ORIGINAL ARTICLE

Two Randomized Trials of Neutralizing Antibodies to Prevent HIV-1 Acquisition

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ABSTRACT

BACKGROUND

Whether a broadly neutralizing antibody (bnAb) can be used to prevent human immunodeficiency virus type 1 (HIV-1) acquisition is unclear.

METHODS

We enrolled at-risk cisgender men and transgender persons in the Americas and Europe in the HVTN 704/HPTN 085 trial and at-risk women in sub-Saharan Africa in the HVTN 703/HPTN 081 trial. Participants were randomly assigned to receive, every 8 weeks, infusions of a bnAb (VRC01) at a dose of either 10 or 30 mg per kilogram (low-dose group and high-dose group, respectively) or placebo, for 10 infusions in total. HIV-1 testing was performed every 4 weeks. The VRC01 80% inhibitory concentration (IC_{80}) of acquired isolates was measured with the TZM-bl assay.

RESULTS

Adverse events were similar in number and severity among the treatment groups within each trial. Among the 2699 participants in HVTN 704/HPTN 085, HIV-1 infection occurred in 32 in the low-dose group, 28 in the high-dose group, and 38 in the placebo group. Among the 1924 participants in HVTN 703/HPTN 081, infection occurred in 28 in the low-dose group, 19 in the high-dose group, and 29 in the placebo group. The incidence of HIV-1 infection per 100 person-years in HVTN 704/HPTN 085 was 2.35 in the pooled VRC01 groups and 2.98 in the placebo group (estimated prevention efficacy, 26.6%; 95% confidence interval [CI], −11.7 to 51.8; P=0.15), and the incidence per 100 person-years in HVTN 703/HPTN 081 was 2.49 in the pooled VRC01 groups and 3.10 in the placebo group (estimated prevention efficacy, 8.8%; 95% CI, −45.1 to 42.6; P=0.70). In prespecified analyses pooling data across the trials, the incidence of infection with VRC01-sensitive isolates (IC_{80} <1 μg per milliliter) per 100 person-years was 0.20 among VRC01 recipients and 0.86 among placebo recipients (estimated prevention efficacy, 75.4%; 95% CI, 45.5 to 88.9). The prevention efficacy against sensitive isolates was similar for each VRC01 dose and trial; VRC01 did not prevent acquisition of other HIV-1 isolates.

CONCLUSIONS

VRC01 did not prevent overall HIV-1 acquisition more effectively than placebo, but analyses of VRC01-sensitive HIV-1 isolates provided proof-of-concept that bnAb prophylaxis can be effective. (Supported by the National Institute of Allergy and Infectious Diseases; HVTN 704/HPTN 085 and HVTN 703/HPTN 081 ClinicalTrials.gov numbers, NCT02716675 and NCT02568215.)

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*Full lists of the investigators in the HVTN 704/HPTN 085 and HVTN 703/HPTN 081 trials are provided in the Supplementary Appendix, available at NEJM.org.

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The global pandemic of human immunodeficiency virus (HIV) infection began 40 years ago and continues unabated, with 1.5 million new infections each year, including 35,000 new cases in the United States and 150,000 new cases in infants globally.[1,2] Although the spread of HIV has been blunted by risk-reduction measures, treatment as prevention, and oral antiretroviral preexposure prophylaxis, longer-acting antiviral agents are needed to increase adherence and effectiveness of biomedical prevention approaches.

Immune-based interventions may also prevent infection. VRC01 is an IgG1 broadly neutralizing antibody (bnAb) directed at the HIV type 1 (HIV-1) envelope protein CD4-binding site; the bnAb was isolated from an HIV-1–infected person and shares sequence and structural similarities with other CD4-binding site bnAbs.[3,4] It has wide coverage in vitro against subtype B and subtype C viral strains.[5-7] VRC01 had an acceptable side-effect profile in early clinical trials[8-11] at serum and mucosal levels similar to those that were found to protect nonhuman primates from lentivirus challenge.[12-15]

We report the results of the Antibody Mediated Prevention trials (HIV Vaccine Trials Network [HVTN] 704/HIV Prevention Trials Network [HPTN] 085 and HVTN 703/HPTN 081), which were designed as proof-of-concept trials to determine whether VRC01 is capable of preventing HIV-1 acquisition and whether its ability to prevent HIV-1 acquisition is defined by the in vitro susceptibility of circulating strains.[16,17]

Methods

Trial Designs and Populations

We conducted two parallel phase 2b, multicenter, randomized, double-blind, placebo-controlled, proof-of-concept efficacy trials of the bnAb VRC01 in persons who were assigned male sex at birth or are transgender and have sex with cisgender men or transgender persons (HVTN 704/HPTN 085, conducted in North America, South America, and Europe) and in at-risk heterosexual women (HVTN 703/HPTN 081, conducted in sub-Saharan Africa). The details of the trial designs,[16] statistical analysis plans (see the protocol, available with the full text of this article at NEJM.org), and demographic and sexual risk factors of the populations have been described previously.[18,19] Participants were randomly assigned in a 1:1:1 ratio to receive intravenous VRC01 at a dose of 10 mg per kilogram of body weight (low-dose group), VRC01 at a dose of 30 mg per kilogram (high-dose group), or saline placebo, at 8-week intervals for 10 infusions (20 months). The primary efficacy end point was documented HIV-1 infection by the week 80 trial visit.

