

HIV

Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection

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Passive immunization with HIV-1–neutralizing monoclonal antibodies (mAbs) is being considered for prevention and treatment of HIV-1 infection. As therapeutic agents, mAbs could be used to suppress active virus replication, maintain suppression induced by antiretroviral therapy (ART), and/or decrease the size of the persistent virus reservoir. We assessed the impact of VRC01, a potent human mAb targeting the HIV-1 CD4 binding site, on ART-treated and untreated HIV-1–infected subjects. Among six ART-treated individuals with undetectable plasma viremia, two infusions of VRC01 did not reduce the peripheral blood cell–associated virus reservoir measured 4 weeks after the second infusion. In contrast, six of eight ART-untreated, viremic subjects infused with a single dose of VRC01 experienced a 1.1 to 1.8 log₁₀ reduction in plasma viremia. The two subjects with minimal responses to VRC01 were found to have predominantly VRC01-resistant virus before treatment. Notably, two subjects with plasma virus load <1000 copies/ml demonstrated virus suppression to undetectable levels for over 20 days until VRC01 levels declined. Among the remaining four subjects with baseline virus loads between 3000 and 30,000 copies, viremia was only partially suppressed by mAb infusion, and we observed strong selection pressure for the outgrowth of less neutralization-sensitive viruses. In summary, a single infusion of mAb VRC01 significantly decreased plasma viremia and preferentially suppressed neutralization-sensitive virus strains. These data demonstrate the virological effect of this neutralizing antibody and highlight the need for combination strategies to maintain virus suppression.

INTRODUCTION

Although highly active antiretroviral therapy (ART) has been used successfully to manage HIV-1 infection for 20 years, additional prevention and cure strategies are needed to further reduce the global incidence of new infections (1). Potent HIV-1–neutralizing antibodies could play a role in both prevention of infection and augmentation of current treatment approaches, including limiting the seeding of the reservoir during acute infection, maintaining virus suppression induced by ART, and reducing the cell-associated virus reservoir during chronic infection (2–5). HIV-1 infection induces a robust polyclonal antibody response target-

ing numerous virus antigens, including the surface-exposed virus envelope glycoprotein (Env) that is composed of surface unit gp120 and membrane-anchored gp41 molecules. Although Env-specific antibodies usually neutralize the autologous infecting virus, rapid emergence of neutralization-resistant variants limits the ability of antibodies to control HIV-1 infection. During the course of chronic infection, the neutralizing antibody response broadens in response to the antigenic diversification of the circulating virus quasispecies (6–9). Consequently, the sera of some HIV-1–infected donors contain potent, broadly neutralizing antibodies (bNAbs), although their own virus has escaped antibody-mediated clearance (10–12).

In recent years, such bNAbs have been isolated from HIV-1–infected donors, and their potency, breadth, and structural mode of recognition have been characterized (6–8, 13, 14). After passive infusion, several of these bNAbs have demonstrated efficacy as both preventive and therapeutic agents in humanized mouse and simian-human immunodeficiency virus (SHIV) rhesus macaque animal models (3, 15–23). Each of the bNAbs identified to date targets one of several relatively conserved epitopes on the virus Env spike (7, 24–32). A subset of these antibodies, the VRC01-class, is composed of antibodies that target the conserved CD4 receptor–binding site (CD4bs) on gp120 and neutralize HIV-1 by partially mimicking the binding of CD4 to this site (33–39). The human immunoglobulin G1 (IgG1) monoclonal antibody (mAb) VRC01 neutralizes about 90% of diverse virus strains with a geometric mean 50% inhibitory concentration (IC₅₀) of 0.33 μg/ml and IC₈₀ of

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1.0 µg/ml (26). Thus, VRC01 is considered to be both potent and broadly reactive. Furthermore, VRC01 and its clonal variants have demonstrated complete protection against infection in several animal models (18–23), and passive VRC01 infusion conferred complete protection against high-dose vaginal and rectal SHIV challenge of macaque monkeys (22). As a therapeutic agent, VRC01 has demonstrated the ability to suppress virus replication during acute SHIV infection (5). In humans, the safety of passive infusion of HIV-1 mAbs into HIV-1-infected subjects has been established in previous studies of less potent, first-generation mAbs 2F5, 4E10, and 2G12. These studies demonstrated small decreases in plasma viremia or delayed virus rebound during treatment interruption from antiretrovirals (ARVs). Notably, the rebound virus contained escape mutations only from 2G12, suggesting that only this mAb exerted selection pressure on the virus (40–43). Recently, mAb 3BNC117, which is a VRC01 class antibody but not a clonal relative of VRC01, was assessed in a phase 1 clinical trial and was shown, after a single infusion, to lower plasma viremia in HIV-1-infected individuals not receiving ART (44).

The VRC01 drug product was developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH) and has been found to be safe in healthy, uninfected adults (45). Here, we assessed the virologic impact of short-term administration of VRC01 on cell-associated virus load in HIV-1-infected subjects with plasma virus load suppressed on ART, and on plasma viremia in subjects not on ART in clinical trial VRC 601. Our results demonstrate the ability of the VRC01 neutralizing mAb to suppress plasma viremia and provide initial correlations of in vitro neutralization and in vivo virologic effects.

RESULTS

Infusion with VRC01 is safe, well tolerated, and long-lasting in subjects on and off ART

A total of 15 subjects on ART received one or two infusions of VRC01 by intravenous (IV) or subcutaneous (SC) administration (table S1 and Fig. 1). An additional eight viremic subjects not on ART received a single VRC01 IV infusion of 40 mg/kg (table S1 and Fig. 1). Demographics of study participants were 81.5% male with a mean age of 35.4 years, 59.3% were Black or African-American, and 77.8% had a

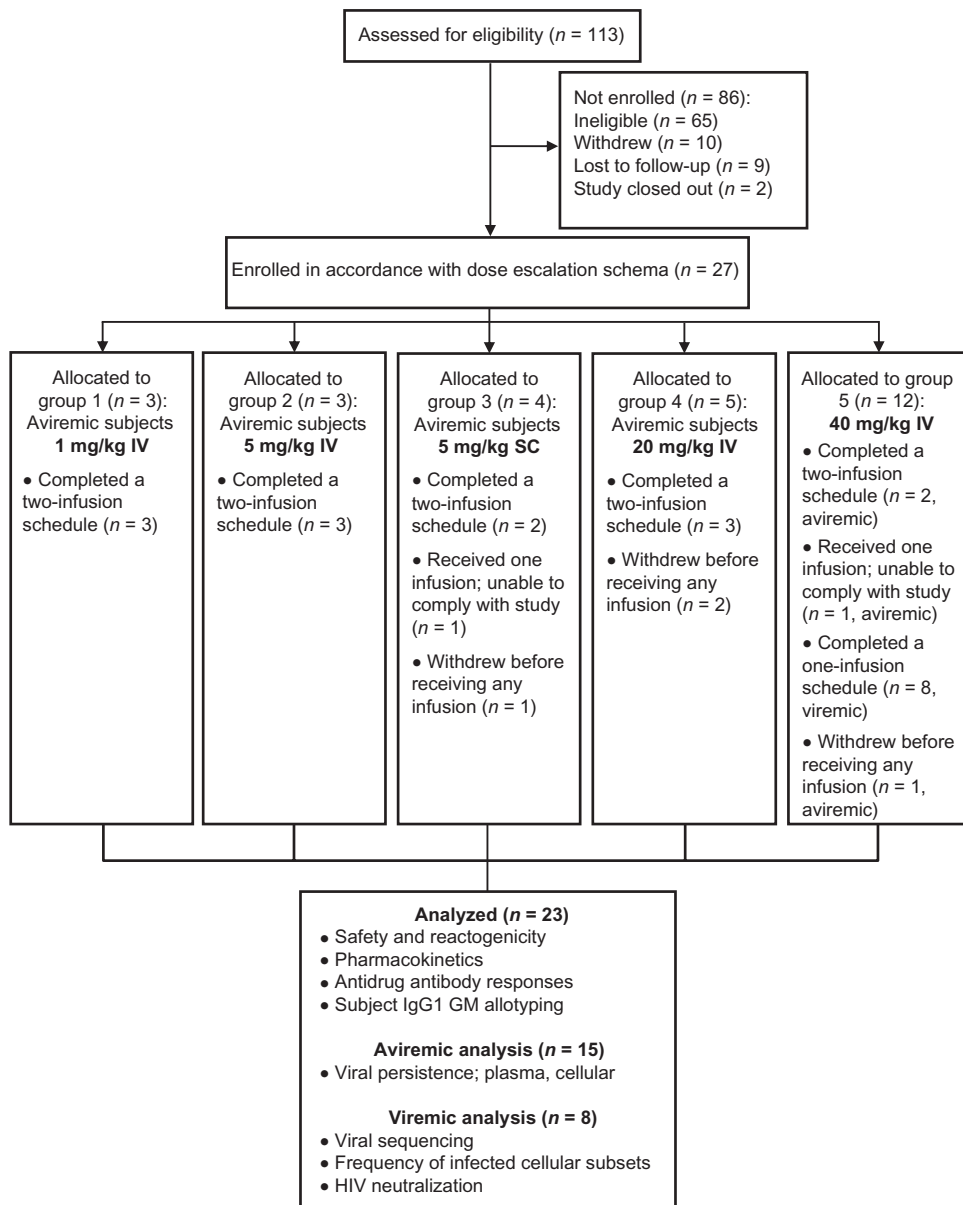


Fig. 1. Consolidated Standards of Reporting Trials flow diagram of VRC trial. Consolidated Standards of Reporting Trials (CONSORT) diagram delineates the study enrollment of 27 subjects who were allocated to five groups. After VRC01 dose escalation in groups 1 to 5, enrollments to group 5 were restricted to viremic subjects on a one-infusion schedule. GM, gamma marker.

college/university or advanced degree (table S2). Overall, the 36 infusions of VRC01 administered throughout the study were safe and well tolerated with no dose-limiting toxicities (table S3).

After VRC01 infusion, the maximum serum concentration of the antibody increased as the administered dose increased (Fig. 2, A to F). The terminal half-life of VRC01 in HIV-1-infected subjects was 12 days for IV and 11 days for SC infusions (table S4). This half-life was slightly lower than the half-life reported for IV infusion of VRC01 into healthy uninfected subjects ($n = 18$) of 15 days (45). Anti-VRC01 antibody responses were not detected at days 28, 84, and 112 (fig. S1). Furthermore, infused VRC01 was functional in the presence of autologous antibodies after infusion as evidenced by achieving the expected increase in serum

HIV neutralization breadth and potency measured on a selected virus panel (fig. S2). Population model pharmacokinetic (PK) analysis using data from this study as well as previously published data generated from VRC01 infusion in HIV-uninfected adults (45) showed no correlation between subject IgG gamma marker allotype and VRC01 half-life, nor between subject baseline virus load and VRC01 half-life (fig. S3).

