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Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults

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Summary

VRC-HIVMAB060-00-AB (VRC01) is a broadly neutralizing HIV-1 monoclonal antibody (mAb) isolated from the B cells of an HIV-infected patient. It is directed against the HIV-1 CD4 binding site and is capable of potently neutralizing the majority of diverse HIV-1 strains. This Phase I dose-escalation study in healthy adults was conducted at the National Institutes of Health (NIH) Clinical Center (Bethesda, MD, USA). Primary objectives were the safety, tolerability and pharmacokinetics (PK) of VRC01 intravenous (i.v.) infusion at 5, 20 or 40 mg/kg, given either once (20 mg/kg) or twice 28 days apart (all doses), and of subcutaneous (s.c.) delivery at 5 mg/kg compared to s.c. placebo given twice, 28 days apart. Cumulatively, 28 subjects received 43 VRC01 and nine received placebo administrations. There were no serious adverse events or dose-limiting toxicities. Mean 28-day serum trough concentrations after the first infusion were 35 and 57 μg/ml for groups infused with 20 mg/kg ($n = 8$) and 40 mg/kg ($n = 5$) doses, respectively. Mean 28-day trough concentrations after the second infusion were 56 and 89 μg/ml for the same two doses. Over the 5–40 mg/kg i.v. dose range ($n = 18$), the clearance was 0·016 l/h and terminal half-life was 15 days. After infusion VRC01 retained expected neutralizing activity in serum, and anti-VRC01 antibody responses were not detected. The human monoclonal antibody (mAb) VRC01 was well tolerated when delivered i.v. or s.c. The mAb demonstrated expected half-life and pharmacokinetics for a human immunoglobulin G. The safety and PK results support and inform VRC01 dosing schedules for planning HIV-1 prevention efficacy studies.

Keywords: HIV-1, monoclonal antibody, pharmacokinetics, Phase I clinical trial, passive immunization

Introduction

Many human viral infections and effective vaccines induce a humoral immune response that protects against future infection. This response is detected typically by *in-vitro* assays that demonstrate binding antibodies to viral surface proteins or by the prevention of viral infection at a cellular level mediated by neutralizing antibodies. Vaccine-induced virus-specific neutralizing antibodies are often considered a mechanistic correlate of protective immunity [1]. To date, clinical trials of HIV-1 vaccine candidates have failed to show robust induction of neutralizing antibodies capable of recognizing the most commonly transmitted HIV-1 isolates [2–4]. However, the sera from most HIV-1 infected

individuals displays virus-neutralizing activity, and some sera are able to potently neutralize diverse viral strains [2,4,5].

In the early 1990s a few cross-reactive HIV-1 human neutralizing monoclonal antibodies (mAbs) were isolated. These mAbs targeted epitopes on the viral surface envelope glycoprotein (Env), a trimeric protein made up of three identical gp120 molecules associated non-covalently with three gp41 molecules. These first-generation human mAbs were limited in either breadth or potency of virus neutralization [6,7]. Infusion of three mAbs (2G12, 2F5 and 4E10) into humans demonstrated, at best, a transient delay in rebounding virus in acutely infected individuals after antiretroviral (ARV) treatment interruption, with rebounding virus often containing escape mutations [8–10]. During the last 10 years, the development of panels of diverse HIV-1 isolates, along with reproducible Env-pseudovirus-based neutralization assays and testing of large clinical cohorts, has led to the identification of HIV-1 patients whose sera contain broadly reactive antibodies [11–16]. Using new techniques for antigen-specific B cell sorting and recovery of immunoglobulin genes by polymerase chain reaction (PCR) [17,18], many new broadly reactive antibodies (bNAbs) have been isolated during the last 5–6 years [5,19,20]. These antibodies target diverse epitopes on the HIV-1 Env [19,21], including the functionally conserved CD4 binding site (CD4bs) [22–25]. Viral attachment to CD4 on a host target cell is an early requirement in the process of viral entry, thus antibody to this region can block HIV-1 entry. VRC-HIVMAB060-00-AB (VRC01) is representative of a class of bNAbs that interact with the CD4bs of HIV-1 Env and have been isolated from numerous donors [22–28]. The ontogeny and structural mode of recognition of the VRC01 class of antibodies have been defined through genetic sequencing crystal structures. Members of this antibody class include VRC01, VRC07, 3BNC117, 12A12, VRC-PG04 and VRC-CH31 [19,23]. While the VRC01 class of antibodies are genetically diverse, with antibody sequence differences of more than 50%, their structural mode of recognition is similar, including reliance upon the antibody CDR H2 interaction with the CD4 binding site region of gp120. Thus, all VRC01 class antibodies contain heavy chain mimicry of the CD4 receptor, and have a heavy chain-derived from the IGHV1-2 germline gene and a light chain with a relatively short 5 amino acid CDR L3 [23,26,29]. Because they can neutralize more than 80% of diverse HIV-1 strains and target a conserved region of the virus necessary for function, candidates from the VRC01 class have been manufactured and advanced into clinical development for the prevention and treatment of HIV-1 infection [30,31].

VRC01 was isolated originally from an HIV-1-infected individual with controlled viral infection for more than 15 years in the absence of anti-retroviral therapy, using protein probes that select B cells with the appropriate binding specificity [25]. VRC01 is highly somatically mutated from the germline precursor, with a nucleotide VH mutation frequency of 32% and VK mutation frequency of 17% [22,24]. VRC01 is not self-reactive and lacks antiphospholipid antibody activity, further supporting its clinical use [27]. The B cell lineage of VRC01, as well as autologous virus, has been interrogated by evaluating longitudinal samples from the original donor [29,32]. It is now understood that germline VRC01 can bind original Env sequence from the donor and that subsequent virus escape produced a fitness cost for virus replication [33]. Subsequent somatic hypermutation (SHM) that occurred in B cells for more than 15+ years led to the expansion of a large VRC01 lineage.

Using *in-vitro* testing, the VRC01 bNAb has a half-maximal inhibitory concentration (IC_{50}) of $< 50\,\mu g/ml$ against 91% and an IC_{50} of $< 1\,\mu g/ml$ against 72% of HIV-1 primary isolates in a panel of 190 Env-pseudotyped viral strains, representing all major circulating HIV-1 genetic subtypes including clades A, B, C, D, G and AG, AE and BC recombinants [25]. Based on preclinical and *in-vitro* data, VRC01 may have the potential to prevent infection in those at risk of HIV-1, including in the setting of mother-to-child HIV-1 transmission during the intrapartum period and breastfeeding [34]. VRC01 neutralized 78% of virus isolates obtained from infected infants in Zambian mother–infant pairs with HIV-1 transmission [35] and neutralized five of six clade C founder viruses cloned from HIV-1-infected infants in Malawi [36]. Additionally, challenge studies have demonstrated the ability of VRC01 to protect non-human primates (NHP) from virulent chimeric simian–human immunodeficiency virus (SHIV) [37].

Based on preclinical protection and therapeutic data, the VRC01 drug product (VRC-HIVMAB060-00-AB) was developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH) for evaluation in a series of clinical trials. The VRC 602 Phase I clinical trial represents the first evaluation of the safety, tolerability, pharmacokinetics (PK) and neutralizing potential of VRC01 administration in healthy uninfected adults.

