Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption


BACKGROUND
The discovery of potent and broadly neutralizing antibodies (bNAbs) against human immunodeficiency virus (HIV) has made passive immunization a potential strategy for the prevention and treatment of HIV infection. We sought to determine whether passive administration of VRC01, a bNAb targeting the HIV CD4-binding site, can safely prevent or delay plasma viral rebound after the discontinuation of antiretroviral therapy (ART).

METHODS
We conducted two open-label trials (AIDS Clinical Trials Group [ACTG] A5340 and National Institutes of Health [NIH] 15-I-0140) of the safety, side-effect profile, pharmacokinetic properties, and antiviral activity of VRC01 in persons with HIV infection who were undergoing interruption of ART.

RESULTS
A total of 24 participants were enrolled, and one serious alcohol-related adverse event occurred. Viral rebound occurred despite plasma VRC01 concentrations greater than 50 μg per milliliter. The median time to rebound was 4 weeks in the A5340 trial and 5.6 weeks in the NIH trial. Study participants were more likely than historical controls to have viral suppression at week 4 (38% vs. 13%, P=0.04 by a two-sided Fisher’s exact test in the A5340 trial; and 80% vs. 13%, P<0.001 by a two-sided Fisher’s exact test in the NIH trial) but the difference was not significant at week 8. Analyses of virus populations before ART as well as before and after ART interruption showed that VRC01 exerted pressure on rebounding virus, resulting in restriction of recrudescent viruses and selection for preexisting and emerging antibody neutralization–resistant virus.

CONCLUSIONS
VRC01 slightly delayed plasma viral rebound in the trial participants, as compared with historical controls, but it did not maintain viral suppression by week 8. In the small number of participants enrolled in these trials, no safety concerns were identified with passive immunization with a single bNAb (VRC01). (Funded by the National Institute of Allergy and Infectious Diseases and others; ACTG A5340 and NIH 15-I-0140 ClinicalTrials.gov numbers, NCT02463227 and NCT02471326.)
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THERAPEUTIC ADMINISTRATION OF MONOCLONAL ANTIBODIES has revolutionized treatment options in oncology, rheumatology, endocrinology, gastroenterology, neurology, and the field of infectious diseases.1,2 The use of broadly neutralizing antibodies (bNAbs) against human immunodeficiency virus (HIV) is a potential approach to the prevention of HIV infection and its therapy and cure.3,4 VRC01 is a bNAb that targets the CD4-binding site of the HIV envelope glycoprotein. VRC01 has been shown to neutralize approximately 90% of a broad panel of 190 group M HIV envelope pseudotyped viruses with a mean 50% inhibitory concentration (IC50) of 0.33 µg per milliliter.5 Passive administration of bNAbs, including VRC01, has been shown to prevent HIV transmission in animal models6,9 and is now being tested in clinical trials of vertical and horizontal transmission in humans.

Combination antiretroviral therapy (ART) potentially suppresses HIV replication; however, it does not eradicate the persistent viral reservoir.10 In most HIV-infected persons, plasma viral rebound predictably occurs within days after treatment interruption.11-14 HIV-specific bNAbs hold potential advantages over current ART. First, bNAbs can be administered as long-acting agents by means of antibody engineering6 or vectored delivery.15,16 Second, unlike classic ART, antibody Fc effector functions enable the killing of HIV-infected cells, which may assist in the clearance of the persistent viral reservoir.4,17 Finally, bNAbs engage the host immune system and may augment antiviral responses.18,19 Preclinical studies of single and combination bNAbs in animal models have shown virus suppression, enhanced viral killing, augmented anti-HIV immune responses, and reduction of the cellular reservoir.18,20,21 In clinical trials involving humans, no safety concerns have been identified thus far with passive administration of bNAbs targeting the CD4-binding site in healthy uninfected persons and in participants with chronic HIV infection who have either viremia or viral suppression.22-24 Passive administration of VRC01 to HIV viremic persons led to a reduction of 1.1 to 1.8 log10 in plasma HIV viremia, although it was ineffective in the quarter of study participants who had baseline resistance to the antibody.24 In addition, we previously found that HIV isolated from the latent viral reservoir of many, but not all, infected persons was inhibited by VRC01 ex vivo.17 Collectively, these findings suggest that passive immunotherapy with VRC01 could potentially prevent plasma viral rebound in HIV-infected persons after analytic treatment interruption.