VRC01 does not reduce cell-associated virus load in individuals receiving effective ART

We evaluated the effect of two VRC01 infusions given 28 days apart on HIV-1 persistence in individuals treated with effective ART by measuring residual plasma virus load and cell-associated virus load. Analysis was restricted to the highest dose groups (group 4, 20 mg/kg and group 5A, 40 mg/kg), where treatment effect would be expected to be greatest. At baseline, plasma virus loads measured by single-copy assay (SCA) were near or below the limit of detection in all but one individual (Fig. 3A and table S5). The one individual with a higher baseline plasma virus load (donor #16; 3,433 copies/ml) showed no decrease 7 days after the second of two VRC01 infusions. Because no single assay has yet been shown to measure total replication-competent cell-associated HIV-1 with high sensitivity and specificity in individuals receiving ART, we used several assays to quantify CD4 T cell-associated virus at baseline and after VRC01 infusion. Levels of total (Fig. 3B and table S5) and integrated HIV DNA (Fig. 3C and table S5) in CD4 T cells were measured as were frequencies of CD4 T cells expressing multiply spliced *tat/rev* RNA [*tat/rev* inducible limiting dilution assay (TILDA); Fig. 3D and table S5] and replication-competent HIV (Fig. 3E and table S5) after stimulation in vitro. We observed no consistent decrease in any of these measures at 7 days after the second VRC01 infusion. Furthermore, measurement of total cell-associated HIV DNA (Fig. 3, F and G, and table S5), unspliced *gag* RNA (Fig. 3, H and I, and table S5), and multiply spliced *rev* RNA (Fig. 3, J and K, and table S5) in sorted central memory (CM) and effector memory (EM) CD4 T cell subsets from blood (fig. S4) showed no consistent decrease after one or two infusions of VRC01. Levels of

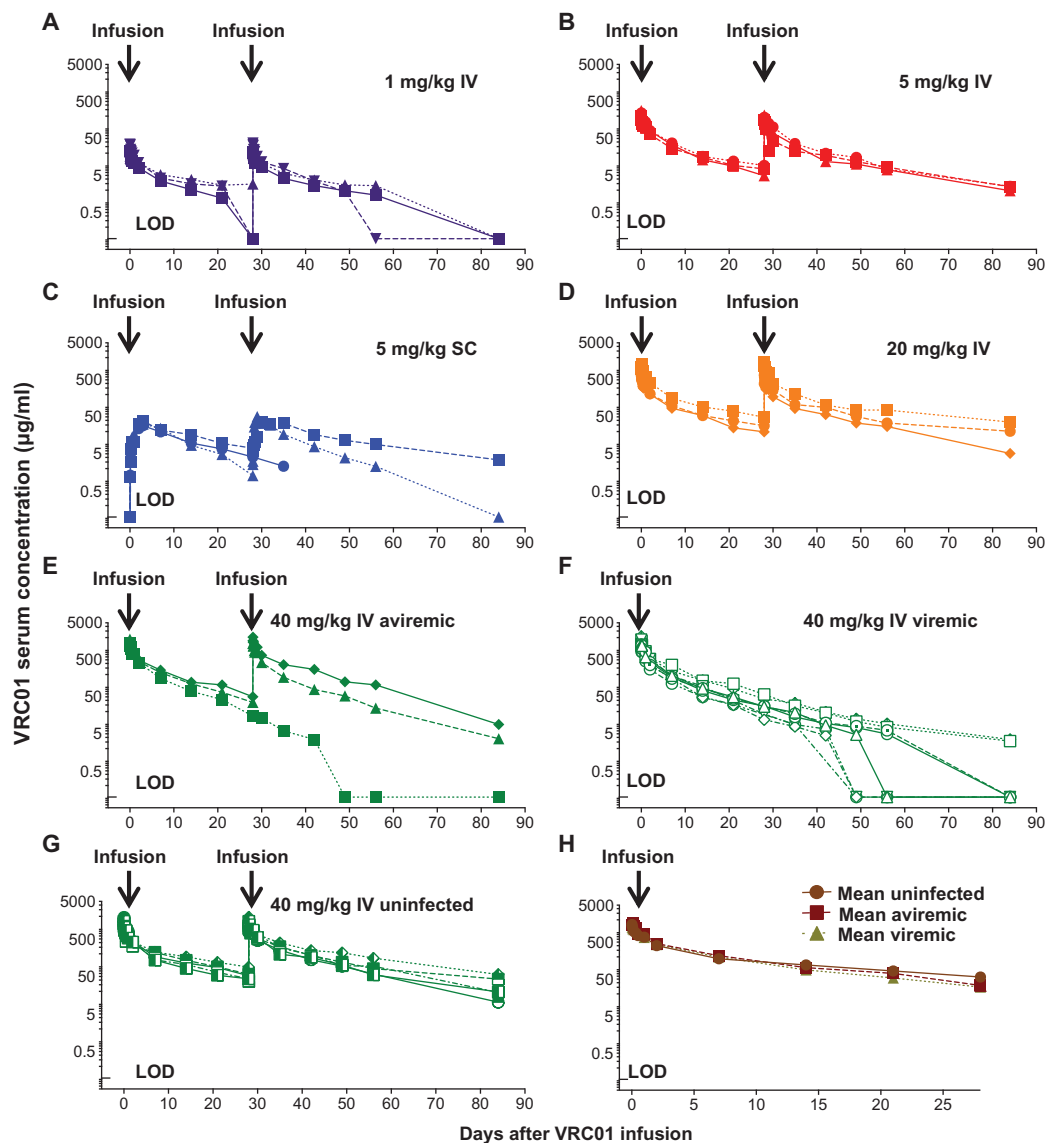


Fig. 2. Detection of antibody VRC01 in sera. (A to G) Serum VRC01 concentration in subjects after infusion with VRC01 is graphed over time for HIV-infected subjects enrolled in trial VRC 601 (Fig. 1 and table S1) as well as for HIV-1-uninfected subjects previously enrolled in trial VRC 602 (45). Day of VRC01 infusion IV or SC is indicated with black arrows and dose is labeled on the graphs. Limit of detection (LOD) of the assay is marked at 0.98 µg/ml. (H) Mean VRC01 serum concentration is graphed after a single infusion with 40 mg/kg IV in HIV-1-uninfected subjects (light brown; $n = 5$), aviremic subjects (maroon; $n = 3$), and viremic subjects (yellow; $n = 8$). Error bars are derived from SEM.

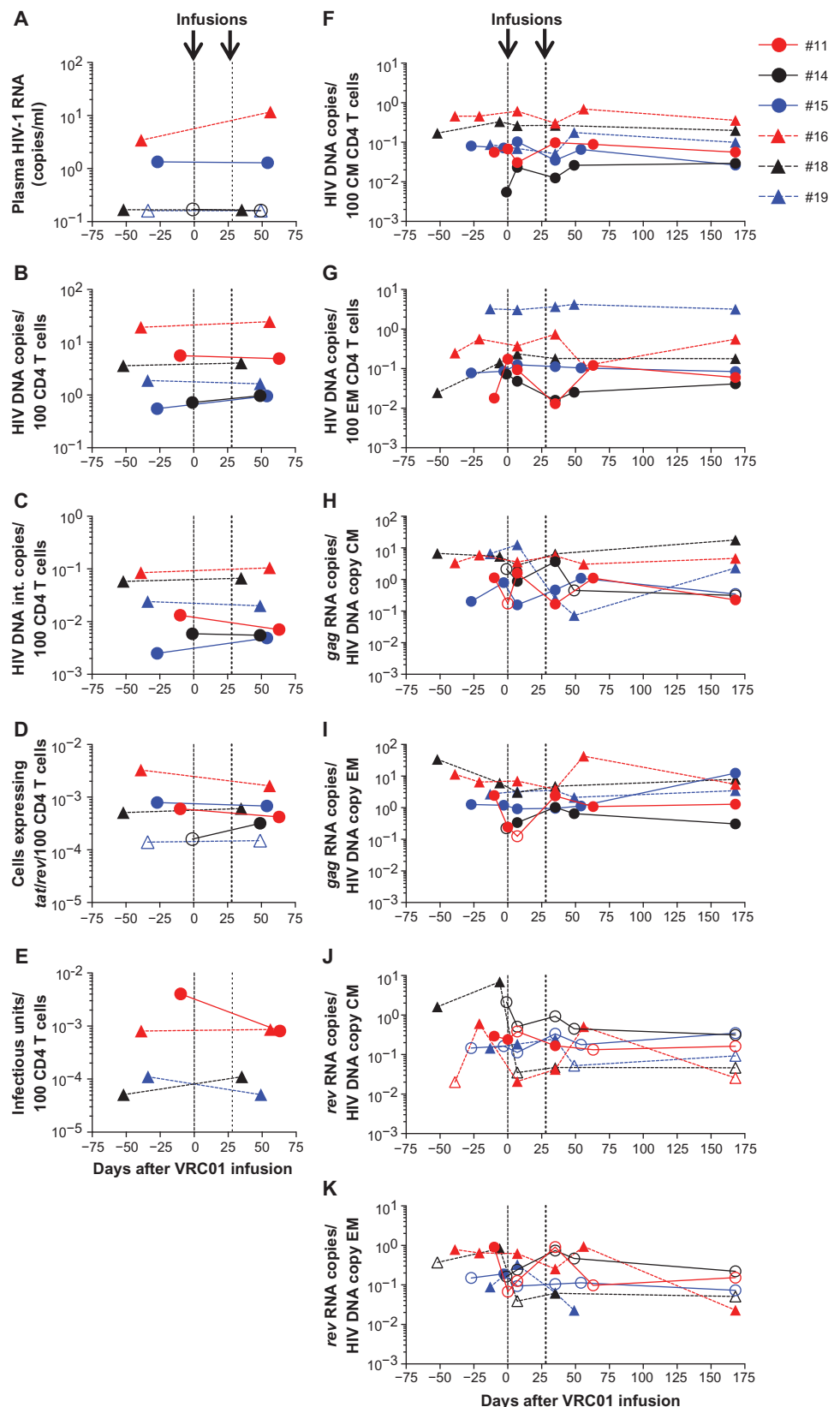
gag RNA were on the order of one copy per HIV DNA copy, and levels of *rev* RNA were frequently below the limits of detection, consistent with a quiescent cellular reservoir minimally expressing the virus even before VRC01 infusion.

Plasma virus load significantly decreases in individuals with circulating VRC01-sensitive virus

Eight study participants not currently receiving ART and with detectable baseline virus loads ranging from 237 to 27,894 copies/ml (Fig. 4A and table S1) received a single high dose of VRC01 (40 mg/kg IV). To analyze the role of the preexisting autologous circulating virus, we amplified 19 to 42 virus *env* genes from the plasma of each subject by

Fig. 3. The effect of VRC01 infusion on virus load in plasma and blood CD4 T cells during ART.

(A) Plasma viremia was measured by SCA ($n = 5$). (B and C) The percentage of total peripheral CD4 T cells in each sample containing total HIV DNA (B) or integrated HIV DNA (C) was measured by quantitative polymerase chain reaction (qPCR) ($n = 6$). (D) The percentage of cells inducibly transcribing multiply spliced *tat/rev* RNA was quantified by TILDA ($n = 6$). (E) The percentage of cells producing replication-competent HIV after in vitro stimulation was measured by quantitative coculture ($n = 4$). (F to K) The percentages of sorted CM and EM CD4 T cell subsets containing total HIV DNA were measured by fluorescence-assisted clonal amplification (FCA) (F and G) ($n = 6$). Copies of unspliced *gag* RNA (H and I) and multiply spliced *rev* RNA (J and K) per HIV DNA copy in CM (H and J) and EM (I and K) CD4 T cell subsets were measured by qRT-PCR ($n = 6$). Vertical dashed lines in each plot indicate the days of the two VRC01 infusions. Participants who received VRC01 doses of 20 mg/kg ($n = 3$) are indicated with circular symbols and solid lines; those who received 40 mg/kg ($n = 3$) are indicated with triangular symbols and dashed lines. Where samples yielded values below the assay detection limit, this detection limit is plotted as an open symbol.



single-genome amplification (SGA) on a time point within 7 days before infusion for each subject. Virus *env* sequences indicated that all eight subjects were infected with genetically distinct subtype B viruses; furthermore, one subject (#23) was infected with two genetically distinct subtype B strains (fig. S5). After infusion with VRC01, plasma virus load declined 12- to 59-fold in six of the eight subjects, and all subjects returned to baseline virus load (within $0.5 \log_{10}$ of baseline) within 56 days after VRC01 infusion (Fig. 4B and table S6). Notably, subjects 22 and 27 who entered the study with the lowest average baseline plasma virus loads (745 and 237 copies/ml, respectively) maintained undetectable virus loads (<20 copies/ml) for more than 20 days after infusion. Compared to baseline, the average virus load of all eight viremic subjects together was significantly decreased between days 3 and 21 after infusion, with the nadir at day 9 (Fig. 4C). These eight individuals were also examined for reduction in levels of total and integrated HIV DNA within peripheral blood CD4 T cells after a single VRC01 infusion (group 5B, 40 mg/kg). Compared to a single preinfusion measurement for each participant, these levels were not consistently decreased at days 7, 35, or 56 after infusion (fig. S6).