Eligibility criteria and details of the schedule and trial visit procedures are included in the Supplementary Appendix, available at NEJM.org. In the HVTN 704/HPTN 085 trial, participants were enrolled at 19 sites in the United States, 5 in Peru, 1 in Brazil, and 1 in Switzerland. In the HVTN 703/HPTN 081 trial, participants were enrolled at 11 sites in South Africa, 3 in Zimbabwe, 2 in Malawi, and 1 each in Botswana, Kenya, Mozambique, and Tanzania.

Counseling about the availability of preexposure prophylaxis was provided as part of the HIV risk counseling that took place at every infusion visit. Participants were given access to oral tenofovir–emtricitabine as preexposure prophylaxis free of charge in the United States through an HVTN-sponsored program in collaboration with Gilead Sciences and in sub-Saharan Africa through an HVTN-established program in collaboration with the South African Medical Research Council (see the Supplementary Appendix).[20]

Trial Oversight

The trials were designed by academic authors and the National Institutes of Health sponsors and collaborators. All data were collected and analyzed by the Statistical and Data Management Center at the Fred Hutchinson Cancer Research Center. The authors had access to the data, critically reviewed the manuscript, and vouch for the accuracy and completeness of the reported results and for the fidelity of the trials to the protocols. The first draft of the manuscript was written by the first, second, and last authors. A protocol safety review team conducted biweekly blinded reviews of safety data, including infusion reactions and adverse events. An independent multinational data and safety monitoring board, convened by the Division of AIDS, National Institute of Allergy and Infectious Diseases, monitored unblinded trial data every 6 months. The data presented in this report were complete through April 3, 2020.

Central and site-specific institutional review

boards and ethics committees reviewed and approved the initial protocol and each subsequent version. All the participants provided written informed consent, and new consent was obtained for each version of the protocol.

ANTIBODY ADMINISTRATION

VRC-HIVMAB060-00-AB in formulation buffer at a pH of 5.8 and saline placebo (sodium chloride for injection 0.9%, USP) were administered in visually identical 150-ml pouches. The enrollment infusion was administered over a period of 60 minutes; subsequent infusions were administered over periods of 15 to 60 minutes. Participants were observed in the clinic for local reactions for 25 to 60 minutes after each infusion and were asked to record their signs and symptoms on the evening of each infusion and for 3 days thereafter. For the first 3 days after each infusion visit, participants were contacted by clinic staff. No preinfusion medications were used.

HIV TESTING

HIV testing was conducted at each 4-week trial visit. Positive results required confirmation with a second, separately drawn blood sample. HIV-1 infections included in the analysis of the primary efficacy end point were identified at centralized laboratories with the use of predefined fourth-generation Food and Drug Administration (FDA)–validated assays and were confirmed by an end-point adjudication committee of experts who were unaffiliated with the trials (Supplementary Appendix).

TESTING OF SENSITIVITY OF HIV-1 ISOLATES TO VRC01

We used the TZM-bl assay to assess the in vitro sensitivity to VRC01 of each HIV-1 strain isolated during the trial. The *rev–env* portion of the HIV-1 genome encoding the complete Env glycoprotein of the identified founder virus of the infection was sequenced and synthesized, and plasmids containing complete *rev–env* cassettes were cotransfected together with an Env-defective backbone vector in 293T/17 cells (see the Supplementary Appendix). Neutralization of the resulting Env-pseudotyped viruses was measured after a single round of infection in TZM-bl cells,[21,22] with titers expressed as the 50% or 80% inhibitory concentration (IC_{50} or IC_{80}) (see the Supplementary Appendix). The IC_{80} was used preferentially for analysis.

STATISTICAL ANALYSIS

All analyses were prespecified in the statistical analysis plans.[16] Prevention efficacy was measured as 1 minus the ratio (VRC01:control) of cumulative incidences of HIV-1 infection diagnosis between enrollment and the week 80 visit for assessment of the primary efficacy end point. Cumulative incidence was estimated with the Nelson–Aalen estimator for the cumulative hazard function, with stratification according to VRC01 dose and trial.[23,24]

In prespecified secondary analyses, the analysis of prevention efficacy was repeated for each of three prespecified categories of in vitro susceptibility of the infecting strain (IC_{80} <1 μg per milliliter, 1 to 3 μg per milliliter, or >3 μg per milliliter) with the use of the Aalen–Johansen estimator (see the Supplementary Appendix for the rationale for the prespecified category cutoff values).[25]

Each trial was designed to have 90% power to detect 60% prevention efficacy (pooled VRC01 doses vs. placebo) at the week 80 visit for assessment of the primary efficacy end point. Pooling of data from the trials was planned to enhance precision in a correlates analysis involving the prespecified in vitro susceptibility of circulating strains. There was no prespecified plan to adjust for multiple comparisons across secondary analyses; 95% confidence intervals are reported without P values. The 95% confidence intervals have not been adjusted for multiple comparisons and should not be used to infer definitive treatment effects.