Here, we report the results of two phase 1 clinical trials designed to investigate the feasibility of achieving sustained suppression of plasma viremia (virologic remission) in HIV-infected persons by means of multiple infusions of VRC01 after the discontinuation of ART that had been successfully suppressing plasma viremia below the detectable concentration. Our goals were to ascertain whether the passive administration of VRC01 is safe and has an acceptable adverse-event and side-effect profile, leads to high VRC01 plasma concentrations, can suppress plasma viremia after the discontinuation of ART, and could inform our understanding of recrudescent viruses after immunologic intervention.

STUDY DESIGN AND OVERSIGHT
We conducted two clinical trials with similar designs to evaluate the safety, adverse-event and side-effect profile, pharmacokinetic characteristics, and antiviral activity of the human monoclonal antibody VRC-HIVMAB060-00-AB (VRC01) in HIV-infected persons who were undergoing analytic treatment interruption. The first trial, AIDS Clinical Trials Group (ACTG) A5340, was conducted at the clinical research sites of the University of Pennsylvania and University of Alabama (see the protocol, available with the full text of this article at NEJM.org). The second trial (NIH 15-I-0140, hereafter referred to as the National Institutes of Health [NIH] trial) was conducted at the National Institute of Allergy and Infectious Diseases, NIH, in Bethesda, Maryland (see the protocol).

Both trials had similar entry criteria and recruited participants who had chronic HIV infection with fully suppressed plasma viremia while receiving ART (details are provided in the Supplementary Methods section in the Supplementary Appendix, available at NEJM.org). Study participants were not prescreened for sensitivity of the virus to neutralization by VRC01 in either trial.

The trials were reviewed by the institutional review boards of each participating institution. All participants provided written informed consent. All the authors vouch for the accuracy and completeness of the data and analyses and the fidelity of the trial to the respective protocol. There was no commercial support for these trials.
Effect of HIV Antibody VRC01 on Viral Rebound

Treatment Procedures, Study Objectives, and Study Outcomes

In step 1 of the A5340 trial, 14 participants received up to three doses of VRC01 (40 mg per kilogram of body weight) administered intravenously at 3-week intervals (Fig. 1). One week after the first dose of VRC01, participants discontinued ART and were followed at weekly intervals until they had a confirmed CD4 T-cell count of less than 350 cells per cubic millimeter or a return of HIV viremia, which was defined as an HIV RNA level of 200 copies or more per milliliter followed by a confirmation level of 1000 copies or more per milliliter or three consecutive measurements of 200 copies or more per milliliter. On confirmation of viral rebound or a decrease in CD4 T cells, participants entered step 2, at which point ART was reinitiated and participants were followed until the HIV viral load was less than 50 copies per milliliter.

The primary objectives of the A5340 trial were to assess the safety and side-effect profile of multiple doses of VRC01 administered to persons with plasma viremia suppressed to below detectable levels and to estimate the proportion of participants with a return of viremia in the presence of high plasma levels of VRC01 at 8 weeks of analytic treatment interruption. Secondary objectives were the frequency of rebound viremia at 4 weeks and the evaluation of the pharmacokinetic characteristics of the product. Key exploratory objectives were the frequency of development of antibodies against VRC01 and the genetic and phenotypic characterization of the rebound virus.

In the NIH trial, 10 participants received infusions of VRC01 (at a dose of 40 kg per kilogram) intravenously 3 days before and 14 to 28 days after discontinuation of ART, and monthly thereafter. Infectious viral isolates (orange circles) were generated from samples obtained before VRC01 infusion and after plasma viral rebound. HIV env sequencing (gray circles) was performed with the use of plasma samples obtained before the initiation of ART and after viral rebound.

Figure 1. Trial Designs.