Virus load returns to baseline as VRC01 concentration decreases and as VRC01 selects for less- sensitive viruses

To test for neutralization sensitivity of baseline virus, representative gp160 *env* sequences from each subject were cloned and expressed as pseudoviruses. As we investigated virus characteristics over time in these eight viremic subjects, we observed three main patterns of antibody and virus kinetics. In pattern 1, seen in subjects 21 and 26, the predominant circulating preinfusion virus was relatively resistant to VRC01. The geometric mean IC_{80} of the 10 autologous preinfusion Envs we tested for subject 21 was $30.3 \mu\text{g/ml}$, and the geometric mean IC_{80} was $17.1 \mu\text{g/ml}$ for the 15 preinfusion Envs isolated from subject 26 (Fig. 5A). Consequently, these two subjects only had a twofold decrease in virus load (table S6). In pattern 2, seen in subjects 22 and 27, the low baseline plasma virus load (745 and 237 copies/ml, respectively) was reduced to undetectable levels after infusion and began to return to baseline on days 56 and 42, respectively, as VRC01 sera concentration waned (Fig. 5B and table S6). In pattern 3, seen in subjects 20, 23, 24, and 25, the representative preinfusion Envs tested for neutralization (7 to 11 clones per subject) were all sensitive to VRC01 with geometric mean IC_{80} s ranging from 0.361 to $1.93 \mu\text{g/ml}$ (Fig. 5A). In these subjects, plasma virus loads declined 14- to 59-fold but were not fully suppressed, and virus loads began increasing to baseline 10 days after infusion (Fig. 5B and table S6).

To better understand the mechanism of virus load increase in the presence of VRC01, we amplified plasma virus sequences from 1-month postinfusion (day 28 or 35), and a subset of these amplicons was cloned. Neutralization sensitivity to VRC01 was compared between pre- and postinfusion Env clones for the six subjects whose viremia was >20 copies/ml at this time. We observed significant decreases in sensitivity to VRC01 for all subjects except 26, whose virus was already relatively resistant (Fig. 6A). Phylogenetic trees of longitudinal full-length gp160 *env* genes from days 2, 7, 28, and 35 after infusion revealed no overall changes in virus diversity, and that for all subjects, except subject 23, sequences remained intermingled over time (figs. S7 to S12). In subject 23, we observed preferential growth of a minor, genetically distinct population that was present at baseline, expanded at day 7, and represented the major population by day 28 (fig. S11).

To determine whether postinfusion reduction in VRC01 sensitivity resulted from development of a small number of resistance mutations

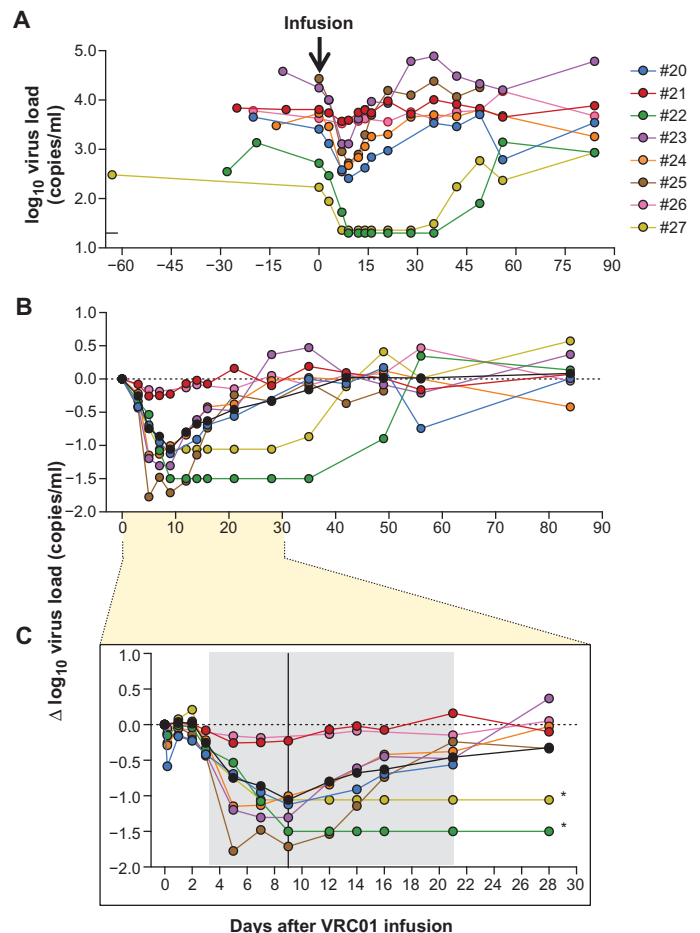


Fig. 4. The effect of VRC01 on plasma virus load after infusion. (A) Plasma virus load over time for eight viremic subjects before and after infusion with VRC01 (day 0, indicated by arrow). Limit of detection of the assay is indicated with a tick at 20 copies/ml. **(B)** \log_{10} change in virus load from average individual baseline for each of the eight subjects longitudinally for 90 days after infusion. Black line indicates mean \log_{10} change in virus load for all viremic subjects. **(C)** Early changes in virus load are magnified by graphing all collected data points from day of infusion (day 0) to 28 days after infusion. Gray box highlights days 3 to 21, at which times there was a statistically significant decline in mean virus load from baseline by paired *t* test. Mean virus load nadir occurred on day 9 after infusion, indicated by a black vertical line. Asterisks denote subjects for whom virus became undetectable (<20 copies/ml) and thus have limited decline in virus load.

or selection against residues that confer VRC01 sensitivity, we generated additional sequences from the preinfusion and 1-month postinfusion time points using a modified FCA assay to specifically amplify the CD4bs (HXB2 positions 141 to 477) (46). Resulting sequences were aligned with SGA sequences to identify positions within V1 to V5 at which amino acid sequences differed between preinfusion and 1-month postinfusion virus strains. We then used a neutralization-based epitope prediction (NEP) algorithm (<http://exon.niaid.nih.gov/nep/>) (47, 48) to predict mutational differences that could be associated with VRC01 selection. Subjects 23 and 26 were not included in this analysis because subject 23 was likely infected with two genetically distinct viruses, and one virus was predominantly circulating before infusion, whereas the other was dominant after infusion, precluding meaningful analysis.

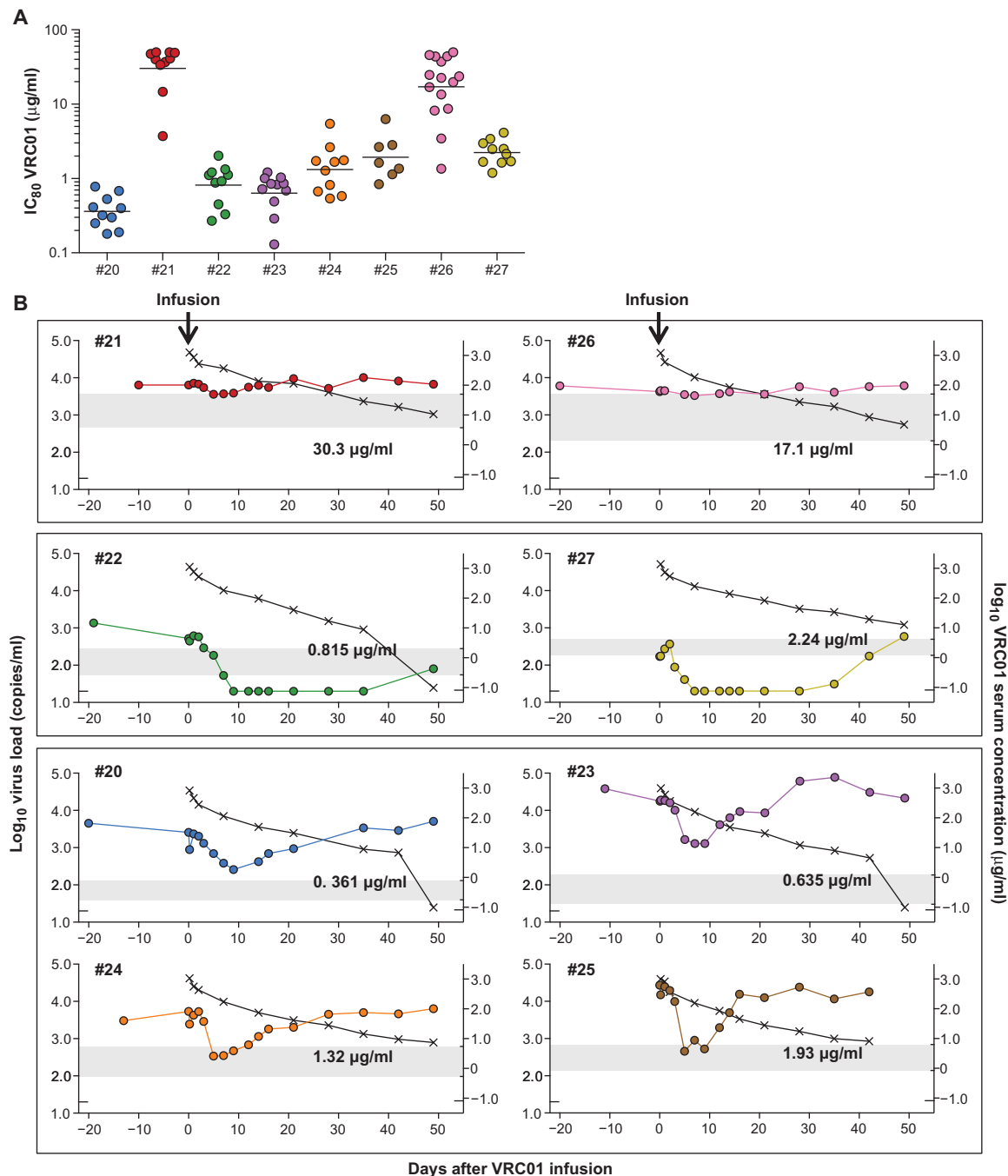


Fig. 5. Kinetics of virus and antibody clearance. (A) About 10 representative preinfusion *env* genes were cloned and expressed as pseudoviruses for each subject. Sensitivity to VRC01 is plotted as IC₈₀ values for each virus, and line indicates geometric mean. (B) Plasma virus load (left y axis) and serum VRC01 concentration (right y axis) are graphed over time for

each viremic individual. Limit of detection of each assay (20 copies/ml and 0.098 µg/ml) is indicated on the respective axes with a tick. Solid gray boxes outline the range of preinfusion IC₈₀s as shown in (A), and geometric mean IC₈₀ values are labeled. Arrows indicate day of VRC01 infusion (day 0).

Subject 26 exhibited no effect on virus load after VRC01 infusion and subsequently had no detectable changes in sequence or neutralization phenotype (Fig. 6A and fig. S12). In subjects for whom virus selection was detected, the top-ranked residue for all subjects was found within or near VRC01 contact sites (table S7) and, interestingly, all residues being selected for postinfusion were found in preinfusion sequences (Fig. 6B).

In subjects 20 and 21, overall selection occurred in or near the β20/β21 portion of the VRC01 epitope as well as within loop D and loop V5 (table S7). In particular, at positions 280 and 429, selection appeared to be for residues that were relatively rare in the preinfusion virus. In subjects 24 and 25, by contrast, the predominant selection occurred within loop V5 at position 462, and selection expanded the dominant

preinfusion population, which was perhaps more resistant to VRC01 than minor populations. Finally, the length of loop V5 was analyzed before and after infusion. The three subjects for whom multiple residue changes in V5 were predicted to result from VRC01 selection indeed had significant differences in V5 loop length before and after infusion (Fig. 6C and table S7), and these length changes were significantly correlated to VRC01 IC_{80} within the subset of cloned Envs (fig. S13). In subjects 24 and 25, selection was for longer V5 loops, whereas in subject 20, selection was for shorter V5 loops.