Methods

Study design and procedures

VRC 602 was a single-site, Phase I dose-escalation study examining the safety and pharmacokinetics of the human monoclonal antibody VRC-HIVMAB060-00-AB (VRC01) in healthy, HIV-uninfected adults, aged 18–50 years, with a weight of 53–115 kg. The study was conducted at the NIH Clinical Center (CC) by the Vaccine Research Center (VRC) Clinical Trials Program (CTP), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD (Clinicaltrials.gov

NCT01993706). The Investigational New Drug (IND) application was sponsored by the NIAID Division of AIDS. The protocol was reviewed and approved by the NIAID Institutional Review Board. US Department of Health and Human Services guidelines for conducting clinical research were followed. All subjects gave written informed consent prior to participation.

Three open-label groups received intravenous VRC01 (group 1: 5 mg/kg, group 2: 20 mg/kg and group 3: 40 mg/kg) under a dose escalation plan (Fig. 1). The dosages in the trial were determined based on preclinical studies performed with VRC01, as well as results from human clinical trials with other mAbs developed for prevention or treatment of viral pathogens. In particular, data from the licensed product Synagis® (palivizumab) directed against the viral pathogen respiratory syncytial virus (RSV) helped to inform VRC01 dosing [38,39]. Subjects were first randomized to the 5 mg/kg dose level with open-label intravenous (i.v.) administration, or blinded subcutaneous (s.c.) administration of VRC01 5 mg/kg or placebo. Safety reviews were conducted by a Data and Safety Monitoring Board at protocol-specified intervals for the two dose escalations. After five subjects were enrolled into each of the groups that specified days 0 and 28 product administration, an additional three subjects were enrolled into group 2 (20 mg/kg i.v.) to assess PK following a single infusion.

All product administrations were monitored by a study clinician. Safety laboratory tests were obtained prior to product administration and 2, 7, 14 and 28 days after each administration. Subjects kept a diary card of solicited systemic symptoms for 3 days after each dose and clinicians assessed the local site on the day of administration and on days 1, 2 and 7. All adverse events (AEs) were reported for 56 days after the second infusion, while serious adverse events and new chronic medical conditions were recorded for the duration of the study. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), and severity was graded using the DAIDS table for Grading the Severity of Adult and Pediatric Adverse Events, version 1·0, December 2004 (with clarification in August 2009). Subjects were followed for safety for 12 weeks following their final administration of study product.

PK blood samples were collected pre-dose and timed from the end of product administration at 0, 1, 2, 4, 8, 12 and 24 h, as well as days 2, 7, 14, 21 and 28 after each administration and 56 days after the second administration. An additional PK blood draw was performed at 72 h post-administration in s.c. subjects. Blood samples for anti-VRC01 antibody evaluation were collected at study day 0 (baseline), day 28 (pre-second infusion), day 56 (28 days post-second infusion) and day 112 (12 weeks post-second infusion) and samples for VRC01 virus neutralization were collected at study days 0 (baseline), 2 (48 h post-first infusion), 28 (pre-second infusion), day (48 h post-second infusion) and 56 (28 days post-second infusion). Subject HIV enzyme immunoassay (EIA) response

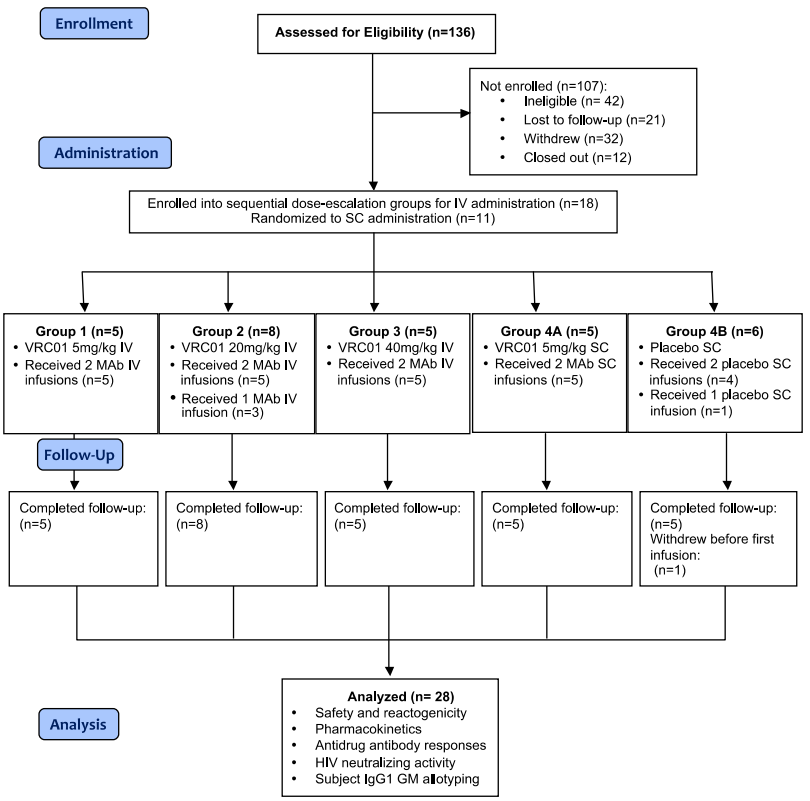

Fig. 1. VRC602 Consolidated Standards of Reporting Trials (CONSORT) diagram with study enrolment, VRC-HIVMAB060-00-AB (VRC01) administration, subject follow-up and data analysis for the four study groups.

was monitored by standard diagnostic test (Ortho VITROS anti-HIV 1 + 2 assay; Ortho Clinical Diagnostics, Rochester, NY, USA) at screening and on study days 7 and 84.

Subject IgG1 GM (gamma marker) allotyping

In the present study subjects were evaluated for the GM3/17 IgG1 allotypes to determine allotype-specific effects on VRC01 (GM3) pharmacokinetics. In the human population there are four GM allotypes in the constant region of IgG1: GM1, 2, 3, and 17. Allelic GM3 and GM17 determinants are expressed in the Fd region (portion of the heavy chain included in the Fab fragment), and GM1 and GM2 are expressed in the Fc region [40]. The presence of CH1 arginine at position 214 correlates with GM3, while CH1 lysine at 214 correlates with GM17 [40]. This panel was chosen based on the availability of quality GM molecular markers as well the likelihood of immunological impact. IgG1 markers GM3 and 17 (arginine to lysine) were determined by a predesigned TaqMan® genotyping assay from Applied Biosystems Inc. (Carlsbad, CA, USA) employing the following primers and probes: forward: 5′-CCCAGACCTACATCTGCAACGTGA-3′, reverse: 5′-CTGCCCTGGACTGGGACTGCAT-3′; reporter 1 (GM 17-specific): VIC-CTCTCACCAACTTTCTTGT-NFQ and reporter 2 (GM 3-specific): FAM-CTCTCACCAACTCT CTTGT-NFQ, as described previously [41].