As shown in Panel A, the A5340 trial had two steps. In step 1, participants received an intravenous infusion of VRC01 (at a dose of 40 mg per kilogram of body weight) (triangles) 1 week before and 2 and 5 weeks after discontinuation of antiretroviral therapy (ART). ART was discontinued 1 week after the first VRC01 infusion. The treatment interruption was open-ended, and participants were monitored weekly until viral rebound. On confirmed plasma viral rebound, participants entered step 2 and ART was reinitiated. Participants were then followed until plasma viremia decreased below 50 copies per milliliter. HIV env sequencing (gray circles) was performed with the use of plasma samples obtained before the initiation of ART and after viral rebound. As shown in Panel B, in the National Institutes of Health (NIH) trial, participants received infusions of VRC01 (at a dose of 40 kg per kilogram) (triangles) 3 days before and 14 to 28 days after discontinuation of ART, and monthly thereafter. Infectious viral isolates (orange circles) were generated from samples obtained before VRC01 infusion and after plasma viral rebound. HIV env sequencing (gray circles) was performed with the use of plasma samples obtained before the initiation of ART and after viral rebound.
Table 1. Characteristics of the Participants at Baseline.†

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AS340 Trial (N = 14)</th>
<th>NIH Trial (N = 10)</th>
<th>Historical Controls from Previous ACTG Studies (N = 61)</th>
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<td>0</td>
<td>6 (10)</td>
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Effect of HIV Antibody VRC01 on Viral Rebound after the discontinuation of ART and monthly thereafter (Fig. 1). Plasma viremia and CD4 T-cell counts were measured at baseline (day −3) and subsequently (Fig. 1). Participants who met any of the following criteria discontinued VRC01 infusions and resumed ART: a decrease of more than 30% in the baseline CD4 T-cell count or an absolute CD4 T-cell count below 350 cells per cubic millimeter, a sustained (≥2 weeks) HIV plasma viremia greater than 1000 copies per milliliter, any HIV-related symptoms, or pregnancy.

The primary end point of the NIH trial was safety, as defined according to the rate of occurrence of grade 3 or higher adverse events, including serious adverse events, that were possibly related to infusion of VRC01. The secondary end point was virologic efficacy, as defined according to the number of participants who met protocol-defined, virologic, immunologic, or clinical criteria to discontinue VRC01 infusions and restart ART. Post hoc analyses of the sequence diversity at the time of plasma viral rebound and the neutralization capacity of VRC01 and other bNAbs against autologous HIV before and after antibody infusions were performed.

**Statistical Analysis**

Statistical analysis of the time to the first confirmed HIV RNA level of 200 copies or more per milliliter during the analytic treatment interruption was performed in both trials (post hoc in the NIH trial) with the percentage of historical controls with viral rebound in previous treatment-interruption trials conducted by the ACTG. In the A5340 trial, the proportion of participants with adverse events was estimated with an exact 95% confidence interval. The proportion of participants who had return of viremia at 8 weeks of analytic treatment interruption and who had data that could be evaluated was estimated with a prespecified exact 90% confidence interval. Details of the historical control group, participant monitoring, and laboratory and statistical methods are outlined in the Supplementary Methods section in the Supplementary Appendix.

**Results**

**Study Participants**

The A5340 trial enrolled 14 participants, all of whom were male, with a median CD4 T-cell count at enrollment of 896 cells per cubic millimeter (interquartile range, 579 to 1053) and a median duration from the initiation of ART to study entry of 4.7 years (interquartile range, 3.8 to 6.0). One participant was excluded from the analyses of time to viral rebound because he discontinued ART before the administration of VRC01.

The NIH trial enrolled 10 participants (8 men and 2 women) with a median CD4 T-cell count of 724 cells per cubic millimeter (interquartile range, 579 to 1053) and a median duration from the initiation of ART to study entry of 4.7 years (interquartile range, 3.8 to 6.0). One participant was excluded from the analyses of time to viral rebound because he discontinued ART before the administration of VRC01.

