798_170_RE_p01s	0.450	1.560	>10	>10
H703_2038_260_RE_e5C3s	4.320	>10	>10	>10
H703_2769_050_RE_p01s	1.020	2.260	>10	>10
V703_0015_110_RE_pblib002_s	>10	>10	>10	>10
V703_0132_200_RE_pblib002_s	0.420	0.820	>10	>10
V703_0510_260_RE_pblib003_s	0.240	0.940	>10	>10
V703_0514_150_RE_pblib004_s	1.700	3.010	>10	>10
V703_0790_190_RE_pblib002_s	0.090	0.310	>10	>10
V703_1255_260_RE_pblib003_s	0.050	0.200	>10	>10
V703_1515_120_RE_pblib002_s	0.005	0.010	>10	>10
V703_1586_210_RE_sga3E8_s	1.460	3.060	>10	>10
V703_1714_080_RE_pblib002_s	0.090	0.200	>10	>10
H703_0013_090Es	0.240	0.580	>10	>10
H703_0203_081_RE_e8A5s	0.290	0.920	>10	>10
H703_0217_050e_2A3	0.010	0.030	>10	>10
H703_0279_110s	0.110	0.420	>10	>10
H703_0472_030s	0.005	0.010	>10	>10
H703_0537_011s_4H1	0.030	0.090	>10	>10
H703_0566_160s	0.030	0.100	>10	>10
H704_1835_150_RE_p001s_2484A	0.037	0.127	>10	>10
H704_0445_180_RE_con_s	0.005	0.023	>10	>10
H704_1835_150_RE_p002s_2484A	0.020	0.087	>10	>10
H703_0514_150Es_F4_V03_024	0.280	0.870	10.000	>10
H703_0406_240_RE_e11E4s	2.190	7.320	9.150	>10
H703_0944_180s	1.090	2.290	8.609	>10
V703_1586_210_RE_pblib002_s	0.770	2.090	8.349	>10
H703_0646_051sN	0.780	2.020	8.081	>10
H703_0629_150_RE_e5A2s	0.040	0.160	7.803	>10
H703_2934_110_RE_cs	0.050	0.320	7.357	>10
H703_0739_110s	0.020	0.090	6.410	>10
H704_0726_080sN	0.054	0.223	5.635	>10
H704_2981_150_RE_p001s_2559A	2.331	8.185	5.211	>10
H704_2544_140eN02	0.207	0.559	5.058	>10
H704_0496_040sN	0.028	0.085	4.769	>10
V703_0510_260_RE_pblib002_s	0.690	1.810	4.549	>10
H703_0790_190_RE_e5G1s	0.590	2.340	4.388	>10
H703_1357_230Es_H1	0.600	2.520	4.368	>10