Env clones from pre- and postinfusion were tested for neutralization sensitivity against an expanded panel of mAbs, and overall, postinfusion viruses were more resistant to VRC01-class antibodies with no corresponding change in sensitivity to membrane proximal region-targeting antibody 10E8 (table S8 and fig. S14). These data suggest that selection for certain residues within the VRC01 epitope may be sufficient to decrease the VRC01 sensitivity of circulating virus, thereby allowing increased circulating plasma virus load.

DISCUSSION

The rationale to evaluate the bNAb VRC01 as a therapeutic agent in humans was based on an emerging body of animal model data suggesting the ability of HIV-1 bNAbs to prevent virus infection and to suppress viremia during chronic infection (3, 15, 16, 22, 49–55). Here, we assessed the impact of VRC01 administration (two infusions given 28 days apart) on the size of the persistent HIV-1 reservoir and the ability of a single VRC01 infusion to suppress plasma viremia.

As candidate therapeutic agents, antibodies differ substantially from current antiretroviral drugs. In addition to their ability to bind free virus and inhibit new rounds of infection (that is, virus neutralization), antibodies can potentially target infected cells expressing virus antigens and mediate cell killing by Fc receptor-mediated effector mechanisms. To assess the potential impact of VRC01 infusion on the cell-associated virus reservoir, we studied HIV-1-infected subjects whose plasma viremia was suppressed by ART. We observed no difference in several measures of the frequency of infected cells in the peripheral blood compartment after two infusions of VRC01. Our PK studies indicated that two infusions at these

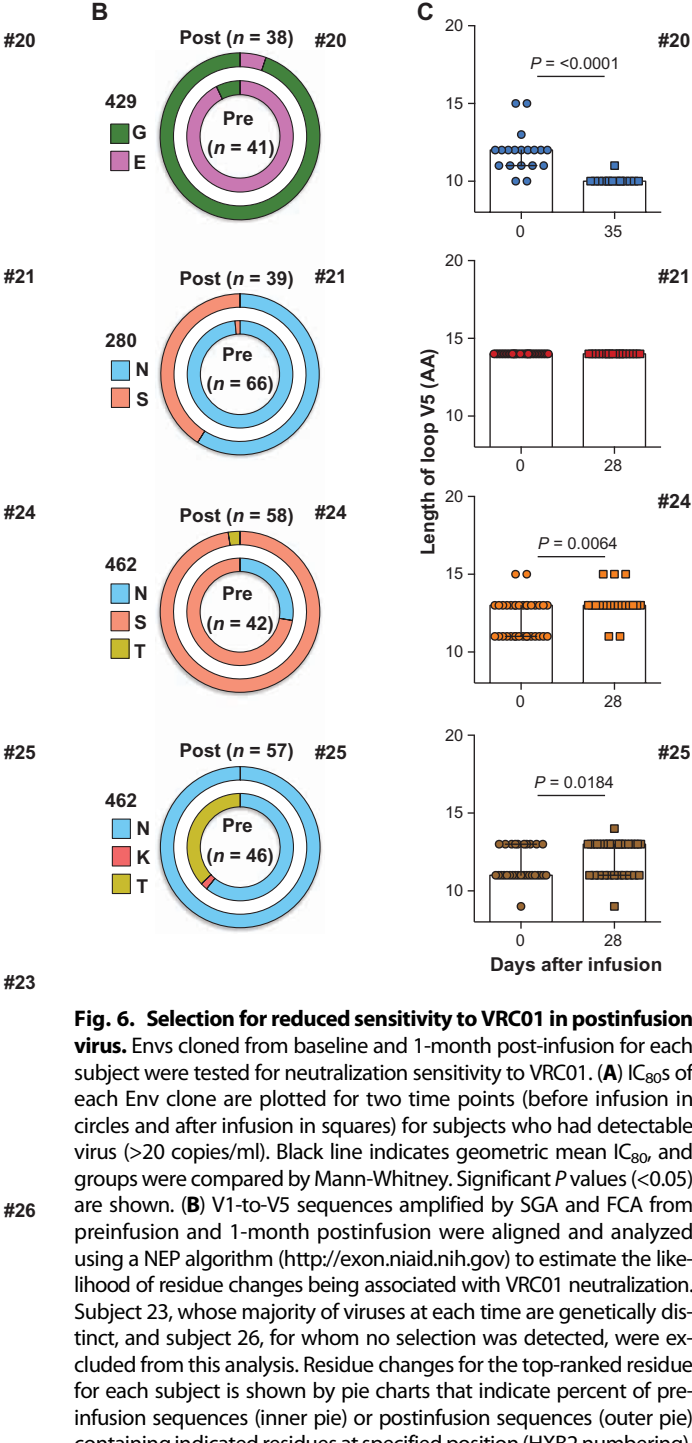


Fig. 6. Selection for reduced sensitivity to VRC01 in postinfusion HIV-1 virus. Envs cloned from baseline and 1-month post-infusion for each subject were tested for neutralization sensitivity to VRC01. (A) IC_{80} s of each Env clone are plotted for two time points (before infusion in circles and after infusion in squares) for subjects who had detectable virus (>20 copies/ml). Black line indicates geometric mean IC_{80} , and groups were compared by Mann-Whitney. Significant P values (<0.05) are shown. (B) V1-to-V5 sequences amplified by SGA and FCA from preinfusion and 1-month postinfusion were aligned and analyzed using a NEP algorithm (<http://exon.niaid.nih.gov>) to estimate the likelihood of residue changes being associated with VRC01 neutralization. Subject 23, whose majority of viruses at each time are genetically distinct, and subject 26, for whom no selection was detected, were excluded from this analysis. Residue changes for the top-ranked residue for each subject is shown by pie charts that indicate percent of pre-infusion sequences (inner pie) or postinfusion sequences (outer pie) containing indicated residues at specified position (HXB2 numbering). (C) Length of V5 sequences amplified by SGA is plotted for preinfusion (circles) and postinfusion (squares) sequences. Lines indicate median and interquartile range, and significance was calculated by Mann-Whitney. Significant P values (<0.05) are shown. AA, amino acid.

dose levels of 20 and 40 mg/kg would produce peak plasma VRC01 levels of about 1000 μ g/ml and a concentration of more than 10 μ g/ml would be maintained over the 56-day time frame. Thus, the lack of observed effect seems unlikely to be due to insufficient antibody

levels. Rather, it is possible that prolonged treatment with bNAbs such as VRC01, perhaps several months, and antibodies with augmented Fc-mediated effector functions, such as those that enhance antibody-dependent cellular cytotoxicity, may be required to affect the cell-associated HIV-1 reservoir during ART. Alternatively, the bulk of the cell-associated reservoir may be in a truly latent state and may not express viral antigens that could be recognized by antibodies. In this regard, sustained levels of antibody in conjunction with latency reversal agents that stimulate virus expression on the cell surface may be required to potentiate the ability of mAbs to kill infected cells under ART (56, 57). We found that levels of cell-associated HIV DNA were stable after VRC01 infusion even in the eight untreated, viremic study participants. Although this may, in part, reflect the imperfect association between levels of cell-associated HIV DNA and levels of virus replication in untreated individuals, it is also possible that VRC01 was more effective at clearing virions from plasma than at interrupting virus transmission between cells in the tissues where most replication occurs. Further studies will be required to determine whether different bNAbs or engineered bNAbs will more effectively target the virus in compartments outside peripheral blood.

In these untreated viremic individuals, we observed a clear effect on plasma viremia in six of eight subjects after a single VRC01 infusion at 40 mg/kg. Subjects were not screened for virus sensitivity to VRC01 before entry into the study. We noted the likely relevance of *in vitro* measurement of virus neutralization resistance (IC_{80} values >15 $\mu\text{g/ml}$). Thus, the two subjects with predominantly resistant virus did not respond to VRC01 infusion, whereas all four subjects with neutralization-sensitive virus displayed a >1 \log_{10} reduction in plasma viremia. These data aid in our understanding of *in vitro* neutralization data and *in vivo* efficacy that should be useful in future therapeutic clinical studies. Notably, the two subjects with plasma virus load <1000 copies/ml at baseline achieved undetectable levels of virus for about 20 days following VRC01 infusion, with a slow return to baseline between days 42 and 56 after infusion. Because the circulating viruses in these two subjects were not more neutralization-sensitive than those found in the other four subjects, these data suggest that this more marked response was due to the lower levels of circulating virus. Furthermore, our data suggest that this return to baseline occurred because of decreased antibody concentration as opposed to selection of resistant strains, although this possibility is currently being explored by sequencing from later time points.

We also investigated phenotypic and genetic changes in the virus after VRC01 infusion among the four donors in whom plasma viremia was only partially suppressed. Here, we found evidence for preferential selection and outgrowth of viruses that were less sensitive to VRC01, a finding that is not surprising given that ARVs have long been used in combination to prevent virus escape. Residues associated with VRC01 selection (significantly changed between the pre- and postinfusion populations) were identified and could all be found in the preinfusion population at varying levels. Thus, the return of baseline viremia between days 16 and 35 in these subjects appears to be primarily related to an outgrowth of preexisting virus less sensitive to VRC01. Our data on the impact of mAb infusion on viremic subjects are similar to the recent phase 1 study in humans that reported that VRC01-class antibody 3BNC117 reduced plasma virus load by 0.8 to 2.5 \log_{10} after infusion into chronically HIV-1-infected subjects (44).

A caveat to our study is the small number of subjects, which allowed in-depth analyses but cannot fully address the roles that antibody con-

centration, virus selection, and preinfusion virus load play in the return of virus load to baseline. The impact of these factors will be investigated in future studies and, together with the data presented here, will inform future combination strategies that will likely be needed for maintenance of virus suppression.

In summary, the data from this phase 1 study indicate that a single antibody infusion can achieve full virus suppression in chronic HIV-1-infected subjects with low levels of plasma viremia. In the absence of full suppression, virus replication allows outgrowth of virus less sensitive to antibody neutralization—a finding that is consistent with our understanding of partial virus suppression observed with antiretroviral drug therapy. These data provide proof of concept for the virologic effect of the VRC01 mAb and highlight the potential relevance of *in vitro* measurements of neutralization resistance. These data inform the future use of HIV-1 mAbs for both prevention and treatment of HIV-1 infection.

MATERIALS AND METHODS

Study design

VRC 601 was a single-site, phase 1, open-label, dose escalation study examining the safety and pharmacokinetics of the human mAb VRC-HIVMAB060-00-AB (VRC01) in HIV-infected adults. The study was conducted at the NIH Clinical Center by the VRC Clinical Trials Program, NIAID, NIH (ClinicalTrials.gov NCT01950325). The Investigational New Drug application was sponsored by NIAID Division of AIDS (DAIDS). The protocol was reviewed and approved by the NIAID Institutional Review Board. U.S. Department of Health and Human Services guidelines for conducting clinical research were followed. All subjects gave written informed consent before participation.

Four study groups were enrolled under a dose escalation schedule and were administered VRC01 at enrollment and 4 weeks later with follow-up through week 24. Dose escalation groups included group 1: 1 mg/kg IV; group 2: 5 mg/kg IV; group 3: 5 mg/kg SC; group 4: 20 mg/kg IV; and group 5: 40 mg/kg IV. Subjects were enrolled sequentially into open dose levels, and at the 5 mg/kg dose level, subjects were randomized to IV or SC administration groups to avoid selection bias. After dose escalation was completed, viremic subjects were enrolled to receive one administration of VRC01 at 40 mg/kg IV with follow-up through week 12 (Fig. 1). Target accrual was at least 3 subjects per dose level and no more than 10 viremic subjects, with total accrual allowance for 15 to 30 subjects.