Study product

To produce VRC-HIVMAB060-00-AB (VRC01), the heavy and light chains encoding VRC01 were cloned and sequenced allowing for the synthetic production of a codon-optimized variable region that was inserted into a proprietary immunoglobulin (Ig)G1 background sequence [25]. The mammalian Glutamine Synthetase Gene Expression System developed by Lonza Biologics (Slough, UK) was used to produce VRC01 under cGMP using a stably transfected Chinese hamster ovary (CHO) cell line. The formulation buffer contains 25 mM sodium citrate, 50 mM sodium chloride and 150 mM L-arginine hydrochloride at pH 5·8. The placebo for s.c. administration was VRC-PLAMAB068-00-AB, a sterile, buffered aqueous solution of 25 mM sodium citrate, 50 mM sodium chloride, 150 mM L-arginine hydrochloride, 10% dextran 40 (w/w) and 0·005% polysorbate 80 (w/w) at pH 5·8. The purified product vials at 100 ± 10 mg/ml and the placebo vials were filled and labelled at the VRC Vaccine Pilot Plant operated by Leidos Biomedical Research, Inc. (Frederick, MD, USA).

A pharmacist prepared individual i.v. doses for subjects by adding the calculated volume of VRC01 needed to achieve the assigned mg/kg dose to a 100-ml bag of 0·9% sodium chloride injection USP; i.v. infusions were administered over at least 60 min; s.c. doses were administered using a s.c. infusion pump into one site in the abdomen or by direct needle and syringe injection with up to 2·5 ml per injection site.

Pharmacokinetic analysis

Quantification of VRC01 concentrations in subject serum was performed in 96-well plates on a Beckman Biomek-based automation platform (Beckman Coulter, Brea, CA, USA) utilizing the monoclonal antibody 5C9 for VRC01 detection. 5C9 is a mouse monoclonal antibody developed from mice immunized with VRC01. The 5C9 antibody was coated onto Immulon-4HXB microtitre plates overnight at 4°C. Plates were then washed and blocked [10% fetal bovine serum (FBS) in phosphate-buffered saline (PBS)] for 2 h at room temperature. Duplicate serial threefold dilutions covering the range of 100–24300 of the test sample were incubated for 2 h at 37°C followed by horseradish peroxidase-labelled goat anti-human antibody (1 h, 37°C) and 3,3′,5,5′-tetramethylbenzidine (TMB substrate) (15 min, room temperature). Colour development was stopped by the addition of sulphuric acid and plates were read within 30 min at 450 nm via the Molecular Devices Paradigm plate reader (Molecular Devices, Sunnyvale, CA, USA). Four-parameter logistic curve regression of a standard curve of VRC01 covering the range from 0·98 to 1000 ng/ml was utilized to quantitate sample concentrations based upon the average of sample dilutions within the range of the assay.

Individual-subject non-compartmental pharmacokinetic analysis was performed using Phoenix (version 6·3, Pharsight-Centara, Princeton, NJ, USA) with VRC01 concentration data from each subject. Calculated parameters included area under the curve (AUC), maximum concentration (C_{max}), time to C_{max} (T_{max}), clearance (CL), terminal elimination rate constant (λ_z) and the terminal half-life ($t_{1/2}$). C_{max} and T_{max} were taken directly from the observed concentration–time data. The terminal slope, λ_z, was determined from the log-linear portion of the curve and the $t_{1/2}$ was calculated as $0{\cdot}693/\lambda_z$ utilizing the following terminal phase equation to determine half-life: $C_{(t2)} = C_{(t1)} \times e^{[-\lambda z \times (t2-t1)]}$, solved for C_2; $C_2 = 0{\cdot}5 \times C_1$ and $t_{1/2} = \ln(2)/\lambda_z$, where $\ln(2) = 0{\cdot}693$. The linear trapezoidal method was used to determine AUC following the first dose to day 28 ($AUC_{0\text{-}D28}$) and AUC following the second dose to the final concentration ($AUC_{0\text{-}Clast}$). The AUC after the final measured concentration (C_{last}) was estimated as C_{last}/λ_z. CL was calculated over both doses administered as $(dose_1 + dose_2)/(AUC_{dose\,1(0\text{-}D28)} + AUC_{dose\,2(0\text{-}inf)})$. For subjects receiving only a single dose, CL was estimated as $dose_1/AUC_{dose1(0\text{-}inf).}$

Neutralizing antibody assay

Serum samples were evaluated to determine the relative concentration of HIV-1 neutralizing antibodies by evaluation of the capacity to prevent the infection of TZM-bl cells by single round infection pseudotyped virus. The pseudotyped virus expresses the envelope antigen and the luciferase reporter gene. Neutralization activity was quantitated by relative decrease in the luciferase activity compared to

Table 1. Demographic characteristics of study participants

Category	Subcategory	Group 1 ($n = 5$)	Group 2 ($n = 8$)	Group 3 ($n = 5$)	Group 4A ($n = 5$)	Group 4B ($n = 6$)	Overall ($n = 29$)
				n (%)			
Gender	Male	4 (80)	7 (88)	2 (40)	5 (100)	4 (67)	22 (76)
	Female	1 (20)	1 (13)	3 (60)	0 (0)	2 (33)	7 (24·1)
Age (years)*	21–30	1 (20)	3 (38)	4 (80)	1 (20)	5 (83)	14 (48)
	31–40	3 (60)	2 (25)	0 (0)	4 (80)	1 (17)	10 (35)
	41–50	1 (20)	3 (38)	1 (20)	0 (0)	0 (0)	5 (17)
Race	Black/African	1 (20)	1 (13)	0 (0)	1 (20)	1 (17)	4 (14)
Ethnicity	Non-Hispanic/Latino	5 (100)	8 (100)	5 (100)	5 (100)	5 (83)	28 (97)
Mean Weight	kg (s.d.)	68 (8·7)	83 (12)	72 (8·8)	82 (9·2)	77 (14)	77 (12)
Education†	Secondary	1 (20)	1 (13)	0 (0)	2 (40)	1 (17)	5 (17)
	College/university	2 (40)	2 (38)	4 (80)	2 (40)	5 (83)	16 (55)
	Advanced degree	2 (40)	4 (50)	1 (20)	1 (20)	0 (0)	8 (28)

*There were no participants aged 18–20. †There were no participants with only a primary education level; s.d. = standard deviation.

infection of TZM-Bl cells in the absence of samples. Pseudotyped viruses were generated by transfection of 293T/17 cells with optimized ratios of envelope-expressing plasmid and backbone vector (pSG3ΔEnv). A panel of six viruses was tested for neutralizing activity spanning a known range of VRC IC_{80}s as well as negative controls. The tested viruses included Q23·17 subtype A, PVO·04 subtype B, MW965·26 subtype C, THRO·18 subtype B (poorly neutralized by VRC01), CAP210 subtype C (VRC01 resistant) and Moloney murine leukaemia virus (MuLV) (negative control). Neutralization assays were performed in 384-well plates using a Beckman Biomek liquid handling system [42]. Additional assay details can be found in the supplemental methods in Supporting information.