Figure 8

Antibody Mediated Prevention Trial Panel

Virus	04_A06		VRC01	
	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)
H703_0842_200Es	0.330	1.020	4.151	>10
V703_1034_190_RE_pbli002_s	0.230	0.690	4.146	>10
H703_1034_190_RE_e5D5s	0.210	0.660	4.070	>10
V703_1453_240_RE_pbli002_s	0.110	0.310	4.050	>10
H703_0926_070s_2H2	0.240	0.700	4.048	>10
H703_1060_080s	0.800	1.980	3.872	>10
V703_0510_260_RE_pbli001_s	0.210	0.710	3.683	>10
V703_1240_201_RE_sga3D1_s	>10	>10	3.241	>10
H703_0406_240_RE_e12B2s	0.740	2.540	3.124	>10
H703_1689_110_RE_e4E6s	0.010	0.040	0.887	>10
H704_3008_040EsN	0.315	4.185	3.419	9.892
H704_2767_070sN	0.088	0.270	2.019	8.698
H704_1109_140_RE_cs	0.841	2.203	3.307	8.658
H704_0847_030_EsN_02C	0.021	0.096	2.710	8.503
V703_0712_250_RE_pbli001_s	0.090	0.270	3.268	7.693
V703_1034_190_RE_pbli003_s	0.017	0.070	2.164	7.652
H704_1528_240_RE_pbli001_s	0.204	0.610	2.390	7.130
H703_1453_240_RE_e9B7s	0.020	0.090	2.569	7.038
V703_1194_150_RE_pbli002_s	0.190	0.760	2.494	6.928
V703_1855_162_RE_pbli003_s	0.015	0.060	2.106	6.831
H704_0575_060_EsN1A	0.174	0.669	2.150	6.694
H704_0575_060_EsN2T	0.180	0.867	2.022	6.428
H704_0847_030_EsN_01T	0.023	0.083	1.908	6.399
H704_2981_150_RE_pbli005_s	0.546	1.940	2.181	6.343
H703_1313_040s	0.210	0.530	1.712	6.265
H704_0575_060_RE_p002s	0.240	0.877	2.027	6.087
H703_1807_240_RE_cs	0.040	0.170	1.883	5.714
H704_2834_210_RE_pb002_s	0.074	0.270	1.881	5.546
H703_0948_170_RE_e4D2s	0.130	0.650	1.620	5.323
H704_2544_150_RE_p001s	0.158	0.638	1.840	5.310
H704_2834_210_RE_pb001_s	0.112	0.383	1.952	5.254
H704_2981_150_RE_pbli003_s	0.411	1.500	1.687	5.241
H704_3000_240_RE_pbsga002_s	0.026	0.119	1.920	5.220
H703_1471_190s	0.210	0.360	1.332	5.212
H704_2981_150_RE_p002s_2559A	0.922	4.752	1.899	4.934
H703_1194_150_RE_cs	0.150	0.550	1.365	4.842
H703_0795_040s	1.850	3.100	1.340	4.770
V703_1240_201_RE_sga4F1_s	0.870	1.870	1.859	4.756
H703_0322_130s_M1I	0.050	0.210	1.605	4.619
H704_0886_250_RE_p001s	0.040	0.265	1.745	4.601
V703_1240_201_RE_pbli003_s	0.230	0.630	1.343	4.525
H703_0109_210s	0.180	0.630	1.405	4.462
H703_1675_G613s	0.040	0.110	1.444	4.393
H704_2839_140_RE_cs	0.049	0.174	1.658	4.337
H703_1750_140Es	0.480	1.080	1.766	4.252
H703_1764_250_RE_cs	0.040	0.120	1.037	4.222
H704_1991_230_RE_p001s_1194T	0.096	0.366	1.605	4.218

Figure 8 (continued)