Eligibility was limited to clinically stable, HIV-infected subjects. Age eligibility was 18 to 50 years old during the dose escalation and 18 to 70 years old for the subsequent enrollment of viremic subjects. During dose escalation, eligibility required no change in ART status in the 24 weeks before enrollment as well as plasma virus load ≤ 50 copies/ml and CD4 count $\geq 350/\mu\text{l}$ on at least two measurements performed within 24 weeks before enrollment. The eligibility for viremic subjects enrolling after the dose escalation required at least one detectable virus load ≥ 50 copies/ml and at least one CD4 count $\geq 250/\mu\text{l}$ within 84 days before enrollment. Other screening laboratory criteria were absolute neutrophil count $\geq 800/\mu\text{l}$, platelets $\geq 100,000/\mu\text{l}$, hemoglobin ≥ 10.0 g/dl, creatinine ≤ 1.31 mg/dl, and alanine aminotransferase (ALT) ≤ 2.5 times the upper limit of normal. Exclusion criteria included previous receipt of humanized or human mAb; weight less than 53 kg or greater than 130 kg; ongoing AIDS-related opportunistic infection;

active injection drug or alcohol dependence; severe allergic reaction in the previous 2 years; uncontrolled hypertension; receipt of other investigational study agent within previous 4 weeks; or clinically significant physical findings or medical conditions that, in the opinion of investigator, would jeopardize the safety or rights of the volunteer. Pregnant and breastfeeding women were not eligible.

The protocol required that no more than one subject per day per group received a first infusion of VRC01 by IV route, that no more than one subject per week received a first infusion by SC route, and that the first subject in each dose group had a virus load ≤ 50 copies/ml.

At 2 weeks after the third subject in group 1 (1 mg/kg IV) completed the day 0 infusion, a safety review was conducted to allow group 2 (5 mg/kg IV) and group 3 (5 mg/kg SC) to enroll. At 2 weeks after the third subject in group 2 completed the first infusion, a safety review was conducted to allow group 4 (20 mg/kg IV) to enroll. At 2 weeks after the third subject in group 4 completed the first infusion, a safety review was conducted to allow group 5 (40 mg/kg IV) to enroll. Each dose group included at least three evaluable subjects.

All product administrations were monitored by a study clinician. Safety laboratory samples were collected at baseline and at 2, 7, 14, and 28 days after each administration. Systemic symptoms were solicited from subjects for 3 days after each administration, and clinicians assessed the local site on the day of administration and on study days 1, 2, and 7. During dose escalation, all adverse events (AEs) were recorded through 56 days after the second infusion, whereas serious adverse events (SAEs) and new chronic medical conditions were recorded for the duration of the study (134 days after second infusion). Subsequently, for viremic subjects enrolling after the dose escalation was complete, all AEs were recorded through 28 days after administration and SAEs were recorded for the duration of the study (84 days after administration). AEs were coded using the Medical Dictionary for Regulatory Activities, 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 August 2009). The study design criteria for pausing all enrollments and product administration were a grade 4 or grade 5 AE assessed as related to study agent or two grade 3 AEs assessed as related to study agent.

For the two-dose schedule, PK blood samples were collected before infusion and timed from the end of product administration at 0, 1, 2, 4, 8, 12, and 24 hours, as well as days 2, 7, 14, 21, and 28 after each administration and days 56, 85, and 140 after the second administration. An additional PK blood draw was performed at 72 hours after administration in SC subjects. For the single-dose viremic subject schedule, PK blood samples were collected before infusion and timed from the end of product administration at 0, 1, 2, 4, and 24 hours, as well as days 2, 7, 14, 21, 28, 35, 42, 49, 56, and 84 after infusion. Blood samples for anti-VRC01 antibody evaluation were collected at study days 0 (baseline), 28, and 84, with an additional day 112 for subjects on a two-dose schedule. Samples for research assays such as VRC01 virus neutralization, virus sequencing analysis, and latent virus quantification were collected at study days 0 (baseline), 7, 35, and 168 for subjects on a two-dose schedule and study days 0, 2, 7, and 28 for single-dose viremic subjects. Additional blood volume was collected for further research analysis via apheresis at baseline as well as 3 to 6 weeks after second infusion in subjects on the two-dose schedule and 3 to 6 weeks after infusion for single-dose viremic subjects. Subject HIV virus load and CD4 count were tested before infusion and on study days 14, 21, 28, 42, 49, 56, 84, 112, and 168 for subjects on a two-dose schedule. For vire-

mic subjects on a single-dose schedule, virus load was tested at preinfusion and at all study time points 4 hours after infusion, and CD4 count was tested before infusion and on study days 7, 14, 21, 28, 35, 42, 49, 56, and 84.

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, which was inserted into a proprietary IgG1 background sequence (26). The mammalian Glutamine Synthetase Gene Expression System developed by Lonza Biologics was used to produce VRC01 under current good manufacturing practice using a stably transfected Chinese hamster ovary 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 purified product vials were filled at a concentration of 100 ± 10 mg/ml and labeled at the VRC Vaccine Pilot Plant operated by Leidos Biomedical Research Inc., Frederick National Laboratory (Frederick, MD). A pharmacist prepared individual IV doses for subjects by adding the calculated volume of VRC01 needed to achieve the assigned milligram-per-kilogram dose to a 100-ml bag of 0.9% sodium chloride injection USP (United States Pharmacopeia) (normal saline). IV infusions were administered over at least 60 min. SC doses were administered using an SC infusion pump into one site in the abdomen with up to 2.5 ml per injection site at a rate of 15 ml/hour.

Plasma virus load

Longitudinal virus load counts for all enrolled subjects before and after infusion were performed by the NIH Clinical Center using the standard diagnostic assay COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0 (Roche).

Serum VRC01 concentrations

Quantification of VRC01 concentrations in subject serum and individual subject noncompartmental PK analysis were performed as previously described (45). Briefly, VRC01 serum concentration quantification was performed in 96-well plates on a Beckman Biomek-based automation platform using the anti-idiotype mAb 5C9.

Isolation of nucleic acids from plasma samples and quantitative reverse transcription PCR and PCR for SCA

Plasma samples were clarified at 25°C, centrifuging at 2000g for 10 min. Supernatants then were processed to collect virions and isolate nucleic acids as described (58).

Plasma samples were assessed for unspliced HIV *gag* RNA copy numbers using gSCA and HMMCGag assays in a multiplexed assay format, as described previously (58). Samples were tested undiluted and diluted at 1:10, with spike controls incorporated into the multiplexed assay to assess any assay inhibition.

Quantification of cell-associated total HIV DNA and integrated HIV from CD4 T cells

Total and integrated HIV DNA in CD4 T cells was determined as previously published (59). Briefly, CD4 T cells were isolated from peripheral blood mononuclear cells (PBMCs) from study subjects by negative magnetic selection (StemCell) and digested by proteinase K. Cell lysates were directly used for HIV DNA quantifications. Total HIV-1 DNA was preamplified with primers annealing within con-

served regions of the 5'LTR (5' long terminal repeat) and the *gag* gene. Integrated HIV-1 DNA was amplified with two *Alu* primers together with a primer annealing the LTR region. In all PCRs, primers specific for the *CD3* gene were added to precisely quantify the cell input. In a second round of PCR, appropriate primers and probes were used to amplify HIV sequences from the first round of amplification. Inner primers specific for the *CD3* gene were used in a separate reaction to determine cell input. The number of copies of total and integrated HIV-1 DNA was calculated by using serial dilutions of proteinase K lysate of ACH-2 cells as a standard curve. Results were expressed as number of HIV copies per 100 CD4 T cells.

Inducible virus quantification by TILDA

The frequency of CD4 T cells with inducible multiply spliced HIV RNA was determined using TILDA as described previously (60). Briefly, CD4 T cells were isolated from PBMCs from study subjects by negative magnetic selection (StemCell) and stimulated with phorbol 12-myristate 13-acetate (100 ng/ml) and ionomycin (1 µg/ml) for 12 hours in the presence of ARVs [(100 nM efavirenz (EFV), 180 nM zidovudine (AZT), and 200 nM raltegravir (RAL)]. Serial dilutions of the stimulated cells were placed in a 96-well plate directly in RT-PCR buffer using one in three dilutions (four times) and with 22 replicates at each dilution. Multiply spliced (ms) HIV RNAs were quantified by seminested real-time PCR with previously described primers in *tat* and *rev* with minor modifications (61). The frequency of positive cells was calculated using the maximum likelihood method, and this number was then expressed as a frequency of cells with inducible msHIV RNA per 100 CD4 T cells.

Quantitative coculture assay

To determine the frequency of CD4 T cells carrying replication-competent HIV, quantitative coculture assays were carried out as described previously (62).

Flow cytometric sorting of cell subsets from PBMCs

PBMC suspensions were stained with LIVE/DEAD Aqua stain (Molecular Probes) and the following fluorescently conjugated antibodies: CD27-Cy5PE (cyanine5-phycoerythrin) (Coulter), CD45RO-PE-Texas Red (Coulter), CD14-PE (Pharmingen), CD11c-PE (Pharmingen), CD3-H7APC (H7-allophycocyanin) (BD), T cell receptor-γδ-APC (Pharmingen), CD4-BV785 (Brilliant Violet 785) (BioLegend), and CD8-QD655 (quantum dot 655) (Invitrogen). Stained PBMCs were sorted on a FACSria into four subsets for each sample, as shown in fig. S4.

Nucleic acid extractions from sorted cell subsets

Cell subsets from PBMCs sorted into growth medium were pelleted by centrifugation at 420g and lysed in RNAzol RT (Molecular Research Center). Total cellular RNA was extracted from the aqueous phase of each lysate according to the manufacturer's protocol. The organic phase of each lysate was solubilized in DNAzol (Molecular Research Center), and total cellular DNA was extracted according to the manufacturer's protocol.

Quantitative RT-PCR for HIV *gag* and *rev*

Unspliced HIV *gag* and multiply spliced HIV *rev* RNAs were quantified in total RNA from sorted cellular subsets by amplification using the RNA UltraSense One-Step Quantitative RT-PCR System (Thermo Fisher). For *gag*, the forward primer was GGTGCGAGAGCGTCAGTAT-

TAAG, the reverse primer was AGCTCCCTGCTTGCCATA, and the probe was FAM-AAAATTCGGTTAAGGCCAGGGGAAAGAA-BHQ. For *rev*, the forward primer was AGGACTCGGCTTGCTGAA, the reverse primer was GCTGTCTCCGCTTCTTCCT, and the probe was FAM-CACRGAAGAGGGCGAGGGG-BHQ. Cycling conditions were 45°C for 30 min, 95°C for 2 min, and then 45 cycles of 95°C for 15 s and 60°C for 60 s. Cellular RNA samples were treated with deoxyribonuclease I (Thermo Fisher) before amplification and were loaded at between 4000 and 5500 cell equivalents per reaction. Absolute quantification of RNA molecules was achieved by interpolation within standard curves generated from dilutions of synthetic RNA standard templates.

SGA and cloning of gp160 env genes

Plasma virus *env* genes were amplified and cloned as previously described with the following modifications (63, 64). Virions were concentrated from 4 ml of plasma by centrifugation and resuspended in 140 µl of phosphate-buffered saline before virus RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen). Complementary DNA (cDNA) was generated by first incubating 2.5 µl of 10 mM deoxynucleotide triphosphates and 2.5 µl of 2 µM antisense primer envB3out (TTGCTACTTGTGATTGCTCCATG) with 33.5 µl of template RNA for 10 min at 65°C to denature the RNA, followed by reverse transcription using SuperScript III reverse transcriptase (Invitrogen, Life Technologies) according to the manufacturer's instructions in a 50-µl total reaction volume. Amplification of *env* genes was performed by nested PCR using the Platinum Taq High Fidelity polymerase (Invitrogen) as previously described (64). To ensure that a majority of amplicons would be generated from a single cDNA template, cDNA was diluted so that PCR-positive wells were fewer than 30% of the reactions. Single cDNA templates were amplified in a 35-cycle first-round PCR with forward and reverse primers envB5out (TAGAGCCCTGGAAGCATCCAGGAAG) and envB3out (TTGCTACTTGTGATTGCTCCATG), followed by a 45-cycle second-round PCR containing 1 µl of first-round product and forward and reverse primers envB5in (caccTTAGGCATCTCCTATGGCAGGAAGAAG) and envB3in (GTCTCGAGACTACTGCTCCACCC). All PCR procedures were performed in PCR clean rooms free of post-PCR or plasmid DNA. Amplicons were run on precast 1% agarose gels (Embi Tec) and sequenced by ACGT Inc. Sequences that contained stop codons, large deletions, or mixed bases were removed from further analysis. Representative *envs* from pre- and postinfusion time points were cloned by reamplifying 2 µl of first-round PCR template with 0.2 µl of 20 µM forward and reverse primers envB5in and envB3in in a total reaction volume of 20 µl using Phusion Hot Start Polymerase (New England Biolabs) according to the manufacturer's instructions, with an annealing temperature of 64°C and an extension time of 1 min for 26 cycles. Amplicons were cloned using the pcDNA3.1 Directional TOPO kit (Invitrogen) and transformed in XL2-Blue MRF' Ultracompetent Cells (Agilent Technologies), all according to the manufacturers' instructions. Transformations were plated on LB + ampicillin (100 µg/ml) plates (K-D Medical) overnight at 37°C. Colonies were cultured, and DNA was isolated using the 24-well blocks and QIAprep 96 Plus Miniprep Kit (Qiagen). All plasmids were sequence-verified before expression.