Table 1 lists the baseline charac-

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Table 1. (Continued.)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A5340 Trial (N = 14)</th>
<th>NIH Trial (N = 10)</th>
<th>Historical Controls from Previous ACTG Studies (N = 61)</th>
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<td>Stavudine–didanosine–nelfinavir</td>
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<td>Other protease inhibitor–based regimen</td>
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*ACTG denotes AIDS Clinical Trials Group, ART antiretroviral therapy, HIV human immunodeficiency virus, IQR interquartile range, NA not available, and NIH National Institutes of Health.
†Race or ethnic group was self-reported.
or before week 8 (92%; 90% CI, 68 to 100), with a median time to rebound of 4 weeks (interquartile range, 3 to 5) (Fig. 2A, and Fig. S3 in the Supplementary Appendix). This is a small delay in viral rebound as compared with the delay in historical controls from previous ACTG studies; 38% of the participants versus 13% of the controls had viral suppression at week 4 (P = 0.04 by a two-sided Fisher’s exact test) and 8% and 3%, respectively, had viral suppression at week 8 (P = 0.44 by a two-sided Fisher’s exact test) (Fig. 2C). One participant (Participant A07) in the A5340 trial had prolonged viral suppression, and detectable plasma viremia developed at week 11 of the analytic treatment interruption when plasma VRC01 levels had waned significantly (plasma VRC01 concentration, 25 μg per milliliter) (Fig. 2A, and Fig. S3 in the Supplementary Appendix). One participant (Participant A11) was excluded from the evaluation of time to viral rebound, since he had detectable plasma viremia at the time of VRC01 infusion (Fig. S3 in the Supplementary Appendix).

In the NIH trial, viral rebound to more than 40 copies per milliliter occurred during VRC01 treatment in all 10 participants, with a median time to rebound of 39 days (interquartile range, 29 to 39) or 5.6 weeks (interquartile range, 4.1 to 5.6) (Fig. 2B, and Fig. S5 in the Supplementary Appendix). However, as compared with the time to plasma viral rebound (HIV RNA level, ≥200 copies per milliliter) in historical controls, VRC01 infusion led to a longer time to rebound (≥200 copies per milliliter), and 80% of the participants versus 13% of the controls had viral suppression at week 4 (P < 0.001 by a two-sided Fisher’s exact test) and 10% and 3%, respectively, had viral suppression at week 8 (P = 0.37 by a two-sided Fisher’s exact test) (Fig. 2C). Nine of 10 study participants resumed ART because of virologic failure; ART was reinitiated in the remaining participant (Participant N03) because of a significant decrease in the CD4 T-cell count associated with low-level plasma viremia (HIV RNA level, 471 copies per milliliter). One participant (Participant N04) self-administered antiretroviral drugs for 3 days off protocol after the first ART interruption; self-administration of antiretroviral drugs may have contributed to a brief period of aviremia (Fig. S5 in the Supplementary Appendix). However, his plasma
viremia rebounded shortly after the second ART interruption.

**VRC01 PHARMACOKINETIC CHARACTERISTICS**
Plasma levels of VRC01 that were achieved by passive infusion were similar to levels reported in previous trials.23,24 In the A5340 trial, participants received 40 mg of VRC01 per kilogram every 3 weeks for three doses and maintained measured plasma VRC01 levels well above 50 μg per milliliter for 8 weeks (median, 175 μg per milliliter; range, 68 to 1494) (Fig. S6 in the Supplementary Appendix).

In the NIH trial, participants received 40 mg of VRC01 per kilogram every 3 weeks for three doses and maintained measured plasma VRC01 levels well above 50 μg per milliliter for 8 weeks (median, 175 μg per milliliter; range, 68 to 1494) (Fig. S6 in the Supplementary Appendix).

Figure 2. Plasma Viremia and Levels of VRC01 in Trial Participants after Discontinuation of ART.
Panel A shows the plasma viremia of participants in the A5340 trial after the interruption of therapy. The gray dotted line indicates the limit of detection of the assay (HIV RNA level, 20 copies per milliliter). Panel B shows the plasma viremia of NIH trial participants after interruption of therapy. The gray dotted line indicates the limit of detection of the assay (HIV RNA level, 40 copies per milliliter). Panel C shows the Kaplan–Meier curve of plasma viral suppression (<200 copies per milliliter) after VRC01 administration and analytic treatment interruption in A5340 and NIH trial participants as compared with historical participants in AIDS Clinical Trials Group (ACTG) trials who underwent interruption of therapy without other immunotherapeutic interventions. Panel D shows in vivo plasma levels of VRC01 at the first detectable plasma viremia. The limit of detection of VRC01 was less than 0.98 μg per milliliter.
VRC01 per kilogram every 2 weeks for the first three doses, then monthly for up to 6 months. NIH trial participants maintained levels of VRC01 in plasma above 100 μg per milliliter at almost all time points throughout the trial (Fig. S5 in the Supplementary Appendix).