Anti-drug antibody analysis (ADA)

To screen for the presence of VRC01 anti-idiotypic antibodies in the serum, a Meso Scale Discovery (MSD) electrochemiluminescence (ECL) bridging assay was developed. Detection of VRC01 anti-drug antibodies (ADA) was achieved by a homogeneous solution phase overnight incubation of diluted serum sample along with biotinylated and SULFO-TAG-labelled drug (VRC01). Any ADA present in the serum bound to biotinylated and SULFO-TAG-labelled drug and formed a complex. Biotin-labelled VRC01 served as a capture molecule on to a streptavidin precoated MSD plate and the SUFO-TAG-labelled VRC01 was the reporter used for detection. Additional assay details can be found in the supplemental methods in Supporting information.

Results

Study population

A total of 29 subjects were enrolled. Overall, the subject population was 76% male and 24% female, had a mean weight of 77 kg, and all subjects had an educational level of high school or higher, with 83% having a college or advanced degree (Table 1). Twenty-seven subjects completed their scheduled infusions and 28 completed the protocol. One subject in group 4B (placebo) withdrew prior to the first infusion and one subject in group 4B (placebo) received only one infusion due to an intercurrent illness that did not resolve in time to receive the second infusion. Study participants were assessed for IgG GM allotype (Table 2). No significant correlations between GM allotype and measured pharmacokinetic parameters were observed.

Product safety

There were 43 VRC01 and nine placebo administrations during the trial. VRC01 was safe and well tolerated and there were no serious adverse events. When present, local and systemic solicited reactogenicity (Tables 3 and 4) was mild, with no moderate reactions in any group after either infusion.

Four adverse events assessed as possibly related to study product administration were mild in severity and resolved

Table 2. Study participant immunoglobulin IgG gamma marker (GM) allotype

	GM3/3	GM17/17	GM3/17
	2	3	1
	5	10	4
	7	12	6
	11	27	8
	13	22	9
	16		14
	17		15
	19		18
	20		23
	21		26
	25		28
% of Study participants	42·9	17·9	39·3

Table 3. Maximum local reactogenicity up to day 7 post-VRC-HIVMAB060-00-AB (VRC01) infusions*

Symptoms intensity	All i.v. subjects groups 1, 2, 3 ($n = 18$)	s.c. VRC01 subjects group 4A ($n = 5$)	s.c. placebo subjects group 4B ($n = 5$)
	n (%)		
Pain/tenderness			
None	16 (89)	3 (60)	5 (100)
Mild	2 (11)	2 (40)	0 (0)
Bruising			
None	18 (100)	5 (100)	5 (100)
Mild	0 (0)	0 (0)	0 (0)
Swelling			
None	18 (100)	5 (100)	5 (100)
Mild	0 (0)	0 (0)	0 (0)
Redness			
None	18 (100)	4 (80)	5 (100)
Mild	0 (0)	1 (20)	0 (0)
Any local symptom			
None	16 (89)	2 (40)	5 (100)
Mild	2 (11)	3 (60)	0 (0)

*There were no moderate or severe reactions throughout the trial; i.v. = intravenous; s.c. = subcutaneous.

with no residual effects. Two of these events, one in group 4A (5 mg/kg s.c. VRC01) and one in 4B (placebo s.c.), were localized pruritus at the s.c. injection site on the day of infusion that resolved on the same day. One event was flushing (subject reported 'warm sensation' from abdomen to lower extremities) in the s.c. placebo group (4B) that resolved within an hour of the infusion, and one event was an elevated alanine amino transferase (ALT 54 IU/l) in the 5 mg/kg i.v. group (group 1) 28 days following infusion that also resolved with no residual effects within 7 days.

No subjects had a reactive HIV EIA response from the administered antibody throughout the study (tested at days 7 and 84).

Pharmacokinetics

At 20 and 40 mg/kg, the mean [± standard deviation (s.d.)] maximum serum concentrations were 940 ± 320 ($n = 8$) and 1600 ± 230 ($n = 5$) μg/ml, respectively, after the first i.v. infusion and 1100 ± 360 ($n = 5$) and 1500 ± 400 ($n = 5$) μg/ml after the second i.v. infusion (Table 5 and Fig. 2). At 20 and 40 mg/kg, mean 28-day trough serum concentrations were 35 ± 6·5 ($n = 8$) and 57 ± 19 μg/ml ($n = 5$) after the first i.v. dose and 56 ± 17 ($n = 5$) and 89 ± 40 ($n = 5$) μg/ml after the second, respectively, demonstrating a trend observed across dosing groups of higher trough values with repetitive doses (Fig. S1). VRC01 clearance for 20 mg/kg was 0·016 ± 0·0035 l/h, with a terminal half-life of 17 ± 4·0 days. VRC01 clearance for 40 mg/kg ($n = 5$) was 0·017 ± 0·0015 l/h, with a terminal half-life of 14 ± 2·9 days (Table 5 and Fig. 2). For the i.v. groups overall ($n = 18$) the clearance was 0·016 ± 0·0033 l/h and terminal half-life was 15 ± 3·9 days.

Table 4. Self-reported systemic reactogenicity day 3 post-VRC-HIVMAB060-00-AB (VRC01) infusions*

Symptoms intensity	All i.v. subject groups 1, 2, 3 ($n = 18$)	s.c. VRC01 subjects group 4A ($n = 5$)	s.c. placebo subjects group 4B ($n = 5$)
	n (%)		
Malaise			
None	14 (78)	4 (80)	5 (100)
Mild	2 (22)	1 (20)	0 (0)
Myalgia			
None	14 (78)	4 (80)	4 (80)
Mild	4 (22)	1 (20)	1 (20)
Headache			
None	13 (72)	4 (80)	4 (80)
Mild	5 (28)	1 (20)	1 (20)
Chills			
None	18 (100)	5 (100)	5 (100)
Mild	0 (0)	0 (0)	0 (0)
Nausea			
None	17 (95)	5 (100)	5 (100)
Mild	1 (5·6)	0 (0)	0 (0)
Temperature			
None	18 (100)	5 (100)	5 (100)
Mild	0 (0)	0 (0)	0 (0)
Joint pain			
None	18 (100)	5 (100)	5 (100)
Mild	0 (0)	0 (0)	0 (0)
Any systemic symptom			
None	10 (56)	4 (80)	3 (60)
Mild	8 (44)	1 (20)	2 (40)

*There were no moderate or severe reactions throughout the trial; i.v. = intravenous; s.c. = subcutaneous.

Anti-VRC01 antibody responses were not detected in any subject at any time-point (Fig. 3).