RESULTS

PARTICIPANT ENROLLMENT AND BASELINE CHARACTERISTICS

Between April 6, 2016, and October 5, 2018, a total of 2699 participants were enrolled in HVTN 704/HPTN 085, and between May 17, 2016, and September 20, 2018, a total of 1924 participants were enrolled in HVTN 703/HPTN 081. The primary analysis populations numbered 2687 and 1924, respectively (Fig. S6 in the Supplementary Appendix). The median age of the participants enrolled in HVTN 704/HPTN 085 was 28 years. In that trial, 31.6% of participants

Table 1. Demographic Characteristics of the Participants in the Primary Analysis at Enrollment and VRC01 Pharmacokinetics.*

Characteristic	HVTN 704/HPTN 085			HVTN 703/HPTN 081		
	Placebo (N=898)	Low-Dose VRC01 (N=895)	High-Dose VRC01 (N=894)	Placebo (N=637)	Low-Dose VRC01 (N=642)	High-Dose VRC01 (N=645)
Median age (range) — yr	28 (18–50)	28 (18–52)	27 (18–50)	26 (18–45)	25 (17–43)	26 (18–44)
Gender identity — no. (%)†						
Cisgender male	818 (91.1)	814 (90.9)	798 (89.3)	0	0	0
Transgender male	7 (0.8)	5 (0.6)	7 (0.8)	0	0	0
Cisgender female	12 (1.3)	16 (1.8)	15 (1.7)	340 (53.4)	338 (52.6)	341 (52.9)
Transgender female	40 (4.5)	42 (4.7)	54 (6.0)	0	0	0
Other or not assessed	24 (2.7)	26 (2.9)	31 (3.5)	297 (46.7)	304 (47.4)	304 (47.1)
Country or region — no. (%)						
United States	458 (51.0)	462 (51.6)	457 (51.1)	—	—	—
Peru	377 (42.0)	372 (41.6)	375 (41.9)	—	—	—
Switzerland	12 (1.3)	12 (1.3)	12 (1.3)	—	—	—
Brazil	51 (5.7)	49 (5.5)	50 (5.6)	—	—	—
Sub-Saharan Africa‡	—	—	—	637 (100.0)	642 (100.0)	645 (100.0)
Median concentration of VRC01 in serum (range) — μg/ml§						
Day 61	—	65.7 (41.7–116.1)	212 (125.5–287.5)	—	88.9 (38.3–111.1)	257.9 (158.6–327.5)
Midpoint	—	23.1 (8.8–43.9	55.1 (26.6–89.5)	—	19.8 (12.7–27.2)	44.7 (29.4–80.5)
Trough	—	5.8 (1.5–18.3)	15.4 (4.5–29.8)	—	4.7 (1.5–6)	8.3 (2.3–16.7)

* The HVTN 704/HPTN 085 trial was conducted in the Americas and Europe and involved persons who were assigned male sex at birth or are transgender and who have sex with cisgender men or transgender persons, and HVTN 703/HPTN 081 involved at-risk heterosexual women in sub-Saharan Africa. Participants in the low-dose group received 10 mg of VRC01 per kilogram of body weight, those in the high-dose group received 30 mg per kilogram, and those in the placebo group received saline placebo.

† Participants may have reported more than one gender identity. "Other" refers to participants who identified as gender queer, gender variant, or gender nonconforming or who chose not to report a gender identity. Gender identity was not assessed in participants in HVTN 703/HPTN 081 outside of South Africa.

‡ Sub-Saharan Africa includes Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, and Zimbabwe.

§ The pharmacokinetic analyses of VRC01 involved a subgroup of 47 VRC01 recipients who had not acquired HIV-1 infection through the week 88 visit and were not likely to have used preexposure prophylaxis (on the basis of self-report or testing of dried blood-spot samples), randomly sampled among the trials and dose groups. Midpoint and trough concentrations are the participant-level median concentrations at the 4-week and 8-week postinfusion visits, respectively, across all 10 infusion intervals.[11] In HVTN 704/HPTN 085, the analysis included 12 participants in the low-dose group and 12 participants in the high-dose group. In HVTN 703/HPTN 081, the analysis included 12 participants in the low-dose group and 11 participants in the high-dose group.

Table 2. Reactogenicity and Safety Data Pooled Across the Two Trials.

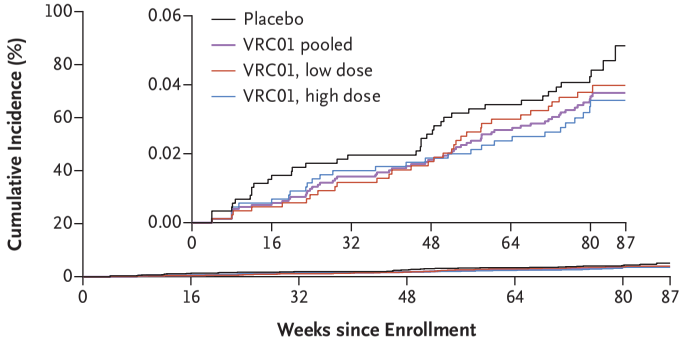
Event	Overall (N=4623)	Placebo (N=1540)	Low-Dose VRC01 (N=1541)	High-Dose VRC01 (N=1542)
		number of participants (percent)		
Pain or tenderness				
None	3552 (76.8)	1171 (76)	1184 (76.8)	1197 (77.6)
Mild	978 (21.2)	341 (22.1)	324 (21)	313 (20.3)
Moderate	89 (1.9)	28 (1.8)	32 (2.1)	29 (1.9)
Severe	4 (0.1)	0	1 (0.1)	3 (0.2)
Potentially life-threatening	0	0	0	0
Maximum systemic symptom severity				
None	2638 (57.1)	875 (56.8)	875 (56.8)	888 (57.6)
Mild	1543 (33.4)	531 (34.5)	506 (32.8)	506 (32.8)
Moderate	431 (9.3)	130 (8.4)	156 (10.1)	145 (9.4)
Severe	11 (0.2)	4 (0.3)	4 (0.3)	3 (0.2)
Potentially life-threatening	0	0	0	0
Increased body temperature				
None	4464 (96.6)	1489 (96.7)	1489 (96.6)	1486 (96.4)
Mild	88 (1.9)	29 (1.9)	25 (1.6)	34 (2.2)
Moderate	58 (1.3)	17 (1.1)	22 (1.4)	19 (1.2)
Severe	12 (0.3)	4 (0.3)	5 (0.3)	3 (0.2)
Potentially life-threatening	1 (<0.1)	1 (0.1)	0	0
Adverse event related to VRC01 or placebo				
Mild	83 (1.8)	22 (1.4)	34 (2.2)	27 (1.8)
Moderate	84 (1.8)	12 (0.8)	28 (1.8)	44 (2.9)
Severe	6 (0.1)	0	4 (0.3)	2 (0.1)
Potentially life-threatening	1 (<0.1)	0	1 (0.1)	0
Death	0	0	0	0