Measured values of plasma VRC01 at the time of rebound were greater than 50 μg per milliliter in all trial participants, except for Participant A07 in the A5340 trial, who had delayed rebound at week 11 of analytic treatment interruption with VRC01 levels of approximately 25 μg per milliliter (Fig. 2D). No anti-VRC01 antibodies were identified in any trial participants.

**SEQUENCE EVIDENCE OF VRC01-MEDIATED VIRUS RESTRICTION**

Different strategies were applied in each trial to elucidate the mechanisms leading to early viral rebound. To characterize rebounding viral populations in the A5340 trial, single-genome sequencing of plasma HIV env genes from pre-ART plasma samples (available in 8 participants) and rebound plasma samples from the first and second weeks of detectable viremia (available in 13 participants) was performed. When analyzed together in a maximum likelihood phylogenetic tree, the pre-ART and rebound sequences of the 13 participants with data that could be evaluated clustered independently, indicating the relatedness of pre-ART and rebound viruses (Fig. S7 in the Supplementary Appendix).

Three independent studies have recently shown that without any additional intervention besides ART, viral rebound after analytic treatment interruption is consistently polyclonal because of the reactivation of multiple latent viruses. Genetic evidence of VRC01-mediated restriction of viral rebound was assessed by analyzing the clonality of rebound virus or by enumerating genetically distinct virus populations that composed rebound viremia. In 3 of 12 participants (25%) in the A5340 trial who had viral rebound in the presence of high concentrations of VRC01, sequence evidence suggested VRC01-mediated restriction of viral rebound. As shown in Participant A04 in Figure 3A and Participants A02 and A12 in Figure S8 in the Supplementary Appendix, pre-ART plasma virus from persons with chronic infection formed characteristic diverse trees, whereas rebound virus clustered into single low-diversity lineages of nearly identical sequences (Figs. S9 and S10 in the Supplementary Appendix).

The remaining 9 of 12 participants (75%) had polyclonal rebound akin to what is reported in historical analytic treatment interruption without intervention, suggesting possible preexisting resistance. As shown in Figure 3B, rebound virus in Participant A05 clustered into multiple genetically distinct rebound lineages that align throughout the pre-ART virus phylogeny, whereas multiple rebound lineages in Participant A03 clustered unevenly within the pre-ART virus population (Fig. 3C). The rebound sequences in Participant
EFFECT OF HIV ANTIBODY VRC01 ON VIRAL REBOUND

A Participant A04

B Participant A05

C Participant A03

D Participant A07

E IC50 Virus Neutralization Titers

F IC50 Virus Neutralization Titers

Participants

IC50 (µg/ml)

Before ART Rebound wk 1 Rebound wk 2

0.005

0.005

0.005

Before/uni0020ART Rebound

0.01

0.1

1

10

100

IC50/uni0020 (µg/ml)

IC80/uni0020 (µg/ml)

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Evidence of selective pressure exerted by VRC01 was also explored at the molecular level in the NIH trial by amplifying env genes by means of single-genome sequencing from pre-ART (a median of 1.8 months before the initiation of ART) and rebound plasma samples. As in the A5340 trial, the majority of NIH trial participants had phylogenetic evidence of multiple viral lineages in rebound plasma virus (Fig. S11 in the Supplementary Appendix). Specific amino acid changes within the VRC01 antibody-binding site were examined with the use of a neutralization-based epitope prediction algorithm.30 In four of the six NIH trial participants, changes were identified in or near the VRC01 epitope, mainly in the V5 loop and the CD4-binding loop (Fig. S12 in the Supplementary Appendix); this outcome suggested VRC01-mediated selective pressure on rebounding virus. Similar patterns were seen in the VRC01-binding site in participants in the A5340 trial (Fig. S10 in the Supplementary Appendix).