HIV-1 neutralizing activity

VRC01 sera concentrations and predicted and experimental reciprocal neutralization serum dilution (ID_{80}) values were assessed for each VRC01 dose group during the course of the study (days 0, 2, 28, 30 and 56) (Table 6). Predicted values across dose groups match the observed experimental values closely, and VRC01 retained broad neutralizing activity across HIV-1 subtypes A, B and C and a range of virus IC_{80} values (0·09–1·6 μg/ml) following i.v. and s.c. administration (Fig. 4). Across virus subtypes, predicted and experimental values show a trend of increasing reciprocal serum dilution values with increasing VRC01 doses and following the second infusion, indicating the increased availability of VRC01 for virus neutralization consistent with changes in VRC01 serum concentrations. As expected, there was no observed neutralizing activity in serum from

Table 5. VRC-HIVMAB060-00-AB (VRC01) mean pharmacokinetic parameter values

Group and dose	C_{max}	T_{max}	CL	$t_{1/2}$	AUC	28-day trough concentration
			Mean (s.d.)			
Group 1 ($n = 5$)i.v. 5 mg/kg	190 (32)	2·3 (0·7)	0·016	14	45 000 (9700)	7·2 (1·5)
Inf 1	210 (50)	1·6 (0·9)	(0·0046)	(3·8)		10 (6·9)
Inf 2						
Group 2i.v. 20 mg/kg	940 (320)	2·1 (1·5)	0·016*	17*	230 000	35 (6·5)
Inf 1 ($n = 8$)	1100 (360)	2·0 (5·9)	(0·0035)	(4·0)	(71 000)	56 (17)
Inf 2 ($n = 5$)						
Group 3 ($n = 5$)i.v. 40 mg/kg	1600 (230)	2·0 (0·6)	0·0170	14	340 000	57 (19)
Inf 1	1500 (400)	1·9 (0·6)	(0·0015)	(2·9)	62 000	89 (40)
Inf 2						
Overalli.v. ($n = 18$)			0·016 (0·0033)	15		
				(3·9)		
Group 4A ($n = 5$)s.c. 5 mg/kg	34 (7·0)	66 (9·4)	0·029	17	30 000	7·54 (1·6)
Inf 1	38 (13)	52 (9·7)	(0·0067)	(2·9)	(6400)	9·4 (1·9)
Inf 2						

*Includes pharmacokinetic (PK) parameters from subjects who received one or two doses of VRC01. C_{max} = maximum concentration (μg/ml); T_{max} = time to C_{max} (h); CL = clearance (l/h); $t_{1/2}$ = terminal half-life (days); AUC = area under the curve, 0-inf (μg × h/ml); λ_z = terminal elimination rate constant (1/days), 28-day trough (μg/ml); i.v. = intravenous; s.c. = subcutaneous.

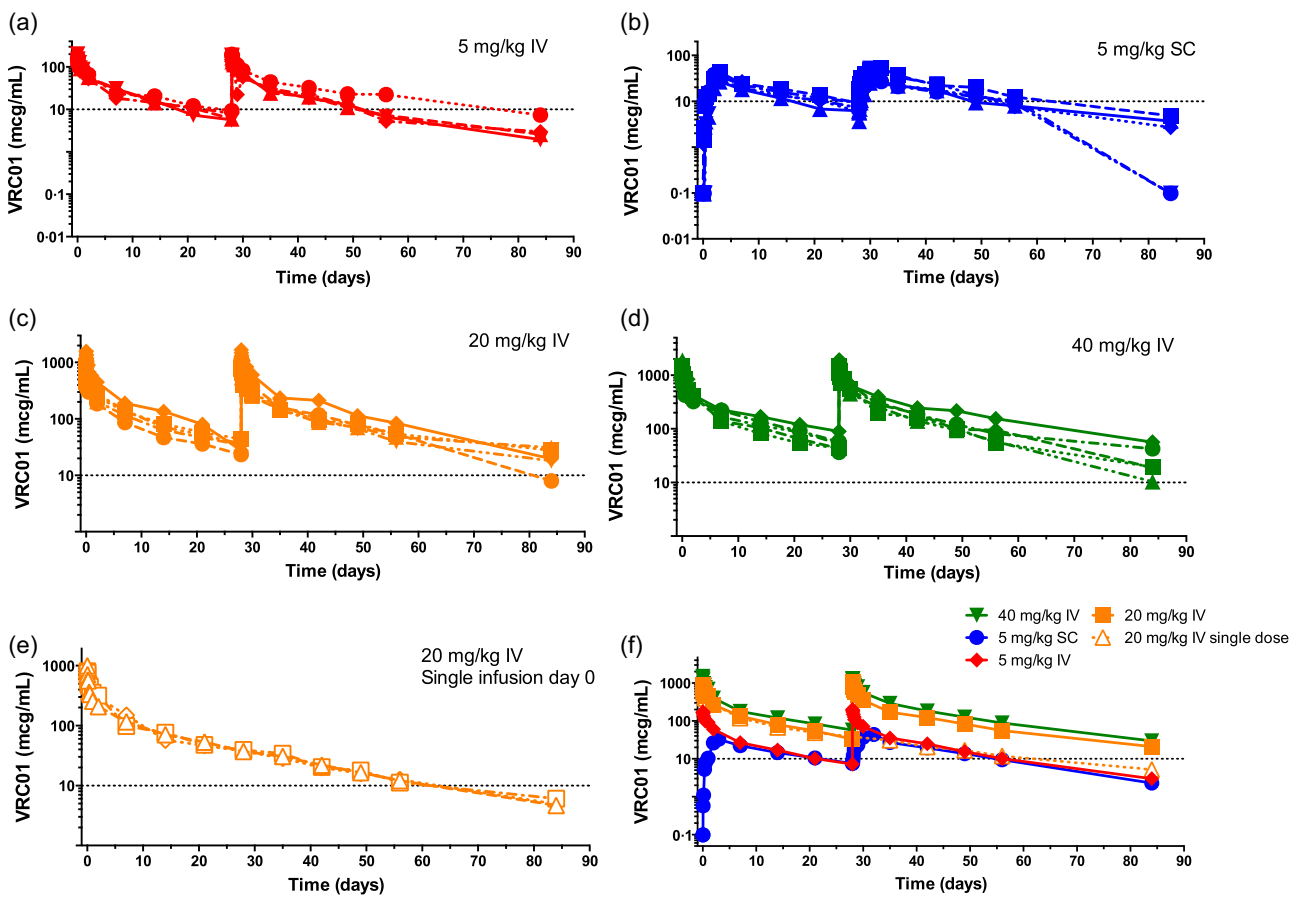

Fig. 2. VRC-HIVMAB060-00-AB (VRC01) concentration (μg/ml) shown per subject over time (days). (a) 5 mg/kg intravenous (i.v.), (b) 5 mg/kg subcutaneous (s.c.), (c) 20 mg/kg i.v., (d) 40 mg/kg i.v., (e) 20 mg/kg i.v. single dose and (f) subject means, all dose groups. A reference level of 10 μg/ml is indicated by the horizontal line on all plots. Doses were administered at days 0 and 28 (a–f) and at day 0 only (e).