reported their race as White, and 15.1% as Black; 57.1% identified as Latinx (Table 1). The median age of participants in HVTN 703/HPTN 081 was 26 years; 98.9% reported their race as Black. Additional details, including sexual risk behavior and the percentages of participants with sexually transmitted infection at enrollment in both trials, are provided in Table S3.

Preexposure prophylaxis use was evenly distributed among the treatment groups in each trial (see Supplementary Text and Figs. S1 through S5). During 4196 person-years of follow-up in HVTN 704/HPTN 085, preexposure prophylaxis was detectable in 39.0% of person-years (95% confidence interval [CI], 35.9 to 42.0) and preexposure prophylaxis was present at effective concentrations in 28.9% of person-years (95% CI, 26.4 to 31.6); during 3300 person-years of follow-up in HVTN 703/HPTN 081, these percentages were 3.8% (95% CI, 2.8 to 4.9) and 0.4% (95% CI, 0.1 to 0.7), respectively.

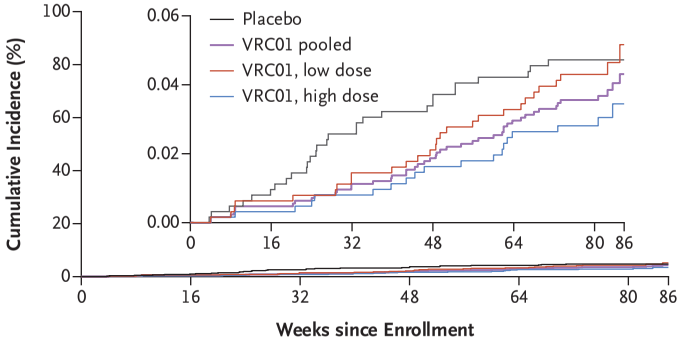
The rate of loss to follow-up was 9.4% per year in HVTN 704/HPTN 085 and 6.3% per year in HVTN 703/HPTN 081, with similar rates among the groups in each trial (Figs. S7 and S8). Adherence to HIV testing across the entire series of 4-week trial visits exceeded 94% in both trials (Tables S1 and S2). In HVTN 704/HPTN 085, the mean number of infusions received was 9.0, with 79% of participants receiving all 10 infusions; in HVTN 703/HPTN 081, these values were 8.9 infusions and 76% of participants (Tables S4 and S5).

A Incidence of HIV-1 Infection in HVTN 704/HPTN 085



No. at Risk							
Placebo	898	852	825	797	773	546	150
VRC01 pooled	1789	1711	1647	1600	1549	1072	340
VRC01, low dose	895	855	826	802	778	533	186
VRC01, high dose	894	856	821	798	771	539	154
Cumulative No. of HIV-1 Infections							
Placebo	0	12	17	22	29	35	38
VRC01 pooled	0	10	23	31	45	59	60
VRC01, low dose	0	4	10	15	25	31	32
VRC01, high dose	0	6	13	16	20	28	28

B Incidence of HIV-1 Infection in HVTN 703/HPTN 081



No. at Risk							
Placebo	637	617	600	582	572	454	157
VRC01 pooled	1287	1241	1211	1176	1146	938	312
VRC01, low dose	642	620	602	587	572	463	154
VRC01, high dose	645	621	609	589	574	475	158
Cumulative No. of HIV-1 Infections							
Placebo	0	6	16	23	26	29	29
VRC01 pooled	0	6	14	23	36	43	47
VRC01, low dose	0	4	9	13	20	26	28
VRC01, high dose	0	2	5	10	16	17	19

C Annualized Incidence and Prevention Efficacy

Trial and Treatment Group	No. of HIV Infections	No. of Person-Yr	Rate per Person-Yr	Prevention Efficacy (95% CI)	P Value
HVTN 704/HPTN 085					
Placebo	38	1275	2.98		
VRC01 pooled	60	2555	2.35	26.6 (−11.7 to 51.8)	0.15
VRC01, low dose	32	1281	2.50	22.4 (−25.5 to 52.0)	
VRC01, high dose	28	1274	2.20	30.9 (−13.9 to 58.0)	
HVTN 703/HPTN 081					
Placebo	29	935	3.10		
VRC01 pooled	47	1889	2.49	8.8 (−45.1 to 42.6)	0.70
VRC01, low dose	28	941	2.98	−9.3 (−85.3 to 35.5)	
VRC01, high dose	19	948	2.00	27.0 (−30.7 to 59.3)	
Pooled trials					
Placebo	67	2207	3.04		
VRC01 pooled	107	4436	2.41	18.1 (−12.2 to 40.2)	0.21
VRC01, low dose	60	2217	2.71	7.2 (−33.3 to 35.3)	
VRC01, high dose	47	2219	2.12	29.0 (−4.0 to 51.6)	