Neutralization assays for plasma and mAbs

Neutralization of viruses by plasma and mAbs were measured using single-round HIV-1 Env-pseudovirus infection of TZM-BL target cells

as previously described (10, 65). Briefly, pseudovirus stocks were generated by cotransfecting 293T cells with the cloned *env* plasmid and an *env*-deficient backbone (pSG3 Δenv) at a 1:3 ratio of DNA. At 72 hours after transfection, culture supernatants were filtered, harvested, and frozen at -80°C until further use. In the neutralization assay, 10 μl of five-fold serially diluted patient serum or mAb was incubated with 40 μl of pseudovirus in a 96-well plate at 37°C for 30 min before addition of TZM-bl cells. After 2 days of incubation, cells were lysed and the viral infectivity was quantified by measuring luciferase activity with a VICTOR Light luminometer (PerkinElmer). Neutralization curves were fit by nonlinear regression using a five-parameter hill slope equation. The 50 and 80% inhibitory concentrations (IC_{50} and IC_{80}) were reported as the antibody concentrations or plasma dilutions required to inhibit infection by 50 and 80%, respectively.

FCA of the CD4bs

Total cellular DNA or plasma HIV-1 *env* cDNA samples were amplified in multiple replicate HIV-1 *env*-specific PCR reaction wells at dilutions anticipated to yield fewer than 30% positive wells. SYBR Green I nucleic acid gel stain (Molecular Probes) was included at a 250,000-fold dilution in the PCRs to serve as a fluorescence tracer, with positive reactions detected by amplification of SYBR Green I fluorescence and confirmed by melt curve analysis. For FCA reactions quantifying HIV DNA copies in total cellular DNA from sorted cell subsets, primers were ESf2 (CCGGCTGGTTTTGCGATCTCTAAARTG) and LSr1 (ATGG-GAGGGGCATACATTGCYYT), and cycling conditions were 94°C for 2 min, followed by 50 cycles of 94°C for 15 s, 63°C for 15 s, and 68°C for 45 s, followed by melt curve analysis. The HIV DNA copy number was determined for each sample according to limiting dilution calculations, as follows: HIV DNA copy number = (number of wells) \times ($-\ln(\text{fraction of negative replicate wells})$). To determine the percentage of cells from each sorted subset containing HIV DNA, the HIV DNA copy number values determined as above were normalized to recovered quantities of total cellular DNA determined by qPCR for the albumin gene (66). For FCA reactions amplifying the full HIV-1 *env* CD4bs region for sequencing, primers were FCabsF (AGCCATAAGC-CATGTGTAAAAYTAAC) and GWra1 (GCTGCGCCCATAGTG-CTTCCTGC), and cycling conditions were 94°C for 2 min, followed by 50 cycles of 94°C for 15 s, 61.5°C for 15 s, and 68°C for 90 s, followed by melt curve analysis. Positive wells from samples calculated to be at limiting dilution were reamplified with primers FCabsF (AGCC-TAAAGCCATGTGTAAAA) and GWra1, and sequences of reamplified products were determined by Sanger sequencing.

Sequence selection by VRC01

To determine sequence changes after infusion that could be associated with antibody selection on the virus, translated sequences generated by FCA and SGA from the preinfusion and 1-month postinfusion time points for each subject were MUSCLE (multiple sequence comparison by log-expectation)-aligned to HXB2 using the Geneious suite version 8.1.7 [www.geneious.com; (67)]. Alignments were hand-edited and trimmed to HXB2 positions 141 to 477. Protein alignments were uploaded to the neutralization-based epitope prediction algorithm (<http://exon.niaid.nih.gov/nep/>). Sequences preinfusion were assigned a value of "0," postinfusion sequences were assigned a value of "50," and potency threshold was set to 25. The result was a rank order of positions that were significantly predicted to be associated with VRC01 selection (47, 48).

Statistical analysis

Magnitude and significance of virus load change from baseline over time was calculated by performing a paired *t* test on the \log_{10} mean change from baseline for all subjects. A virus load that was undetectable at the sensitivity level of the assay (20 copies/ml) was treated as 20. A *P* value <0.05 was considered statistically significant.

SUPPLEMENTARY MATERIALS

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Materials and Methods

- Fig. S1. Assessment of anti-VRC01 response after infusion with monoclonal VRC01.
Fig. S2. Characteristics of serum neutralization after VRC01 infusion.
Fig. S3. Population PK analysis of VRC01 infusion.
Fig. S4. Gating tree for flow cytometric sorting of cellular subsets from blood.
Fig. S5. Genetic diversity of preinfusion autologous virus in viremic subjects.
Fig. S6. The effect of VRC01 infusion on CD4 T cell-associated virus DNA levels in viremic individuals.
Fig. S7. Selection pressure on autologous virus from subject 20 after infusion with VRC01.
Fig. S8. Selection pressure on autologous virus from subject 21 after infusion with VRC01.
Fig. S9. Selection pressure on autologous virus from subject 24 after infusion with VRC01.
Fig. S10. Selection pressure on autologous virus from subject 25 after infusion with VRC01.
Fig. S11. Selection pressure on autologous virus from subject 23 after infusion with VRC01.
Fig. S12. Selection pressure on autologous virus from subject 26 after infusion with VRC01.
Fig. S13. Correlation of virus neutralization sensitivity to VRC01 and V5 loop length.
Fig. S14. Selection for reduced sensitivity to 3BNC117 in postinfusion virus.
Table S1. Clinical characteristics of HIV-1-infected subjects.
Table S2. Demographic characteristics of study participants.
Table S3. Reactogenicity after infusions with VRC01.
Table S4. VRC01 mean PK parameter values.
Table S5. Source data for cell-associated virus in aviremic subjects (Fig. 3).
Table S6. Characteristics of plasma virus kinetics in relation to serum antibody concentration.
Table S7. Sequence changes in Env protein sequences after infusion with VRC01.
Table S8. Sensitivity of pre- and postinfusion autologous virus clones from viremic subjects to mAbs.
Reference (68)

REFERENCES AND NOTES

- P. Piot, S. S. Abdool Karim, R. Hecht, H. Legido-Quigley, K. Buse, J. Stover, S. Resch, T. Ryckman, S. Magedal, M. Dybul, E. Goosby, C. Watts, N. Kilonzo, J. McManus, M. Sidibé; UNAIDS-Lancet Commission, Defeating AIDS—Advancing global health. *Lancet* **386**, 171–218 (2015).
- Y. Voronin, L. M. Mofenson, C. K. Cunningham, M. G. Fowler, P. Kaleebu, E. J. McFarland, J. T. Safrit, B. S. Graham, W. Snow, HIV monoclonal antibodies: A new opportunity to further reduce mother-to-child HIV transmission. *PLoS Med.* **11**, e1001616 (2014).
- D. H. Barouch, J. B. Whitney, B. Moldt, F. Klein, T. Y. Oliveira, J. Liu, K. E. Stephenson, H.-W. Chang, K. Shekhar, S. Gupta, J. P. Nkolola, M. S. Seaman, K. M. Smith, E. N. Borducchi, C. Cabral, J. Y. Smith, S. Blackmore, S. Sanisetty, J. R. Perry, M. Beck, M. G. Lewis, W. Rinaldi, A. K. Chakraborty, P. Pognard, M. C. Nussenzweig, D. R. Burton, Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* **503**, 224–228 (2013).
- J. A. Horwitz, A. Halper-Stromberg, H. Mouquet, A. D. Gitlin, A. Tretiakova, T. R. Eisenreich, M. Malbec, S. Gravemann, E. Billerbeck, M. Dörner, H. Büning, O. Schwartz, E. Knops, R. Kaiser, M. S. Seaman, J. M. Wilson, C. M. Rice, A. Ploss, P. J. Bjorkman, F. Klein, M. C. Nussenzweig, HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16538–16543 (2013).
- D. L. Bolton, A. Pegu, K. Wang, K. McGinnis, M. Nason, K. Foulds, V. Letukas, S. D. Schmidt, X. Chen, J. P. Todd, J. Lifson, S. Rao, N. L. Michael, M. L. Robb, J. R. Mascola, R. A. Koup, HIV-1 monoclonal antibodies suppress acute SHIV viremia and limit seeding of cell-associated viral reservoirs. *J. Virol.* **JVI02454-15** (2015).
- A. P. West Jr., L. Scharf, J. F. Scheid, F. Klein, P. J. Bjorkman, M. C. Nussenzweig, Structural insights on the role of antibodies in HIV-1 vaccine and therapy. *Cell* **156**, 633–648 (2014).
- D. R. Burton, J. R. Mascola, Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat. Immunol.* **16**, 571–576 (2015).