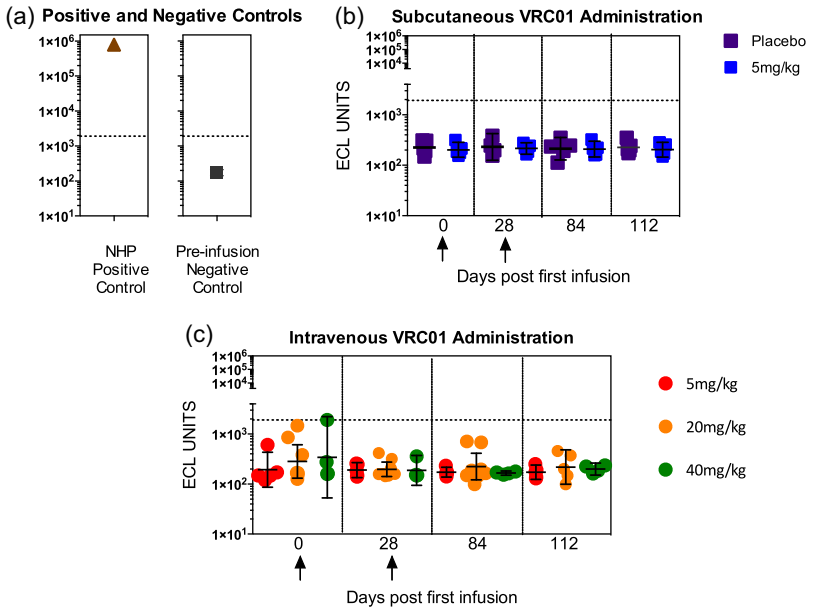

Fig. 3. Evaluation of anti-VRC-HIVMAB060-00-AB (VRC01) antibodies following VRC01 infusion. The anti-drug antibody analysis (ADA) response is measured using a homogeneous bridging electrochemiluminescence (ECL) format. (a) Typical detection of ECL anti-VRC01 activity from a non-human primate 56 days following infusion with VRC01. The geometric mean values from 30 HIV-negative non-infused subjects demonstrate the negative control for the ECL bridging assay. (b) Longitudinal analyses following either subcutaneously delivered VRC01 at 5 mg/kg or placebo on days 0 and 28 (marked with arrows). (c) Longitudinal analyses following intravenous doses (5, 20 or 40 mg/kg) on days 0 and 28 (marked with arrows). All error bars indicate geometric mean with 95% confidence intervals. The horizontal line on panels a, b and c represents the upper bound of all known negative ADA responses from subjects never exposed to VRC01. No anti-VRC01 antibody was detected post-infusion on days 28, 56 or 112.

any subject against the negative control MuLV or the VRC01 resistant virus CAP210, and poor neutralization for THRO·18 (data not shown).

Discussion

Advances in HIV-1 virology and B cell technologies during the past decade have led to the isolation of many broadly neutralizing antibodies which target diverse epitopes on the HIV-1 Env. [5,19,20]. VRC01 is a potent bNAb and is member of a class of HIV-1 mAbs that bind to the conserved CD4 binding site of gp120 by partially mimicking the structural interaction of the cellular CD4 receptor with gp120 [23,26,29]. VRC01 neutralizes up to 91% of HIV-1 primary isolates in a panel representing all major circulating HIV-1 genetic subtypes, and delivery has demonstrated complete protection in NHP SHIV challenge models [37].

In the clinical trial described here, intravenous and subcutaneous administration of VRC01 was safe and was well tolerated in healthy volunteers, without dose-limiting toxicity or serious adverse events. When present, local and systemic reactogenicity events associated with administration were mild and resolved with no residual effects. Subjects did not develop anti-VRC01 antibody responses and all subjects were negative for HIV-1 by EIA throughout the trial.

PK analysis from this clinical trial revealed a VRC01 terminal half-life of 15 days across all i.v.-infused dose groups, and 28-day trough levels after first infusion of 35 μg/ml and 57 μg/ml for 20 and 40 mg/kg dose groups, respectively. Following the second infusion, the 28-day trough values rose to 57 μg/ml and 89 μg/ml for 20 and 40 mg/kg dose, respectively. In non-human primate animal models, passive administration of VRC01 has provided complete protection against a high-dose mucosal challenge with two different SHIVs; SHIV-SF162P3, VRC01 IC_{50}: 1·86 μg/ml and SHIV-BaLP4, VRC01 IC_{50}: 0·02 μg/ml. For reference, in a 170 HIV panel the median VRC01 IC_{50} value is 0·3 μg/ml [25]. In the SHIV infection model, viral challenge was performed 2 days after i.v. VRC01 infusion and plasma VRC01 levels were measured just prior to challenge [37]. Using the more neutralization-sensitive SHIV BaLP4, complete protection against infection was observed after an infusion dose of 1·25 mg/kg and approximately 50% protection was observed after infusion of 0·3 mg/kg. The associated plasma VRC01 concentrations at the time of challenge were 4–5 μg/ml after the 1·25 mg/kg infusion (unpublished data) and 1–2 μg/ml after the 0·3 mg/kg infusion [37]. Using the more neutralization-resistant SHIV SF162P3, complete protection was observed after infusion of 20 mg/kg and 50% protection was seen after 5 mg/kg infusion. The associated VRC01 plasma levels were 52–88 μg/ml on day 2 after 20 mg/kg infusion and were 18–28 μg/ml after the 5 mg/kg [37]. In the trial reported here, following a single infusion of 20 mg/kg the mean VRC01 concentration remained above 20 μg/ml up to 6 weeks post-infusion. In the 20 mg/kg dose group which received two infusions, the mean VRC01 concentration remained above 20 μg/ml up to 8 weeks post-second infusion. Together, these data indicate that potentially protective VRC01 serum levels can be achieved for up to 8 weeks post-infusion. In addition, *in-vitro* virus neutralization assays demonstrated that passive infusion of VRC01 produced plasma viral neutralization across HIV-1 subtypes A, B and C, as expected.

As part of the overall PK assessment, subjects were genotyped for the most common IgG1 GM alleles (GM3 and

Table 6. Predicted and experimental reciprocal neutralization serum titres (ID_{80})