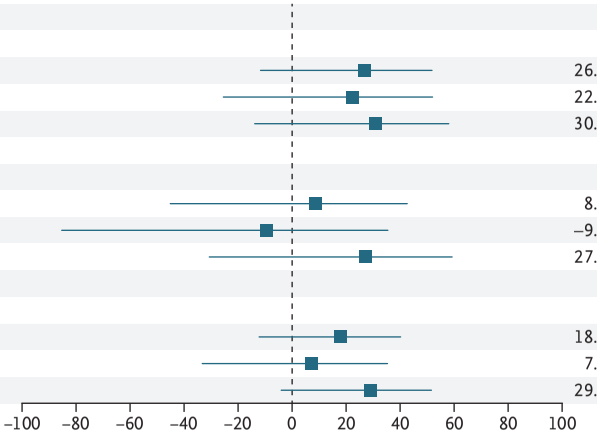


Figure 1 (facing page). Overall Cumulative Incidence of HIV-1 Acquisition and Prevention Efficacy.

Panels A and B show the cumulative incidence of human immunodeficiency virus type 1 (HIV-1) acquisition in the HIV Vaccine Trials Network (HVTN) 704/HIV Prevention Trials Network (HPTN) 085 trial (conducted in the Americas and Europe and involving persons who were assigned male sex at birth or are transgender and who have sex with cisgender men or transgender persons) and HVTN 703/HPTN 081 (involving at-risk heterosexual women in sub-Saharan Africa), respectively. Participants in the low-dose group received 10 mg of VRC01 per kilogram of body weight, those in the high-dose group received 30 mg per kilogram, and those in the placebo group received saline placebo. The insets show the same data on an enlarged y axis. Panel C shows the annualized incidence of HIV-1 acquisition and a forest plot of prevention efficacy at the week 80 visit in each trial and pooled over both trials. CI denotes confidence interval.

SAFETY

The number and severity of adverse events and the percentages of participants with reactogenicity events were balanced among the treatment groups (Tables 2 and S6 through S8). In HVTN 703/HPTN 081, adverse events related to VRC01 were numerically more common than those related to placebo (Table S9). Local and systemic reactions of moderate-to-severe grades were uncommon (Tables S10 through S13). Adverse events that were related to VRC01 and were graded as moderate to severe were observed in 1.2% of the participants in HVTN 704/HPTN 085 and in 3.0% of the participants in HVTN 703/HPTN 081. Six deaths occurred, all of which were judged by the investigators and the data and safety monitoring board to have been unrelated to VRC01 or placebo (Table S14).

OVERALL EFFICACY

VRC01 was not associated with a significantly lower incidence of overall HIV-1 acquisition by the week 80 visit in either trial (Fig. 1A and 1B). In HVTN 704/HPTN 085, the overall estimated prevention efficacy as compared with the placebo group at the week 80 visit was 26.6% (95% CI, −11.7 to 51.8; P=0.15) in the pooled VRC01 group, 22.4% (95% CI, −25.5 to 52.0) in the low-dose group, and 30.9% (95% CI, −13.9 to 58.0) in the high-dose group (Fig. 1C). In HVTN 703/HPTN 081, the overall prevention efficacy as compared with the placebo group at the week 80 visit was 8.8% (95% CI, −45.1 to 42.6; P=0.70) in the pooled VRC01 group, −9.3% (95% CI, −85.3 to 35.5) in the low-dose group, and 27.0% (95% CI, −30.7 to 59.3) in the high-dose group. Although overall prevention efficacy was higher in the high-dose group than in the low-dose group and was higher in HVTN 704/HPTN 085 than in HVTN 703/HPTN 081, the confidence intervals overlapped considerably.

In HVTN 704/HPTN 085, there were 32 infections in the low-dose group, 28 in the high-dose group, and 38 in the placebo group, for incidences of 2.50 per 100 person-years (95% CI, 1.71 to 3.53), 2.20 per 100 person-years (95% CI, 1.46 to 3.18), and 2.98 per 100 person-years (95% CI, 2.11 to 4.09), respectively (Fig. 1C). In HVTN 703/HPTN 081, there were 28 infections in the low-dose group, 19 in the high-dose group, and 29 in the placebo group, for incidences of 2.98 per 100 person-years (95% CI, 1.98 to 4.30), 2.00 per 100 person-years (95% CI, 1.21 to 3.13), and 3.10 per 100 person-years (95% CI, 2.08 to 4.45), respectively (Tables S15 and S16). The point estimate of overall prevention efficacy was approximately 50 to 60% at 16 to 24 weeks (after three infusions) and decreased over time (Fig. S9). The decrease appeared to be greater in HVTN 703/HPTN 081 (Table S17).

ASSOCIATION BETWEEN PROTECTION AT WEEK 80 AND VIRAL STRAIN SENSITIVITY

Our prespecified analyses of the vitro susceptibility of the HIV-1 isolates acquired during the trial revealed several findings regarding the association between the in vitro neutralization sensitivity of the isolates and the efficacy of VRC01 in preventing HIV-1 acquisition. For these analyses, we calculated prevention efficacy against viruses in the prespecified in vitro sensitive, intermediate, and resistant categories, using pooled data from both trials (Fig. 2). The estimated prevention efficacy against sensitive viruses was high in both VRC01 dose groups (Figs. S10 through S12). In analyses of pooled data from the two trials, the incidence of infection with sensitive viruses (IC_{80} <1 μg per milliliter) was 0.20 per 100 person-years among VRC01 recipients, as compared with 0.86 per 100 person-years among placebo recipients (estimated efficacy, 75.4%; 95% CI, 45.5 to 88.9). VRC01 did not prevent infections with intermediate or resistant viruses (prevention efficacy estimates near zero) (Fig. 2B). The frequency of isolates with an IC_{80}