8. J. Overbaugh, L. Morris, The antibody response against HIV-1. *Cold Spring Harb. Perspect. Med.* **2**, a007039 (2012).
9. C. A. Derdeyn, P. L. Moore, L. Morris, Development of broadly neutralizing antibodies from auto-antigenic neutralizing antibody responses in HIV infection. *Curr. Opin. HIV AIDS* **9**, 210–216 (2014).
10. X. Wu, C. Wang, S. O'Dell, Y. Li, B. F. Keele, Z. Yang, H. Imamichi, N. Doria-Rose, J. A. Hoxie, M. Connors, G. M. Shaw, R. T. Wyatt, J. R. Mascola, Selection pressure on HIV-1 envelope by broadly neutralizing antibodies to the conserved CD4-binding site. *J. Virol.* **86**, 5844–5856 (2012).
11. P. L. Moore, E. S. Gray, C. K. Wibmer, J. N. Bhiman, M. Nonyane, D. J. Sheward, T. Hermanus, S. Bajimaya, N. L. Tumba, M.-R. Abrahams, B. E. Lambson, N. Ranchebe, L. Ping, N. Ngandu, Q. Abdool Karim, S. S. Abdool Karim, R. I. Swanstrom, M. S. Seaman, C. Williamson, L. Morris, Evolution of an HIV glycan-dependent broadly neutralizing antibody epitope through immune escape. *Nat. Med.* **18**, 1688–1692 (2012).
12. D. N. Sather, S. Carbonetti, J. Kehayia, Z. Kraft, I. Mikell, J. F. Scheid, F. Klein, L. Stamatatos, Broadly neutralizing antibodies developed by an HIV-positive elite neutralizer exact a replication fitness cost on the contemporaneous virus. *J. Virol.* **86**, 12676–12685 (2012).
13. C. K. Wibmer, P. L. Moore, L. Morris, HIV broadly neutralizing antibody targets. *Curr. Opin. HIV AIDS* **10**, 135–143 (2015).
14. P. D. Kwong, J. R. Mascola, Human antibodies that neutralize HIV-1: Identification, structures, and B cell ontogenies. *Immunity* **37**, 412–425 (2012).
15. M. Shingai, Y. Nishimura, F. Klein, H. Mouquet, O. K. Donau, R. Plishka, A. Buckler-White, M. Seaman, M. Piatak Jr., J. D. Lifson, D. S. Dimitrov, M. C. Nussenzweig, M. A. Martin, Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* **503**, 277–280 (2013).
16. F. Klein, A. Halper-Stromberg, J. A. Horwitz, G. Gruell, J. F. Scheid, S. Bournazos, H. Mouquet, L. A. Spatz, R. Diskin, A. Abadir, T. Zang, M. Dorner, E. Billerbeck, R. N. Labitt, C. Gaebler, P. M. Marcovecchio, R.-B. Incesu, T. R. Eisenreich, P. D. Bieniasz, M. S. Seaman, P. J. Bjorkman, J. V. Ravetch, A. Ploss, M. C. Nussenzweig, HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* **492**, 118–122 (2012).
17. R. Diskin, F. Klein, J. A. Horwitz, A. Halper-Stromberg, D. N. Sather, P. M. Marcovecchio, T. Lee, A. P. West Jr., H. Gao, M. S. Seaman, L. Stamatatos, M. C. Nussenzweig, P. J. Bjorkman, Restricting HIV-1 pathways for escape using rationally designed anti-HIV-1 antibodies. *J. Exp. Med.* **210**, 1235–1249 (2013).
18. M. Veselinovic, C. P. Neff, L. R. Mulder, R. Akkina, Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. *Virology* **432**, 505–510 (2012).
19. S.-Y. Ko, A. Pegu, R. S. Rudicell, Z.-Y. Yang, M. G. Joyce, X. Chen, K. Wang, S. Bao, T. D. Kraemer, T. Rath, M. Zeng, S. D. Schmidt, J.-P. Todd, S. R. Penzak, K. O. Saunders, M. C. Nason, A. T. Haase, S. S. Rao, R. S. Blumberg, J. R. Mascola, G. J. Nabel, Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* **514**, 642–645 (2014).
20. A. B. Balazs, Y. Ouyang, C. M. Hong, J. Chen, S. M. Nguyen, D. S. Rao, D. S. An, D. Baltimore, Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. *Nat. Med.* **20**, 296–300 (2014).
21. A. B. Balazs, J. Chen, C. M. Hong, D. S. Rao, L. Yang, D. Baltimore, Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature* **481**, 81–84 (2012).
22. A. Pegu, Z.-Y. Yang, J. C. Boyington, L. Wu, S.-Y. Ko, S. D. Schmidt, K. McKee, W.-P. Kong, W. Shi, X. Chen, J.-P. Todd, N. L. Letvin, J. Huang, M. C. Nason, J. A. Hoxie, P. D. Kwong, M. Connors, S. S. Rao, J. R. Mascola, G. J. Nabel, Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. *Sci. Transl. Med.* **6**, 243ra88 (2014).
23. R. S. Rudicell, Y. D. Kwon, S.-Y. Ko, A. Pegu, M. K. Louder, I. S. Georgiev, X. Wu, J. Zhu, J. C. Boyington, X. Chen, W. Shi, Z.-Y. Yang, N. A. Doria-Rose, K. McKee, S. O'Dell, S. D. Schmidt, G.-Y. Chuang, A. Druz, C. Soto, Y. Yang, B. Zhang, T. Zhou, J.-P. Todd, K. E. Lloyd, J. Eudaily, K. E. Roberts, B. R. Donald, R. T. Bailer, J. Ledgerwood; NISC Comparative Sequencing Program, J. C. Mullikin, L. Shapiro, R. A. Koup, B. S. Graham, M. C. Nason, M. Connors, B. F. Haynes, S. S. Rao, M. Roederer, P. D. Kwong, J. R. Mascola, G. J. Nabel, Enhanced potency of a broadly neutralizing HIV-1 antibody in vitro improves protection against lentiviral infection in vivo. *J. Virol.* **88**, 12669–12682 (2014).
24. L. Scharf, J. F. Scheid, J. H. Lee, A. P. West Jr., C. Chen, H. Gao, P. N. P. Gnanapragasam, R. Mares, M. S. Seaman, A. B. Ward, M. C. Nussenzweig, P. J. Bjorkman, Antibody BANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. *Cell Rep.* **7**, 785–795 (2014).
25. J. F. Scheid, H. Mouquet, B. Ueberheide, R. Diskin, F. Klein, T. Y. K. Oliveira, J. Pietzsch, D. Fenyo, A. Abadir, K. Velinzon, A. Hurley, S. Myung, F. Boulad, P. Poignard, D. R. Burton, F. Pereyra, D. D. Ho, B. D. Walker, M. S. Seaman, P. J. Bjorkman, B. T. Chait, M. C. Nussenzweig, Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* **333**, 1633–1637 (2011).
26. X. Wu, Z.-Y. Yang, Y. Li, C.-M. Hegerkorp, W. R. Schief, M. S. Seaman, T. Zhou, S. D. Schmidt, L. Wu, L. Xu, N. S. Longo, K. McKee, S. O'Dell, M. K. Louder, D. L. Wycuff, Y. Feng, M. Nason, N. Doria-Rose, M. Connors, P. D. Kwong, M. Roederer, R. T. Wyatt, G. J. Nabel, J. R. Mascola, Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* **329**, 856–861 (2010).
27. J. Huang, G. Ofek, L. Laub, M. K. Louder, N. A. Doria-Rose, N. S. Longo, H. Imamichi, R. T. Bailer, B. Chakrabarti, S. K. Sharma, S. M. Alam, T. Wang, Y. Yang, B. Zhang, S. A. Migueles, R. Wyatt, B. F. Haynes, P. D. Kwong, J. R. Mascola, M. Connors, Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature* **491**, 406–412 (2012).
28. L. M. Walker, M. Huber, K. J. Doores, E. Falkowska, R. Pejchal, J.-P. Julien, S.-K. Wang, A. Ramos, P.-Y. Chan-Hui, M. Moyle, J. L. Mitcham, P. W. Hammond, O. A. Olsen, P. Phung, S. Fling, C.-H. Wong, S. Phogat, T. Wrin, M. D. Simek; Protocol G Principal Investigators, W. C. Koff, I. A. Wilson, D. R. Burton, P. Poignard, Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* **477**, 466–470 (2011).
29. L. M. Walker, S. K. Phogat, P.-Y. Chan-Hui, D. Wagner, P. Phung, J. L. Goss, T. Wrin, M. D. Simek, S. Fling, J. L. Mitcham, J. K. Lehrman, F. H. Priddy, O. A. Olsen, S. M. Frey, P. W. Hammond; Protocol G Principal Investigators, S. Kaminsky, T. Zamb, M. Moyle, W. C. Koff, P. Poignard, D. R. Burton, Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* **326**, 285–289 (2009).
30. E. Falkowska, K. M. Le, A. Ramos, K. J. Doores, J. H. Lee, C. Blattner, A. Ramirez, R. Derking, M. J. van Gils, C.-H. Liang, R. McBride, B. von Bredow, S. S. Shivatare, C.-Y. Wu, P.-Y. Chan-Hui, Y. Liu, T. Feizi, M. B. Zwick, W. C. Koff, M. S. Seaman, K. Swiderek, J. P. Moore, D. Evans, J. C. Paulson, C.-H. Wong, A. B. Ward, I. A. Wilson, R. W. Sanders, P. Poignard, D. R. Burton, Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. *Immunity* **40**, 657–668 (2014).
31. C. Blattner, J. H. Lee, K. Slipeen, R. Derking, E. Falkowska, A. T. de la Peña, A. Cupo, J.-P. Julien, M. van Gils, P. S. Lee, W. Peng, J. C. Paulson, P. Poignard, D. R. Burton, J. P. Moore, R. W. Sanders, I. A. Wilson, A. B. Ward, Structural delineation of a quaternary, cleavage-dependent epitope at the gp41-gp120 interface on intact HIV-1 Env trimers. *Immunity* **40**, 669–680 (2014).
32. J. Huang, B. H. Kang, M. Pancera, J. H. Lee, T. Tong, Y. Feng, H. Imamichi, I. S. Georgiev, G.-Y. Chuang, A. Druz, N. A. Doria-Rose, L. Laub, K. Slipeen, M. J. van Gils, A. T. de la Peña, R. Derking, P.-J. Klasse, S. A. Migueles, R. T. Bailer, M. Alam, P. Pugach, B. F. Haynes, R. T. Wyatt, R. W. Sanders, J. M. Binley, A. B. Ward, J. R. Mascola, P. D. Kwong, M. Connors, Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp120 interface. *Nature* **515**, 138–142 (2014).
33. T. Zhou, J. Zhu, X. Wu, S. Moquin, B. Zhang, P. Acharya, I. S. Georgiev, H. R. Altae-Tran, G.-Y. Chuang, M. G. Joyce, Y. D. Kwon, N. S. Longo, M. K. Louder, T. Luongo, K. McKee, C. A. Schramm, J. Skinner, Y. Yang, Z. Yang, Z. Zhang, A. Zheng, M. Bonsignori, B. F. Haynes, J. F. Scheid, M. C. Nussenzweig, M. Simek, D. R. Burton, W. C. Koff; NISC Comparative Sequencing Program, J. C. Mullikin, M. Connors, L. Shapiro, G. J. Nabel, J. R. Mascola, P. D. Kwong, Multidonor analysis reveals structural elements, genetic determinants, and maturation pathway for HIV-1 neutralization by VRC01-class antibodies. *Immunity* **39**, 245–258 (2013).
34. X. Wu, T. Zhou, J. Zhu, B. Zhang, I. Georgiev, C. Wang, X. Chen, N. S. Longo, M. Louder, K. McKee, S. O'Dell, S. Peretto, S. D. Schmidt, W. Shi, L. Wu, Y. Yang, Z.-Y. Yang, Z. Yang, Z. Zhang, M. Bonsignori, J. A. Crump, S. H. Kapiga, N. E. Sam, B. F. Haynes, M. Simek, D. R. Burton, W. C. Koff, N. A. Doria-Rose, M. Connors; NISC Comparative Sequencing Program, J. C. Mullikin, G. J. Nabel, M. Roederer, L. Shapiro, P. D. Kwong, J. R. Mascola, Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* **333**, 1593–1602 (2011).
35. T. Zhou, I. Georgiev, X. Wu, Z.-Y. Yang, K. Dai, A. Finzi, Y. D. Kwon, J. F. Scheid, W. Shi, L. Xu, Y. Yang, J. Zhu, M. C. Nussenzweig, J. Sodroski, L. Shapiro, G. J. Nabel, J. R. Mascola, P. D. Kwong, Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science* **329**, 811–817 (2010).
36. A. P. West Jr., R. Diskin, M. C. Nussenzweig, P. J. Bjorkman, Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E2083–E2090 (2012).
37. R. Diskin, J. F. Scheid, P. M. Marcovecchio, A. P. West Jr., F. Klein, H. Gao, P. N. P. Gnanapragasam, A. Abadir, M. S. Seaman, M. C. Nussenzweig, P. J. Bjorkman, Increasing the potency and breadth of an HIV antibody by using structure-based rational design. *Science* **334**, 1289–1293 (2011).
38. Y. Li, S. O'Dell, L. M. Walker, X. Wu, J. Guenaga, Y. Feng, S. D. Schmidt, K. McKee, M. K. Louder, J. E. Ledgerwood, B. S. Graham, B. F. Haynes, D. R. Burton, R. T. Wyatt, J. R. Mascola, Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. *J. Virol.* **85**, 8954–8967 (2011).
39. T. Zhou, R. M. Lynch, L. Chen, P. Acharya, X. Wu, N. A. Doria-Rose, M. G. Joyce, D. Lingwood, C. Soto, R. T. Bailer, M. J. Erandes, R. Kong, N. S. Longo, M. K. Louder, K. McKee, S. O'Dell, S. D. Schmidt, L. Tran, Z. Yang, A. Druz, T. S. Luongo, S. Moquin, S. Srivatsan, Y. Yang, B. Zhang, A. Zheng, M. Pancera, T. Kirys, I. S. Georgiev, T. Gindin, H.-P. Peng, A.-S. Yang; NISC Comparative Sequencing Program, J. C. Mullikin, M. D. Gray, L. Stamatatos, D. R. Burton, W. C. Koff, M. S. Cohen, B. F. Haynes, J. P. Casazza, M. Connors, D. Corti, A. Lanzavecchia, Q. J. Sattantau, R. A. Weiss, A. P. West Jr., P. J. Bjorkman, J. F. Scheid, M. C. Nussenzweig, L. Shapiro, J. R. Mascola, P. D. Kwong, Structural repertoire of HIV-1-neutralizing antibodies targeting the CD4 supersite in 14 donors. *Cell* **161**, 1280–1292 (2015).