**SELECTION FOR VRC01-RESISTANT REBOUNDING VIRUSES**

The role of resistance to VRC01 at viral rebound in the presence of high VRC01 concentrations in the A5340 trial was assessed by cloning selected env genes from 46 quasi-species and lineages collected throughout the pre-ART and rebound periods of the trial (median, 3 pre-ART and 3 rebound env genes per participant). These env genes were cloned, expressed as pseudoviruses, and tested for sensitivity to neutralization by VRC01. Notably, nearly all participants who had viral rebound early with high concentrations of plasma VRC01 had rebound Env pseudoviruses with IC50 neutralization titers higher than 1 μg per milliliter (median, 4.1 μg per milliliter; range, 1.9 to >50.0), conferring what is generally perceived to be at least moderate resistance to VRC01.5,31,32 Only Participants A02 and A07, who had rebound at week 8 and 11 after analytic treatment interruption, respectively, had rebound viruses with IC50 neutralization titers below 1 μg per milliliter (Fig. 3E).

Similarly, all participants who had viral rebound early had preexisting resistant virus as either dominant or minor populations in the pre-ART virus, as shown in the phylogenetic trees of the four participants in Figure 3A through 3D. The prevalence of preexisting resistance predicted the pattern of rebound. In participants with VRC01-resistant virus in multiple pre-ART variants (e.g., Participants A05, A06, and A09) (Fig. 3, and Fig. S8 in the Supplementary Appendix), VRC01 therapy was followed by rapid, polyclonal rebound with highly resistant virus. In participants in whom there was a range of neutralization sensitivities in the baseline virus (e.g., Participants A03 and A04 [Fig. 3] and Participant A12 [Fig. S8 in the Supplementary Appendix]), VRC01 therapy led to monoclonal or compartmentalized rebound after variable durations of suppression (range, 2 to 6 weeks). Finally, Participants A02 and A07, who had highly sensitive virus throughout their tested pre-ART populations, had suppression maintained for 7 and 10 weeks, respectively, and had rebound with relatively sensitive monoclonal or oligoclonal virus.

Regardless of the time to rebound, resistance to VRC01 increased almost universally in participants in the A5340 trial during treatment with VRC01. In an exploratory analysis, pre-ART and rebound Env pseudoviruses were compared in the eight participants in whom both samples were available. This analysis showed significantly increased VRC01 resistance at rebound by IC50 (mean increase by a factor of 3.44, P = 0.006 by a two-sided random-effects model) and IC80 (mean increase by a factor of 3.79, P = 0.004 by a two-sided random-effects model) (Fig. 3E and 3F, and Fig. S13 in the Supplementary Appendix).

**NEUTRALIZATION CAPACITY OF VRC01 AND OTHER bNABS AGAINST AUTOLOGOUS HIV BEFORE AND AFTER ANTIBODY INFUSIONS**

In the NIH trial, the role of resistance to VRC01 and other bNAbS was explored by testing fully replication-competent autologous HIV isolates recovered from the CD4 T cells of trial participants before and after antibody infusions. Multiple viral isolates (182 total) were generated from peripheral-blood mononuclear cells obtained from trial participants before infusions of VRC01 (eight participants, 75 isolates) and after infu-
Effect of HIV Antibody VRC01 on Viral Rebound

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In both trials, suggesting that persons with chronic HIV infection who were undergoing analytic treatment interruption in several participants. Participant-to-participant variability prevented us from concluding that the observed decrease in sensitivity was universal (P = 0.08). Nonetheless, in Participants N03, N04, N08, and N10, there was strong evidence of the emergence of HIV isolates that were less sensitive to VRC01, highly resistant to VRC01, or both (Fig. 4B). It is noteworthy that the preinfusion isolates obtained from Participant N09 were already highly resistant to VRC01 and remained resistant after infusion. In contrast, there were no detected changes in susceptibility of pre-ART versus rebound viral isolates to neutralization by 3BNC117, 10-1074, PGT121, and UB-421 (Figs. S14 and S15 in the Supplementary Appendix). The rebound VRC01-resistant isolates from two participants (Participants N04 and N08) were also resistant to the CD4-binding site bNAb 3BNC117 (Figs. S14 and S15 in the Supplementary Appendix). Collectively, the analyses of viral isolates in the NIH trial corroborate the sequence-based analysis observed in the A5340 trial and show selection for preexisting VRC01-resistant virus and the capacity for VRC01 to further drive resistance during analytic treatment interruption.