				Reciprocal neutralization serum titre (ID_{80})					
				Q23·17 (A)*		PVO·04 (B)*		MW965·26 (C)*	
VRC01 Treatment	Subject	Day of study	VRC01 Sera Concentration (ug/ml)	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
5 mg/kg i.v.	1	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	1	2	53·37	269·3	142·8	33·1	20·3	599·3	265·3
	1	28	8·01	40·4	24·1	< 10·0	< 10·0	89·9	32·5
	1	30	58·66	296·0	158·5	36·4	17·7	658·8	294·9
	1	56	5·31	26·8	24·3	< 10·0	< 10·0	59·6	36·1
	2	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	2	2	53·25	268·7	237·8	33·1	21·5	598·1	297·6
	2	28	5·64	28·4	10·8	< 10·0	< 10·0	63·3	33·2
	2	30	58·40	294·7	274·6	36·3	22·2	655·9	634·1
	2	56	6·61	33·3	18·3	< 10·0	< 10·0	74·2	40·3
	3	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	3	2	56·97	287·5	248·5	35·4	34·3	639·8	636·9
	3	28	5·93	29·9	21·5	< 10·0	< 10·0	66·6	42·7
	3	30	68·65	346·5	237·9	42·6	19·7	771·1	406·0
	3	56	7·29	36·8	27·3	< 10·0	< 10·0	81·9	66·5
	4	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	4	2	66·32	334·7	339·6	41·2	26·1	744·8	553·2
	4	28	9·31	47·0	14·6	< 10·0	< 10·0	104·6	55·7
	4	30	83·07	419·2	223·3	51·6	26·8	933·0	461·3
	4	56	22·30	112·5	44·7	13·8	< 10·0	250·5	86·2
	5	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	5	2	29·44	148·6	146·9	18·3	26·9	330·6	247·1
	5	28	7·28	36·8	11·7	< 10	< 10·0	81·8	39·3
	5	30	35·22	177·7	215·7	21·9	25·5	395·6	407·9
	5	56	9·32	47·0	40·9	< 10	< 10·0	104·6	70·1
20 mg/kg i.v.	6	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	6	2	203·03	1024·5	1151·9	126·1	115·3	2280·2	1686·6
	6	28	34·59	174·5	232·4	21·5	11·9	388·5	314·4
	6	30	253·90	1281·3	1382·6	157·7	233·2	2851·5	2209·7
	6	56	47·96	242·0	179·4	29·8	30·8	538·6	464·7
	7	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	7	2	275·98	1392·7	1226·0	171·4	211·3	3099·5	1513·8
	7	28	36·17	182·5	150·1	22·5	22·8	406·2	225·8
	7	30	282·34	1424·8	1228·7	175·3	174·2	3170·9	2211·4
	7	56	39·71	200·4	220·8	24·7	31·2	446·0	367·2
	8	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	8	2	248·80	1255·5	1307·9	154·5	131·2	2794·3	2226·3
	8	28	44·07	222·4	138·3	27·4	30·4	495·0	377·8
	8	30	271·20	1368·6	1308·3	168·4	195·0	3045·8	2994·7
	8	56	54·09	272·9	267·4	33·6	33·5	607·5	421·3
	9	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	9	2	185·76	937·4	763·5	115·4	119·4	2086·3	1418·9
	9	28	23·44	118·3	52·5	14·6	< 10·0	263·2	126·9
	9	30	172·63	871·1	1029·2	107·2	151·1	1938·7	1880·8
	9	56	18·90	95·4	77·8	11·7	11·4	212·2	244·6
	10	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	10	2	220·30	1111·7	869·5	136·8	166·1	2474·2	1744·8
	10	28	26·08	131·6	165·8	16·2	29·1	292·9	233·8
	10	30	201·48	1016·7	1185·8	125·1	166·6	2262·8	2249·0
	10	56	47·33	238·8	87·2	29·4	17·3	531·5	217·3
	11	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0

Table 6. *Continued*

				Reciprocal neutralization serum titre (ID_{80})					
				Q23·17 (A)*		PVO·04 (B)*		MW965·26 (C)*	
VRC01 Treatment	Subject	Day of study	VRC01 Sera Concentration (ug/ml)	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
	11	2	291·975	1473·4	1437·7	181·3	201·5	3279·2	2456·0
	11	28	37·530	189·4	180·1	23·3	19·8	421·5	438·9
	11	56	12·592	63·5	53·1	< 10·0	< 10·0	141·4	180·8
	12	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	12	2	317·500	1602·2	1290·6	197·2	193·7	3565·8	2854·6
	12	28	39·168	197·7	198·8	24·3	11·9	439·9	584·0
	12	56	11·178	56·4	45·9	< 10·0	< 10·0	125·5	182·6
	13	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	13	2	207·325	1046·2	1522·6	128·7	245·1	2328·5	2502·4
	13	28	37·150	187·5	195·5	23·1	23·3	417·2	439·1
	13	56	12·091	61·0	55·2	< 10·0	< 10·0	135·8	148·0
40 mg/kg i.v.	14	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	14	2	425·30	2146·2	1940·8	264·1	240·0	4776·5	3085·4
	14	28	42·97	216·8	181·0	26·7	26·4	482·5	293·5
	14	30	666·17	3361·7	2110·4	413·7	344·0	7481·7	5525·5
	14	56	94·75	478·1	325·0	58·8	48·6	1064·1	734·7
	15	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	15	2	423·83	2138·8	2804·5	263·2	515·2	4760·0	5443·4
	15	28	89·39	451·1	436·5	55·5	51·0	1004·0	853·6
	15	30	648·20	3271·1	1839·0	402·5	272·9	7279·9	4086·6
	15	56	154·88	781·6	548·7	96·2	86·7	1739·5	991·0
	16	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	16	2	434·48	2192·5	1348·4	269·8	190·1	4879·6	2555·7
	16	28	52·56	265·3	168·1	32·6	14·2	590·3	253·1
	16	30	455·65	2299·4	1206·5	283·0	231·4	5117·4	2080·3
	16	56	58·23	293·8	161·9	36·2	33·0	653·9	328·7
	17	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	17	2	324·50	1637·5	2753·8	201·5	339·7	3644·4	4945·2
	17	28	57·53	290·3	660·8	35·7	52·8	646·2	632·0
	17	30	504·70	2546·9	1531·5	313·4	275·5	5668·3	3076·8
	17	56	81·13	409·4	300·2	50·4	35·9	911·1	384·1
	18	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	18	2	415·33	2095·9	1385·6	257·9	230·9	4664·6	2436·7
	18	28	43·60	220·0	225·9	27·1	26·5	489·6	308·7
	18	30	549·23	2771·6	2000·9	341·1	337·5	6168·4	3373·6
	18	56	55·24	278·8	179·1	34·3	23·2	620·4	391·7
5 mg/kg s.c.	19	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	19	2	22·780	115·0	88·8	14·1	< 10·0	255·8	239·1
	19	28	5·911	29·8	12·1	< 10·0	< 10·0	66·4	41·3
	19	30	30·238	152·6	113·5	18·8	12·5	339·6	326·5
	19	56	7·733	39·0	36·2	< 10·0	< 10·0	86·8	55·3
	20	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	20	2	31·330	158·1	299·2	19·5	25·6	351·9	358·7
	20	28	9·024	45·5	68·1	< 10·0	< 10·0	101·3	115·8
	20	30	47·112	237·7	519·0	29·3	58·0	529·1	1089·0
	20	56	12·410	62·6	104·5	< 10·0	< 10·0	139·4	224·7
	21	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	21	2	19·305	97·4	96·2	12·0	13·1	216·8	165·1
	21	28	6·192	31·2	15·7	< 10·0	< 10·0	69·5	37·0
	21	30	25·190	127·1	120·1	15·6	11·5	282·9	260·5
	21	56	7·964	40·2	23·9	< 10·0	< 10·0	89·4	51·5

Table 6. *Continued*

VRC01 Treatment	Subject	Day of study	VRC01 Sera Concentration (ug/ml)	Reciprocal neutralization serum titre (ID_{80})					
				Q23·17 (A)*		PVO·04 (B)*		MW965·26 (C)*	
				Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
	22	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	22	2	20·190	101·9	112·9	12·5	10·4	226·8	170·4
	22	28	7·188	36·3	20·3	< 10·0	< 10·0	80·7	72·0
	22	30	51·937	262·1	267·2	32·3	31·4	583·3	370·1
	22	56	8·780	44·3	38·4	< 10·0	< 10·0	98·6	62·5
	23	0	< 0·098	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0	< 10·0
	23	2	38·100	192·3	180·7	23·7	25·5	427·9	379·6
	23	28	9·383	47·3	25·3	< 10·0	< 10·0	105·4	59·5
	23	30	29·883	150·8	119·9	18·6	13·8	335·6	222·5
	23	56	10·253	51·7	40·2	< 10·0	< 10·0	115·2	79·9

*HIV subtype; i.v. = intravenous; s.c. = subcutaneous; VCR01 = VRC-HIVMAB060-00-AB.