40. C. Armbruster, G. M. Stiegler, B. A. Vcelar, W. Jäger, N. L. Michael, N. Vetter, H. W. D. Katinger, A phase I trial with two human monoclonal antibodies (hMAb 2F5, 2G12) against HIV-1. *AIDS* **16**, 227–233 (2002).
41. G. Stiegler, C. Armbruster, B. Vcelar, H. Stoiber, R. Kunert, N. L. Michael, L. L. Jagodzinski, C. Ammann, W. Jäger, J. Jacobson, N. Vetter, H. Katinger, Antiviral activity of the neutralizing antibodies 2F5 and 2G12 in asymptomatic HIV-1-infected humans: A phase I evaluation. *AIDS* **16**, 2019–2025 (2002).
42. A. Trkola, H. Kuster, P. Rusert, B. Joos, M. Fischer, C. Leemann, A. Manrique, M. Huber, M. Rehr, A. Oxenius, R. Weber, G. Stiegler, B. Vcelar, H. Katinger, L. Aceto, H. F. Günthard, Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat. Med.* **11**, 615–622 (2005).
43. S. Mehandru, B. Vcelar, T. Wrin, G. Stiegler, B. Joos, H. Mohri, D. Boden, J. Galovich, K. Tenner-Racz, P. Racz, M. Carrington, C. Petropoulos, H. Katinger, M. Markowitz, Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection. *J. Virol.* **81**, 11016–11031 (2007).
44. M. Caskey, F. Klein, J. C. C. Lorenzi, M. S. Seaman, A. P. West Jr., N. Buckley, G. Kremer, L. Nogueira, M. Braunschweig, J. F. Scheid, J. A. Horwitz, I. Shmeliovich, S. Ben-Avraham, M. Witmer-Pack, M. Platten, C. Lehmann, L. A. Burke, T. Hawthorne, R. J. Gorelick, B. D. Walker, T. Keler, R. M. Gulick, G. Fätkenheuer, S. J. Schlesinger, M. C. Nussenzweig, Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* **522**, 487–491 (2015).
45. J. E. Ledgerwood, E. E. Coates, G. Yamshchikov, J. G. Saunders, L. Holman, M. E. Enama, A. DeZure, R. M. Lynch, I. Gordon, S. Plummer, C. S. Hendel, A. Pegu, M. Conan-Cibotti, S. Sitar, R. T. Bailer, S. Narpala, A. McDermott, M. Louder, S. O'Dell, S. Mohan, J. P. Pandey, R. M. Schwartz, Z. Hu, R. A. Koup, E. Capparelli, J. R. Mascola, B. S. Graham; VRC 602 Study Team, Safety, pharmacokinetics, and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin. Exp. Immunol.* **182**, 289–301 (2015).
46. S. A. Yukl, E. Boritz, M. Busch, C. Bentsen, T.-W. Chun, D. Douek, E. Eisele, A. Haase, Y.-C. Ho, G. Hütter, J. S. Justement, S. Keating, T.-H. Lee, P. Li, D. Murray, S. Palmer, C. Pilcher, S. Pillai, R. W. Price, M. Rothenberger, T. Schacker, J. Siliciano, R. Siliciano, E. Sinclair, M. Strain, J. Wong, D. Richman, S. G. Deeks, Challenges in detecting HIV persistence during potentially curative interventions: A study of the Berlin patient. *PLOS Pathog.* **9**, e1003347 (2013).
47. G.-Y. Chuang, D. Liou, P. D. Kwong, I. S. Georgiev, NEP: Web server for epitope prediction based on antibody neutralization of viral strains with diverse sequences. *Nucleic Acids Res.* **42**, W64–W71 (2014).
48. G.-Y. Chuang, P. Acharya, S. D. Schmidt, Y. Yang, M. K. Louder, T. Zhou, Y. D. Kwon, M. Pancera, R. T. Bailer, N. A. Doria-Rose, M. C. Nussenzweig, J. R. Mascola, P. D. Kwong, I. S. Georgiev, Residue-level prediction of HIV-1 antibody epitopes based on neutralization of diverse viral strains. *J. Virol.* **87**, 10047–10058 (2013).
49. J. R. Mascola, Defining the protective antibody response for HIV-1. *Curr. Mol. Med.* **3**, 209–216 (2003).
50. B. Moldt, E. G. Rakasz, N. Schultz, P.-Y. Chan-Hui, K. Swiderek, K. L. Weisgrau, S. M. Piaskowski, Z. Bergman, D. I. Watkins, P. Poignard, D. R. Burton, Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 18921–18925 (2012).
51. M. Shingai, O. K. Donau, R. J. Plishka, A. Buckler-White, J. R. Mascola, G. J. Nabel, M. C. Nason, D. Montefiori, B. Moldt, P. Poignard, R. Diskin, P. J. Bjorkman, M. A. Eckhaus, F. Klein, H. Mouquet, J. C. Cetrulo Lorenzi, A. Gazumyan, D. R. Burton, M. C. Nussenzweig, M. A. Martin, Y. Nishimura, Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. *J. Exp. Med.* **211**, 2061–2074 (2014).
52. D. R. Burton, A. J. Hessel, B. F. Keele, P. J. Klasse, T. A. Ketas, B. Moldt, D. C. Dunlop, P. Poignard, L. A. Doyle, L. Cavacini, R. S. Veazey, J. P. Moore, Limited or no protection by weakly or non-neutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11181–11186 (2011).
53. P. W. H. I. Parren, P. A. Marx, A. J. Hessel, A. Luckay, J. Harouse, C. Cheng-Mayer, J. P. Moore, D. R. Burton, Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J. Virol.* **75**, 8340–8347 (2001).
54. J. R. Mascola, M. G. Lewis, G. Stiegler, D. Harris, T. C. VanCott, D. Hayes, M. K. Louder, C. R. Brown, C. V. Sapan, S. S. Frankel, Y. Lu, M. L. Robb, H. Katinger, D. L. Birx, Protection of macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J. Virol.* **73**, 4009–4018 (1999).
55. A. J. Hessel, P. Poignard, M. Hunter, L. Hangartner, D. M. Tehrani, W. K. Bleeker, P. W. H. I. Parren, P. A. Marx, D. R. Burton, Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat. Med.* **15**, 951–954 (2009).
56. N. M. Archin, J. M. Sung, C. Garrido, N. Soriano-Sarabia, D. M. Margolis, Eradicating HIV-1 infection: Seeking to clear a persistent pathogen. *Nat. Rev. Microbiol.* **12**, 750–764 (2014).
57. A. M. Spivak, A. Andrade, E. Eisele, R. Hoh, P. Bacchetti, N. N. Bumpus, F. Emad, R. Buckheit III, E. F. McCance-Katz, J. Lai, M. Kennedy, G. Chander, R. F. Siliciano, J. D. Siliciano, S. G. Deeks, A pilot study assessing the safety and latency-reversing activity of disulfiram in HIV-1-infected adults on antiretroviral therapy. *Clin. Infect. Dis.* **58**, 883–890 (2014).
58. M. Somsouk, R. M. Dunham, M. Cohen, R. Albright, M. Abdel-Mohsen, T. Liegler, J. Lifson, M. Piatak, R. Gorelick, Y. Huang, Y. Wu, P. Y. Hsue, J. N. Martin, S. G. Deeks, J. M. McCune, P. W. Hunt, The immunologic effects of mesalamine in treated HIV-infected individuals with incomplete CD4+ T cell recovery: A randomized crossover trial. *PLOS One* **9**, e116306 (2014).
59. C. Vanderveeten, R. Fromentin, E. Merlini, M. B. Lawani, S. DaFonseca, W. Bakeman, A. McNulty, M. Ramgopal, N. Michael, J. H. Kim, J. Ananworanich, N. Chomont, Cross-clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *J. Virol.* **88**, 12385–12396 (2014).
60. F. A. Procopio, R. Fromentin, D. A. Kulpa, J. H. Brehm, A.-G. Bebin, M. C. Strain, D. D. Richman, U. O'Doherty, S. Palmer, F. M. Hecht, R. Hoh, R. J. O. Barnard, M. D. Miller, D. J. Hazuda, S. G. Deeks, R.-P. Sékaly, N. Chomont, A novel assay to measure the magnitude of the inducible viral reservoir in HIV-infected individuals. *EBioMedicine* **2**, 874–883 (2015).
61. A. O. Pasternak, K. W. Adema, M. Bakker, S. Jurriaans, B. Berkhout, M. Cornelissen, V. V. Lukashov, Highly sensitive methods based on seminested real-time reverse transcription-PCR for quantitation of human immunodeficiency virus type 1 unspliced and multiply spliced RNA and proviral DNA. *J. Clin. Microbiol.* **46**, 2206–2211 (2008).
62. T.-W. Chun, D. Engel, S. B. Mizell, C. W. Hallahan, M. Fischette, S. Park, R. T. Davey Jr., M. Dybul, J. A. Kovacs, J. A. Metcalf, J. M. Mican, M. M. Berrey, L. Corey, H. C. Lane, A. S. Fauci, Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. *Nat. Med.* **5**, 651–655 (1999).
63. J. F. Salazar-Gonzalez, E. Bailes, K. T. Pham, M. G. Salazar, M. B. Guffey, B. F. Keele, C. A. Derdeyn, P. Farmer, E. Hunter, S. Allen, O. Manigart, J. M. Mulenga, J. A. Anderson, R. Swanstrom, B. F. Haynes, G. S. Athreya, B. T. M. Korber, P. M. Sharp, G. M. Shaw, B. H. Hahn, Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. *J. Virol.* **82**, 3952–3970 (2008).
64. B. F. Keele, E. E. Giorgi, J. F. Salazar-Gonzalez, J. M. Decker, K. T. Pham, M. G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J. L. Kirchherr, F. Gao, J. A. Anderson, L.-H. Ping, R. Swanstrom, G. D. Tomaras, W. A. Blattner, P. A. Goepfert, J. M. Kilby, M. S. Saag, E. L. Delwart, M. P. Busch, M. S. Cohen, D. C. Montefiori, B. F. Haynes, B. Gaschen, G. S. Athreya, H. Y. Lee, N. Wood, C. Seoighe, A. S. Perelson, T. Bhattacharya, B. T. Korber, B. H. Hahn, G. M. Shaw, Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 7552–7557 (2008).
65. M. Li, F. Gao, J. R. Mascola, L. Stamatatos, V. R. Polonis, M. Koutsoukos, G. Voss, P. Goepfert, P. Gilbert, K. M. Greene, M. Bilska, D. L. Kothe, J. F. Salazar-Gonzalez, X. Wei, J. M. Decker, B. H. Hahn, D. C. Montefiori, Human immunodeficiency virus type 1 *env* clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J. Virol.* **79**, 10108–10125 (2005).
66. D. C. Douek, J. M. Brenchley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto, J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D. A. Price, M. Connors, R. A. Koup, HIV preferentially infects HIV-specific CD4+ T cells. *Nature* **417**, 95–98 (2002).
67. M. Kearse, R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, A. Drummond, Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
68. J. P. Pandey, N. Kaur, S. Costa, J. Amorim, R. Nabico, P. Linhares, R. Vaz, M. Viana-Pereira, R. M. Reis, Immunoglobulin genes implicated in glioma risk. *Oncimmunology* **3**, e28609 (2014).

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Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection

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Editor's Summary

Passive aggression for HIV

Antibodies that neutralize HIV could add to the therapeutic arsenal to prevent and treat disease. Lynch *et al.* have now tested one such antibody—VRC01—in HIV-infected individuals. Although little difference was observed in viral reservoir in individuals on antiretroviral therapy, plasma viremia was reduced in untreated subjects with a single infusion of VRC01, preferentially suppressing neutralization-sensitive strains. Passive immunization with neutralizing antibodies could therefore aid in viral suppression in HIV-infected individuals.

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