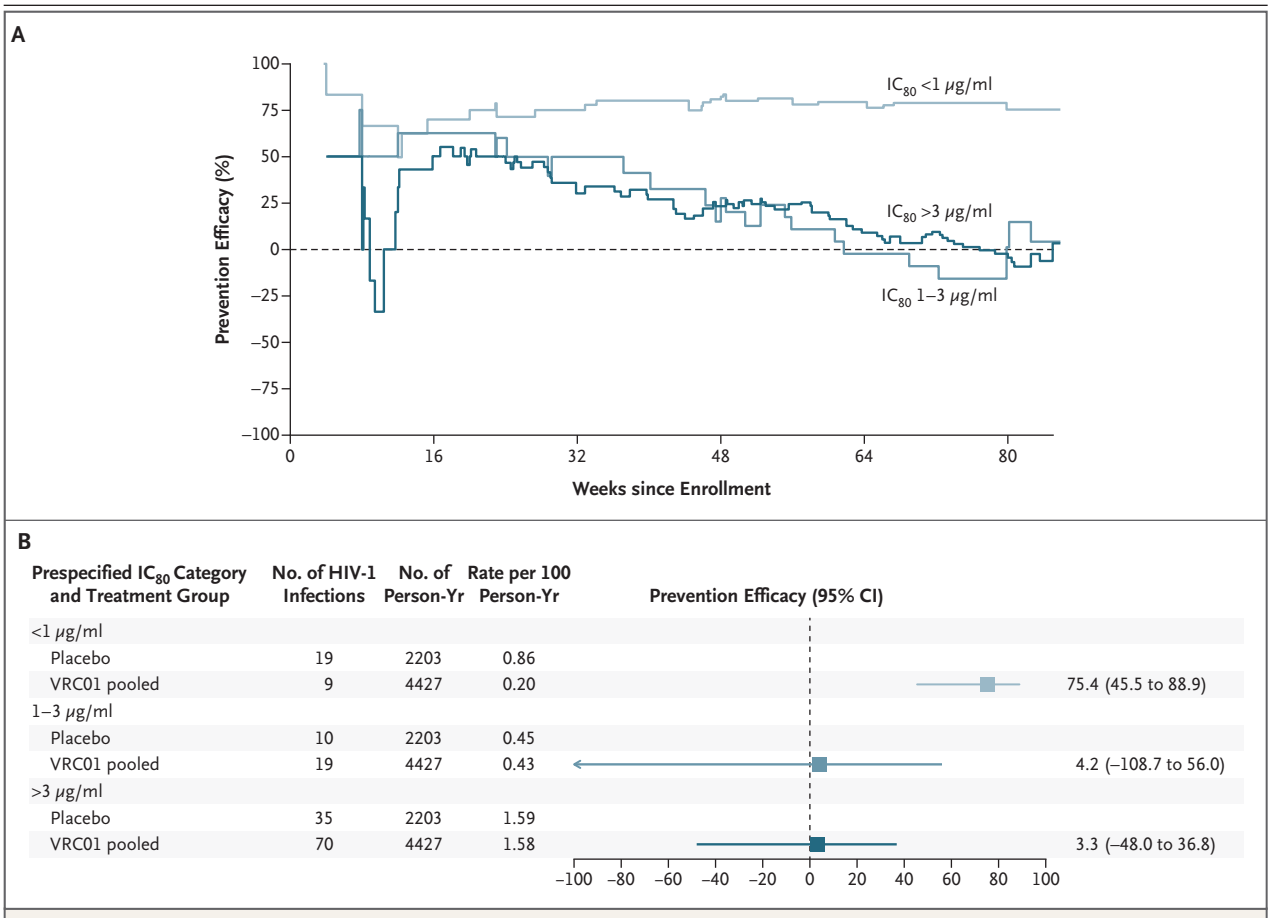


Figure 2. Prevention Efficacy of VRC01 against HIV-1 of Different in Vitro Sensitivities (IC_{80}) to VRC01.

Each acquired virus (162 infected participants) was evaluated as an Env-pseudotyped virus in the TZM-bl target cell assay. Panel A shows prevention efficacy (pooled VRC01 groups vs. placebo) over the 80-week trial period against each virus for the three prespecified 80% inhibitory concentration (IC_{80}) categories: less than 1 μg per milliliter, 1 to 3 μg per milliliter, and greater than 3 μg per milliliter. Panel B shows a forest plot of prevention efficacy (pooled VRC01 groups vs. placebo) at the week 80 visit for the three prespecified IC_{80} categories.

of less than 1 μg per milliliter was 30% (19 of 64) in the placebo groups and 9% (9 of 98) in the VRC01 groups; 55% of isolates in the placebo group had IC_{80} values greater than 3 μg per milliliter, and 49% of these isolates had values greater than 10 μg per milliliter (Table S18); these susceptibilities in the placebo group indicate somewhat more viruses with in vitro resistance than in previous panels of viruses.[6,7,26-28]

Prevention efficacy was also assessed with the use of the entire range of IC_{80} values (0.1 to >10 μg per milliliter) and was found to vary with IC_{80}. The estimated prevention efficacy exceeded 80% against viruses with an IC_{80} of less than 0.3 μg per milliliter (the most sensitive viruses) and decreased with increasing IC_{80}, reaching zero at an IC_{80} of approximately 5 μg per milliliter (Figs. 3A and S14 through S19). Acquired viruses in the VRC01 groups were more resistant to neutralization than acquired viruses in the placebo groups, with a geometric mean IC_{80} of 8.4 μg per milliliter (95% CI, 6.0 to 11.6) in the pooled VRC01 group, as compared with 3.5 μg per milliliter (95% CI, 2.3 to 5.6) in the placebo group (geometric mean ratio, 2.4; 95% CI, 1.4 to 4.1) (Fig. 3A and Table S19).