Antibody Mediated Prevention Trial Panel

Virus	04_A06		VRC01	
	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)
H703_1789_230_RE_e3A3s	0.120	0.270	1.054	4.150
H703_1407_090s_4G4	0.050	0.170	1.463	3.988
H703_1848_190_RE_e6D1s	0.090	0.310	1.391	3.936
V703_0597_190_RE_pb1ib002_s	0.160	0.460	1.062	3.836
H703_1889_100Es	0.130	0.360	1.076	3.790
H703_1026_120Es_A5	0.520	0.870	1.036	3.754
H704_0513_150_eN02C	0.297	0.907	1.422	3.675
H704_0911_150sN	0.346	0.930	1.253	3.641
H703_1945_090s_2A3	0.070	0.290	0.947	3.637
V703_1407_090_RE_pb1ib003_s	0.120	>10	1.139	3.627
H704_2788_060eN_12	0.178	0.686	1.436	3.600
H704_2788_060eN_04	0.163	0.576	1.324	3.457
H704_0513_150_eN01T	0.460	1.358	1.522	3.384
H703_2018_240_RE_e6A1s	0.140	0.540	1.028	3.310
H704_0746_760_RE_p003s	0.206	0.724	1.310	3.284
V703_2372_170_RE_con_s	0.060	0.200	0.944	3.276
H703_1687_100Es	0.030	0.170	0.847	3.184
H704_0746_760_RE_p001s	0.105	0.635	1.150	3.115
V703_3000_090_RE_pb1ib002_s	0.057	0.200	0.853	3.042
V703_2141_160_RE_sga3D6_s	0.820	1.660	1.196	2.887
H703_2117_110_RE_e2A10s	0.040	0.140	0.881	2.855
H703_2149_060_RE_eB10s	0.040	0.190	1.249	2.844
H703_2304_150_RE_cs	0.010	0.040	0.681	2.723
V703_0597_190_RE_pb1ib003_s	0.140	0.340	0.661	2.712
H703_0860_150Es	0.027	0.100	0.797	2.635
H704_0513_150_RE_pb1ib003_s	0.259	0.907	1.092	2.585
H703_2539_070_RE_e6F6s	0.270	>10	0.719	2.515
H703_2631_150_RE_e2F8s	0.140	0.470	0.686	2.473
H704_0644_060sN_prelimSeq	0.074	0.221	1.018	2.459
H704_0746_760_RE_p002s	0.139	0.520	0.952	2.418
H704_2544_140eN01	0.088	0.280	0.949	2.411
H703_0597_190_RE_e5A1s	0.140	0.380	0.650	2.395
H703_2788_030Es_B1	0.040	>10	0.529	2.257
H703_0993_110_RE_cs	0.020	0.060	0.739	2.209
H703_1945_090s_2F1	0.040	0.220	0.451	2.105
H703_1783_170Es	0.180	0.370	0.706	2.062
V704_0128_220_RE_pb1ib002_s	0.025	0.096	0.057	2.011
H704_0128_220_RE_pb001_s	0.017	0.057	0.057	2.011
H703_1945_090s_3D5	0.040	0.200	0.452	1.950
H704_3000_240_RE_pb1sga001_s	0.009	0.063	0.523	1.790
H703_0309_100s	0.110	0.290	0.483	1.781
H703_0636_200Es	0.030	0.120	0.429	1.630
V703_1383_240_RE_pb1ib002_s	0.010	0.040	0.462	1.603
V703_0309_100_RE_pb1ib002_s	0.100	0.240	0.416	1.537
H703_1758_260_RE_cs	0.050	0.180	0.414	1.430
H704_1706_050sN	0.203	0.751	0.514	1.343
V703_0712_250_RE_pb1ib002_s	0.100	0.240	0.317	1.320

Figure 8 (continued)