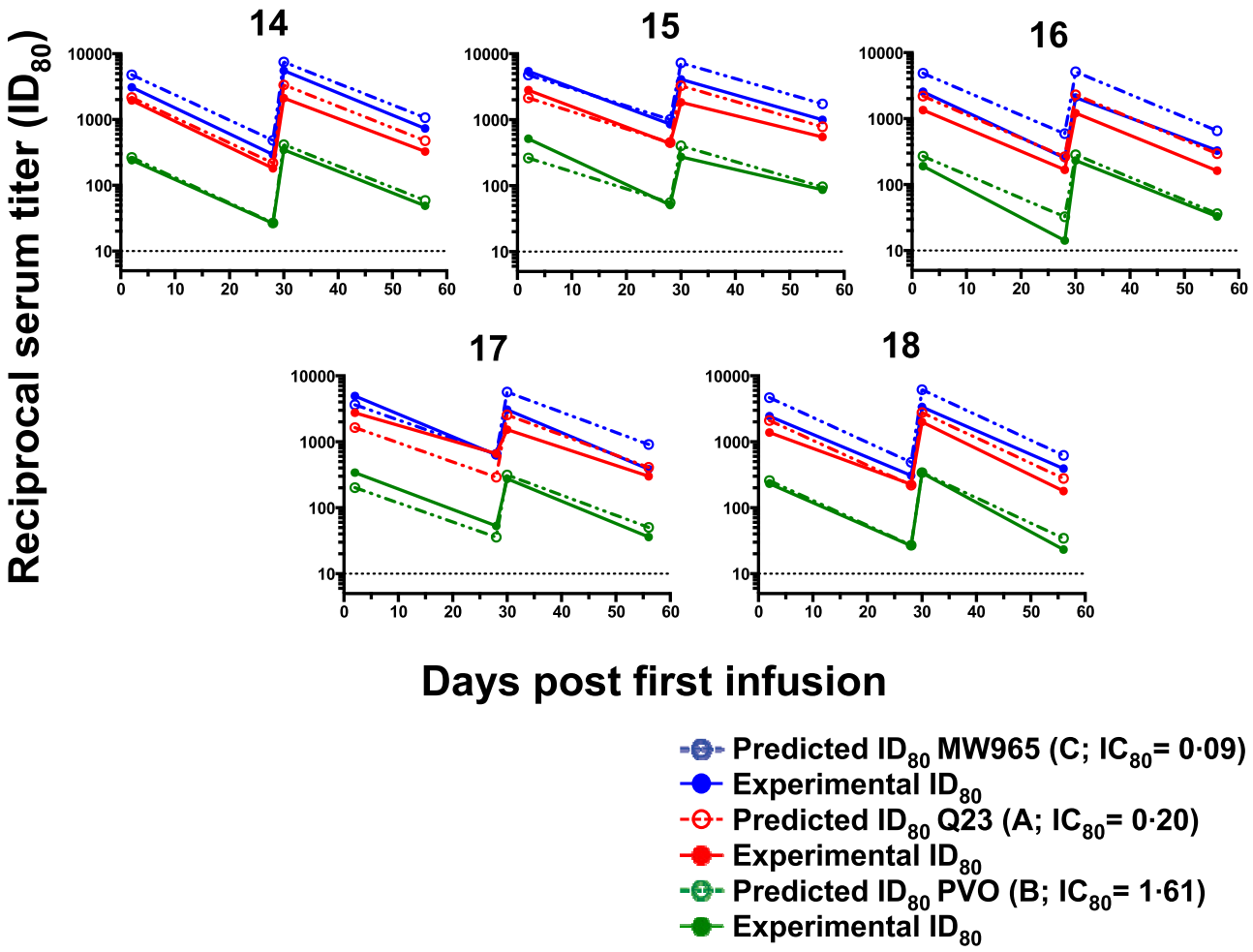

Fig. 4. Serum neutralization titres after VRC-HIVMAB060-00-AB (VRC01) infusion of 40 mg/kg. Data for five subjects are shown. Solid lines and symbols show measured serum dilution that produced 80% neutralization (ID_{80}) against the virus indicated in the legend. Predicted ID_{80} values were calculated based on the measured concentration of VRC01 in each sera and the established inhibitory concentration (IC_{80}) of the monoclonal antibody against each virus. Predicted values are graphed as open circles with dotted lines. The viruses tested are Q23.17, clade A (red), PVO, clade B (green) and MW965, clade C (blue) The VRC01 IC_{80} values against each virus is shown in parenthesis in the legend.

GM17) to determine if subject allotype impacts upon the activity of the VRC01 drug product. VRC01 contains the GM3 allele in the constant region of the heavy chain (γ1), and GM determinants of IgG1 have the potential to be immunogenic and anti-allotype antibodies have been detected when individuals are exposed to an allotype they do not possess in their genome [43–45]. No correlations to any of the PK or clinical parameters were seen based on subject GM allotyping; therefore, subject allotype, or the theoretical potential for an anti-GM3 response, had no influence on PK, virus neutralization or safety outcomes in this study.

Based on the initial clinical data reported here and available *in-vitro* and preclinical data, VRC01 has potential clinical use in three broad areas: (1) prevention of transmission from HIV-1-infected mothers to newborn and breastfeeding infants, (2) prevention of HIV infection by sexual transmission and (3) therapeutic applications in HIV-1-infected individuals. To further define the optimal regimen, an assessment of PK parameters including half-life and trough are being evaluated extensively in an ongoing Phase IB multi-site study in healthy adults using a range of doses, routes and administration intervals. VRC01 is also being evaluated for safety, pharmacokinetics and virological impact in infants at risk of infection and in a series of trials involving HIV-1-infected aviraemic and viraemic subjects. Given its PK profile, human safety data and ability to protect NHPs from infection, VRC01 will be assessed in passive immunization prevention studies in infants [34], adolescents and adults at high risk of infection. Ongoing trials will evaluate multiple dosing regimens, ARV treatment interruption following VRC01 administration and the potential impact of VRC01 on the viral reservoir. Both intravenous and subcutaneous administration routes will continue to be evaluated. Subcutaneous administration has practical advantages; however, for adults, weight-based subcutaneous dosing is limited by tolerability of the injected volume and may therefore be more appropriate for infant administration where the total volume of a weight-based dose would be lower. The data generated in expanded VRC01 trials will help to guide anti-CD4bs nNAb development and may provide a benchmark for the development of next generation HIV-1 vaccines designed to induce bNAbs.

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Disclosure

Some authors are listed as inventors on pending patent applications for VRC01.

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Supporting information

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Day 28 VRC-HIVMAB060-00-AB (VRC01) trough concentrations after first and second infusions by dose group. Across doses a trend of higher day 28 VRC01 trough concentrations is observed following the second VRC01 dose.