Viral load at the time of first detection was lower among VRC01 recipients than among placebo recipients infected with sensitive viruses (estimated geometric mean, 9800 copies per milliliter vs. 176,000 copies per milliliter), showing in vivo inhibition of replication even among

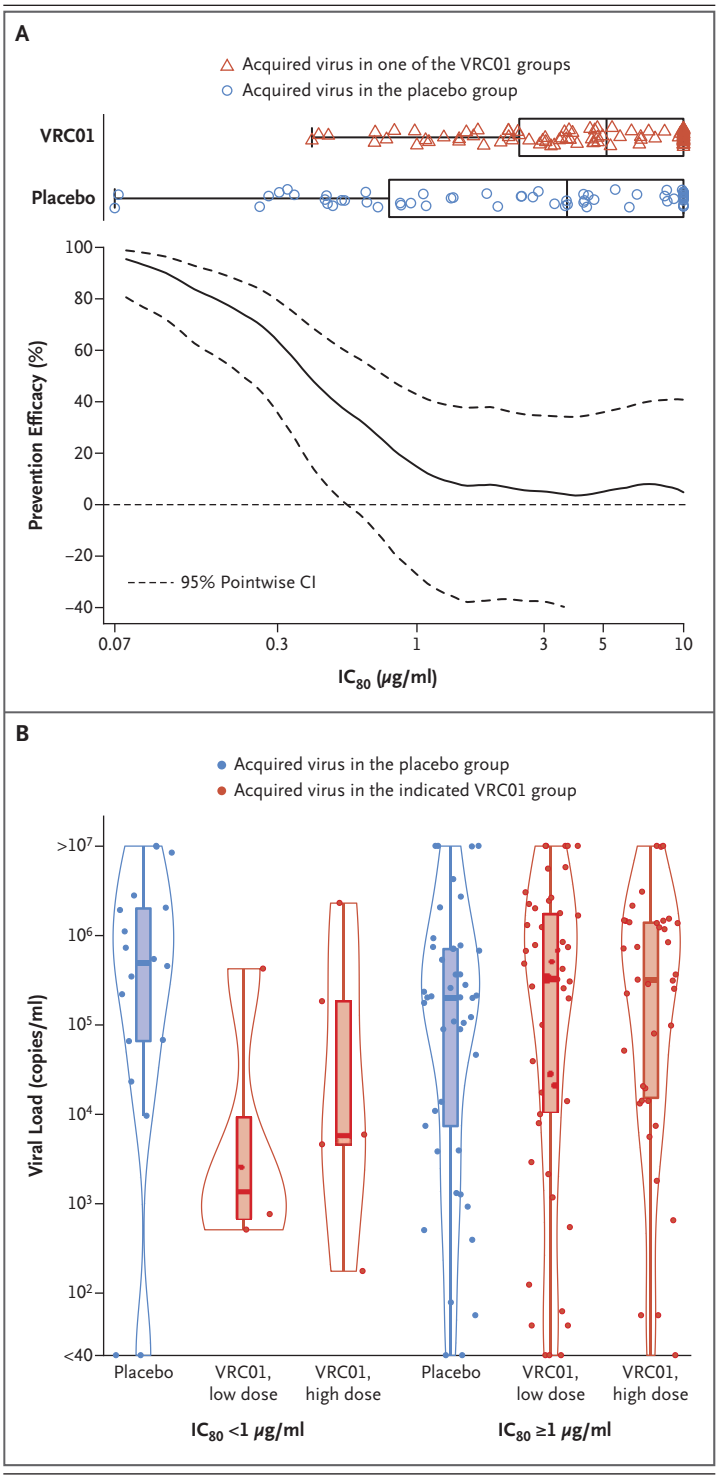


Figure 3. Cumulative Incidence of HIV-1 Infection and Prevention Efficacy According to IC_{80} Category.

Panel A shows the estimated prevention efficacy (pooled VRC01 groups vs. placebo) at the week 80 visit with 95% confidence intervals plotted against quantitative IC_{80}. Box-and-whisker plots above the graph show the distribution of acquired virus IC_{80} values for each primary end-point infection, stratified according to treatment group. A red triangle indicates an acquired virus in the designated VRC01 group, and a blue circle an acquired virus in the placebo group. In the box-and-whisker plots, the right and left sides of the box indicate the interquartile range, the vertical line the median, and the whiskers the range. Data on virus neutralization were missing for eight infections in HVTN 704/HPTN 085 and four infections in HVTN 703/HPTN 081; these cases were excluded. Panel B shows the distribution of viral loads at detection, stratified according to treatment group and IC_{80} category. A red dot indicates an acquired virus in the designated VRC01 group, and a blue dot an acquired virus in the placebo group. In the box-and-whisker plots, the top and bottom of the box indicate the interquartile range, the horizontal line the median, and the whiskers the range. The shapes drawn around the box plots in Panel B are violin plots showing the kernel probability density of the viral load data at different viral load values.

breakthrough isolates. VRC01 did not influence the initial viral load among participants infected with intermediate or resistant viruses (estimated geometric mean, 103,000 copies per milliliter vs. 65,000 copies per milliliter) (Fig. 3B). Thus, on the basis of multiple markers of acquisition and viral breakthrough, the in vivo susceptibility of HIV-1 to VRC01 was predicted by in vitro sensitivity testing with Env-pseudotyped viruses in the TZM-bl assay.