Antibody Mediated Prevention Trial Panel

Virus	04_A06		VRC01	
	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)	IC ₅₀ (µg/ml)	IC ₈₀ (µg/ml)
H704_0856_240_RE_pb001_s	0.053	0.211	0.524	1.320
V704_0372_250_RE_pb1ib001_s	0.033	0.219	0.415	1.320
H704_0026_231_RE_pbsga001_s	0.059	0.266	0.460	1.300
H703_1383_240_RE_e5D3s	0.010	0.040	0.425	1.196
H703_0967_040s	0.020	0.060	0.323	1.109
H704_2541_080EsN	0.101	0.284	0.408	1.082
H703_1828_220Es	0.040	0.150	0.293	1.077
H704_2555_240_RE_con_s_vpuG	0.032	0.138	0.424	1.061
H704_1183_220EsN	0.031	0.126	0.453	1.057
H703_1714_080c	0.037	0.150	0.291	1.034
V703_1383_240_RE_pb1ib003_s	0.010	0.020	0.311	1.002
H703_0805_770_RE_e10F8s	0.050	0.190	0.286	0.995
H704_2095_130_RE_cs	0.115	0.555	0.323	0.949
H704_0907_130sN	0.062	0.185	0.393	0.871
V704_0372_250_RE_pb1ib002_s	0.021	0.119	0.326	0.868
H704_1429_090_eN2G	0.025	0.083	0.373	0.866
H703_1551_143s_2G8	0.020	0.070	0.265	0.823
H703_0805_770_RE_e10C9s	0.780	2.110	0.244	0.811
H704_2541_080_RE_p001s	0.052	0.184	0.353	0.800
H704_1429_090_eN1A	0.038	0.138	0.307	0.746
H704_2065_060_RE_p001s	0.039	0.310	0.233	0.714
H703_0578_130_RE_cs	0.050	0.200	0.209	0.693
H704_2065_060_RE_p002s	0.031	0.243	0.194	0.674
H704_2541_080_RE_p003s	0.019	0.058	0.213	0.611
H704_1775_030cN_SynGtoA_567	0.019	0.139	0.095	0.483
H704_1180_070EsN	0.010	0.075	0.152	0.462
V703_1104_100_RE_pb1ib001_s	0.280	1.000	0.143	0.458
H704_1930_170_RE_c02s_1523G	0.027	0.126	0.119	0.452
H704_1180_070_RE_pb1ib027_s	0.016	0.066	0.144	0.434
H703_0520_030Es	0.060	0.120	0.133	0.428
H704_2448_240_RE_cs	0.044	0.275	0.140	0.412
V703_2769_050_RE_pb1ib002_s	0.050	0.180	0.118	0.410
H704_1930_170_RE_c01s_1523A	0.020	0.083	0.110	0.358
H704_2684_181_RE_p0001s	0.019	0.085	0.119	0.347
H704_1783_150_RE_cs	0.020	0.065	0.114	0.327
H704_2684_181_RE_pb1ib006_s	0.015	0.068	0.093	0.317
H704_1535_030sN	0.121	0.331	0.118	0.304
H704_0944_180_RE_cs	0.008	0.037	0.069	0.278
V703_1298_080_RE_pb1ib002_s	0.100	0.630	0.090	0.257
V703_3000_090_RE_pb1ib001_s	0.010	0.020	0.069	0.249
H703_0514_150Es_A4_V02_028	0.010	0.040	0.037	0.142
V703_0458_260_RE_sga6H5_s	0.025	0.110	0.047	0.088
H704_0855_080_EsN	0.006	0.025	0.031	0.076
V703_1915_250_RE_sgaA3_s	0.090	0.310	0.021	0.073
GeoMean IC _{50/80} of neutralized (µg/ml)	0.084	0.287	0.839	1.907
Breadth (%)	98	96	83	69

Figure 8 (continued)