**DISCUSSION**

In two similarly designed clinical trials, we found that the passive administration of multiple doses of VRC01 monotherapy generated high plasma VRC01 concentrations, and no safety concerns were identified. In persons with chronic HIV infection who were undergoing analytic treatment interruption, as compared with historical controls, VRC01 therapy slightly delayed plasma viral rebound; however, viral suppression beyond 8 weeks was not achieved. Sequence-based and neutralization analyses suggest that VRC01 can restrict the clonality of rebounding virus in some participants, select for preexisting resistance, and drive the emergence of VRC01-resistant virus. Baseline resistance to VRC01 was common in both trials, suggesting that persons with chronic infection may frequently harbor archived resistant virus to this antibody.

Our results suggest that the prevalence of clinically significant archived resistance to VRC01 may present a considerable challenge in the use of bNAbs as therapeutic agents for HIV infection. Preexisting resistance to bNAbs is biologically plausible, since before the initiation of ART, persons with chronic HIV infection have extensive exposure to a polyclonal autologous B-cell response that results in archived escape variants to many antibody specificities, including those of bNAb target epitopes. Indeed, a previous study tested replication-competent viral isolates derived from the latent viral reservoir and showed resistance to VRC01 in a substantial proportion of persons in an autologous culture system. In participants with only sensitive pre-ART virus (tested as infectious isolates or envelope-pseudotyped virus) who had rebound with VRC01-resistant virus, it is unclear whether this rebound indicates selection for low-frequency resistant viruses that were not sampled or the emergence of new VRC01 resistance. The development of methods to characterize the phenotypic characteristics (e.g., neutralization sensitivity) of the persistent replication-competent viral reservoir will be needed to distinguish between these different mechanisms of failure of HIV-specific bNAbs.

Although extensive preexisting resistance limited the efficacy of VRC01 in both trials, it is notable that in a previous study of viral isolates and the present NIH clinical trial, other tested bNAbs appeared to have less prevalent archived resistance (Fig. 4A). The efficacy of any given bNAbs in persons with chronic HIV infection will be dependent in part on whether these persons have resistant virus to that bNAb, even at very low frequencies, in persistent viral reservoirs. Future clinical trials may consider prescreening for resistance, although this is a complex task.

The emergence of VRC01-resistant HIV after infusions of VRC01 and discontinuation of ART was observed in both trials. However, a fraction of infectious HIV isolates in some trial participants remained sensitive to VRC01 despite viral rebound in the presence of high levels of VRC01 in plasma. This could be explained by a possible artifact of culture whereby isolates from the persistent viral reservoirs were induced by the ex vivo conditions needed to stimulate cells into producing replication-competent viral isolates.
but they may not have been actively replicating after discontinuation of ART. In the A5340 trial, virus that rebounded early in the presence of high concentrations of VRC01 was almost universally resistant to VRC01. Only two participants (including Participant A07, who had viral rebound after plasma VRC01 concentrations had waned substantially) had VRC01-sensitive envelope glycoproteins.

Our findings highlight an important consideration for the design of future clinical trials of passive immunotherapy in HIV-infected persons. During the early years of development of antiretroviral drugs for HIV infection, the nucleoside reverse transcriptase inhibitor zidovudine used as a single agent resulted in a decrease of approximately 0.5 log copies per milliliter in plasma viremia that almost invariably rebounded with zidovudine-resistant mutants. The advent of additional antiretroviral drugs directed at different viral targets and used in combinations led to more potent viral suppression for longer durations of time. Analogous to current regimens of highly successful combination ART that targets multiple HIV gene products, our data suggest that immunotherapy will probably require multiple bNabs that target different sites on the HIV envelope glycoprotein.

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APPENDIX

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