DISCUSSION

Our trials were designed to evaluate whether long-term administration of a bnAb (VRC01) could prevent HIV-1 acquisition, whether susceptibility of the circulating viruses in the community to the bnAb would influence prevention efficacy according to subtype or gender, and whether we could determine the in vitro level of VRC01 neutralization sensitivity of viruses as a biomarker of protection. All these questions were answered in our trial.

Although VRC01 administered intravenously at 8-week intervals over 20 months did not have a significant effect on HIV-1 transmission overall in either trial, prespecified secondary analyses suggest that VRC01 was associated with a lower risk of acquisition of HIV-1 isolates that had in vitro sensitivity to the antibody (i.e., an IC_{80} of <1 μg per milliliter). Against this group of highly sensitive viruses, receipt of VRC01 was associated

with 75% protection over the 20-month trial period among women in sub-Saharan Africa who were at risk for HIV-1 infection and were exposed to subtype C variants (HVTN 703/HPTN 081 trial) and at-risk persons in South America, Switzerland, and the United States who were assigned male sex at birth or are transgender and who were exposed to subtype B variants (HVTN 704/HPTN 085 trial). Susceptibility to the antibody was the important determinant of antiviral activity. Our trials were designed with the idea that strains with an IC_{80} of less than 10 μg per milliliter would be effectively inhibited by VRC01, an in vitro cutoff value that was met for 65 to 81% of strains in contemporaneous subtype B and C panels.[3,6,7,26-28] However, we found that only strains with an IC_{80} of less than 1 μg per milliliter — which corresponded to only 30% of the viruses circulating in the trial regions — were effectively inhibited by VRC01, which is likely to explain the lack of an overall significant prevention efficacy over the 20-month trial period. Our randomization scheme resulted in equal distribution of oral preexposure prophylaxis use in our trials, and this factor therefore should not have influenced our findings. The relatively low rate of oral emtricitabine–tenofovir use, despite unrestricted cost-free access and counseling on its availability throughout the trial, illustrates the lack of interest in this prevention strategy among the participants. This seemed especially true among women in sub-Saharan Africa, where, despite on-site access to oral emtricitabine–tenofovir, uptake was very low.

Our data suggest that VRC01 applied pressure on the circulating strains of virus at the earliest stages of acquisition and may have suppressed infection in the tissue, with emergence of resistant isolates over time. Isolates acquired among VRC01 recipients were more resistant to VRC01 than those acquired among placebo recipients (geometric mean IC_{80}, 8.4 μg per milliliter vs. 3.5 μg per milliliter). Viral loads at the time of detection were lower among VRC01 recipients than among placebo recipients for acquired viruses with an IC_{80} lower than 1.0 μg per milliliter. We are evaluating whether molecular signatures provide further insights into these observed effects. Studies to determine whether early transient suppression and late breakthrough occurred over time are also under way. These studies may help to define whether such effects can be precluded by bnAb combinations or the use of Fc receptors that concentrate the virus in tissue and enhance serum and tissue concentrations of antibody. Although formal evaluations of antidrug antibodies in participants, including persons infected with breakthrough isolates, are under way, analyses of VRC01 levels in the pharmacokinetics substudy have revealed no decline in VRC01 concentration or neutralizing activity in serum over successive infusions.[11,29,30]

Highly potent antibodies with dosing intervals of 3 to 6 months are now in clinical development, and early-phase clinical trials of two-bnAb (ClinicalTrials.gov numbers, NCT04173819 and NCT04212091) and two- and three-bnAb (NCT03928821) combinations are being conducted.[31] In addition, both bispecific (NCT03875209) and trispecific (NCT03705169) bnAbs are in clinical development. Our trials suggest a path forward for the use of combination bnAb regimens for effective prevention of HIV infection in high-risk populations. Our results support the idea that in general, the serum neutralization titer against exposing viruses will be the key factor for achieving high prevention efficacy, such that bnAb regimens that provide both high bnAb concentrations for long periods of time and high coverage of circulating strains are most promising. Our data indicate that the likelihood that potent combinations of bnAbs will be effective in inhibiting acquisition of circulating community strains can be evaluated with the use of the IC_{80} cutoff we have defined here (i.e., <1 μg per milliliter). Combinations of bnAbs to provide one- or two-bnAb coverage at levels associated with in vivo protection can be designed with the TZM-bl assays, and animal challenge models can be useful screening tests for such future studies. Studies of the use of bnAbs for treatment of HIV-1 infection indicate that viral rebound results from "escape" of resistant variants from a diverse swarm after bnAb infusion and that such variants often possess such molecular signatures.[32-34]

VRC01 did not prevent overall HIV-1 acquisition in either of our trials. Analyses that were focused on HIV isolates that were sensitive to VRC01 provided proof-of-concept for bnAbs having the potential to prevent HIV-1 acquisition. Similar to what has been seen with first-genera-

tion antiretroviral therapy, innate resistance and selection of resistant isolates over time mitigate the effect of this treatment as a single preventive therapeutic agent, which suggests that there is a need to assess the efficacy of a broader, more potent combination of antibodies.

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APPENDIX

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