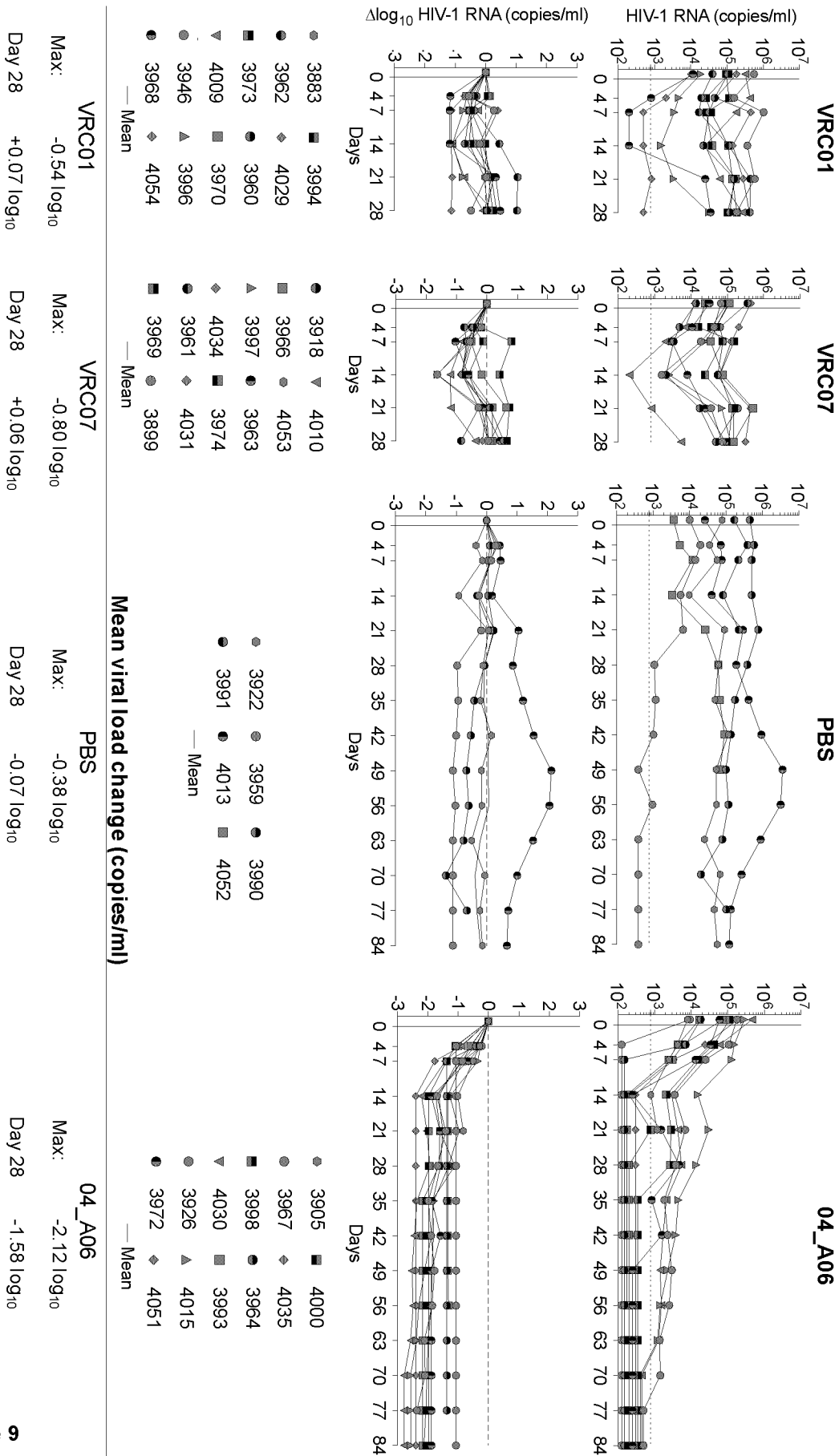


Figure 9

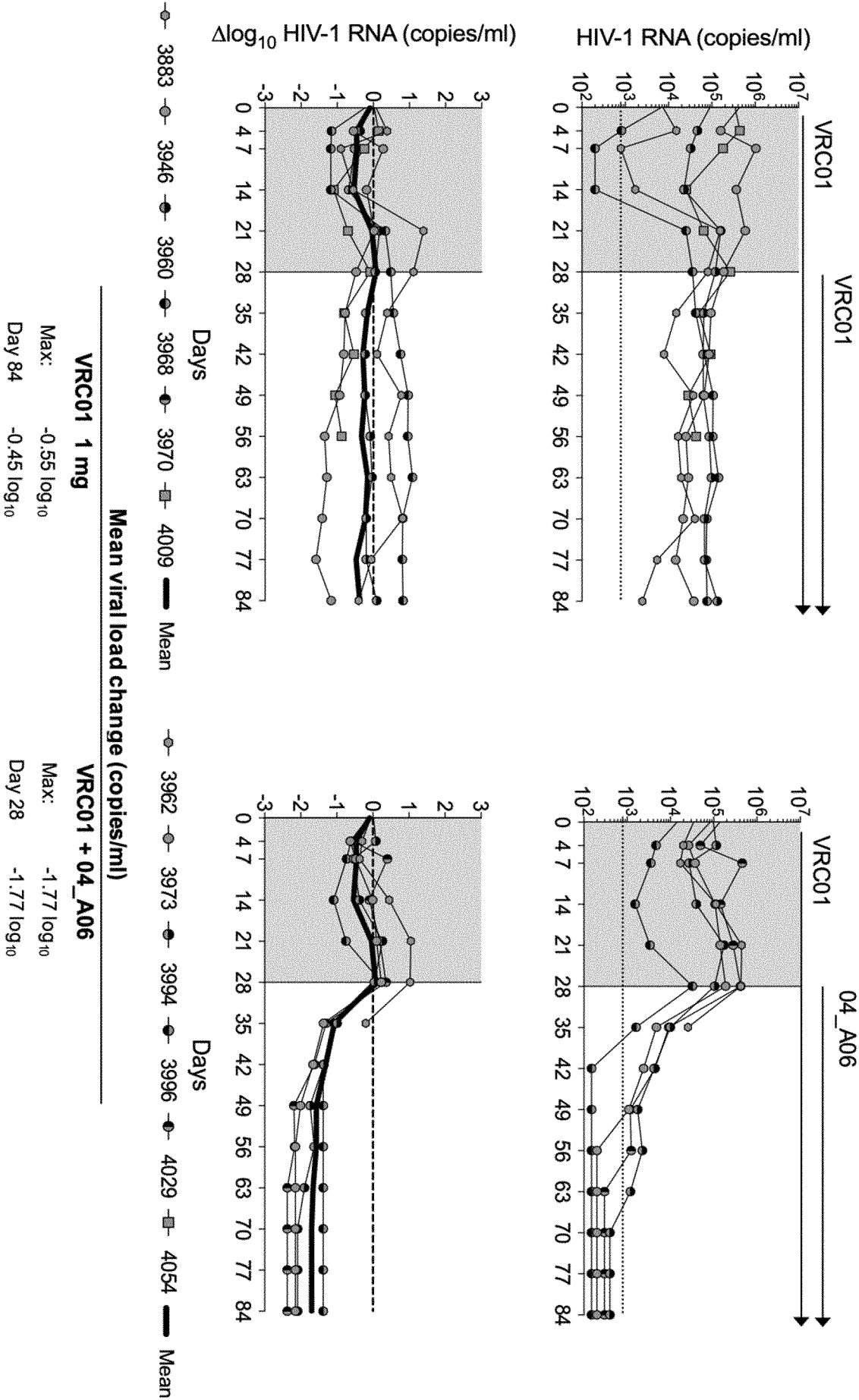


Figure 10

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/065842

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/10 A61P31/18
ADD. A61K39/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61P A61K

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

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

EPO-Internal**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 271 389 B1 (US HEALTH [US]) 12 February 2020 (2020-02-12) figure 1D -----	1 - 15
X	US 10 450 368 B2 (UNIV DUKE [US]) 22 October 2019 (2019-10-22) figure 27B -----	1 - 15
X	US 2020/239551 A1 (SAJADI MOHAMMAD [US] ET AL) 30 July 2020 (2020-07-30) paragraph [0181]; figure 8 -----	1 - 15
X	WO 2021/108761 A1 (UNIV MARYLAND [US]; US DEPT VETERANS AFFAIRS [US]; US HEALTH [US]) 3 June 2021 (2021-06-03) page 6, line 12; figure 1 ----- - / - -	1 - 15



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

13 September 2024

Date of mailing of the international search report

04/10/2024

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/065842

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/196975 A1 (THE US SECRETARY DEPT OF HEALTH & HUMAN SERVICES [US]; UNIV DUKE [US]) 8 December 2016 (2016-12-08) figure 5A -----	1 - 15
X	US 2019/002539 A1 (SHAO YIMING [CN] ET AL) 3 January 2019 (2019-01-03) table 4A -----	1 - 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/065842

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/065842

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