ORIGINAL ARTICLE

Effect of Anti-CD4 Antibody UB-421 on HIV-1 Rebound after Treatment Interruption

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ABSTRACT

BACKGROUND

Administration of a single broadly neutralizing human immunodeficiency virus (HIV)–specific antibody to HIV-infected persons leads to the development of antibody-resistant virus in the absence of antiretroviral therapy (ART). It is possible that monotherapy with UB-421, an antibody that blocks the virus-binding site on human CD4+ T cells, could induce sustained virologic suppression without induction of resistance in HIV-infected persons after analytic treatment interruption.

METHODS

We conducted a nonrandomized, open-label, phase 2 clinical study evaluating the safety, pharmacokinetics, and antiviral activity of UB-421 monotherapy in HIV-infected persons undergoing analytic treatment interruption. All the participants had undetectable plasma viremia (<20 copies of HIV RNA per milliliter) at the screening visit. After discontinuation of ART, participants received eight intravenous infusions of UB-421, at a dose of either 10 mg per kilogram of body weight every week (Cohort 1) or 25 mg per kilogram every 2 weeks (Cohort 2). The primary outcome was the time to viral rebound (≥400 copies per milliliter).

RESULTS

A total of 29 participants were enrolled, 14 in Cohort 1 and 15 in Cohort 2. Administration of UB-421 maintained virologic suppression (<20 copies per milliliter) in all the participants (94.5% of measurements at study visits 2 through 9) during analytic treatment interruption, with intermittent viral blips (range, 21 to 142 copies per milliliter) observed in 8 participants (28%). No study participants had plasma viral rebound to more than 400 copies per milliliter. CD4+ T-cell counts remained stable throughout the duration of the study. Rash, mostly of grade 1, was a common and transient adverse event; one participant discontinued the study drug owing to a rash. A decrease in the population of CD4+ regulatory T cells was observed during UB-421 monotherapy.

CONCLUSIONS

UB-421 maintained virologic suppression (during the 8 to 16 weeks of study) in participants in the absence of ART. One participant discontinued therapy owing to a rash. (Funded by United Biomedical and others; ClinicalTrials.gov number, NCT02369146.)

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N Engl J Med 2019;380:1535-45.
DOI: 10.1056/NEJMoa1802264

Antiretroviral therapy (ART) has successfully transformed human immunodeficiency virus (HIV) infection from a fatal to a manageable chronic disease.[1] Nonetheless, ART alone cannot eradicate HIV because of the persistence of viral reservoirs,[2] and plasma viremia promptly rebounds shortly after discontinuation of ART.[3,4] The likelihood of drug resistance still exists in persons receiving clinically effective ART[5] or those receiving preexposure prophylaxis,[6] and transmission of drug-resistant virus could confer a predisposition to virologic failure.[7] Therefore, treatment options with new mechanisms of action should be explored[8] in order to provide better suppression of HIV.

Monoclonal antibodies (mAbs) have been explored for the treatment of HIV infection. However, attempts to use broadly neutralizing HIV-specific monoclonal antibodies (bNAbs)[9-11] to substitute for ART remain a formidable clinical challenge given the emergence of antibody-resistant virus.[9] Alternatively, mAbs that target CD4 or coreceptors, such as CCR5 or CXCR4, can be used as antiviral agents. Theoretically, an agent that competitively binds and blocks the site where all HIV variants attach for entry into the host cell may provide a wide breadth of viral coverage and reduce the likelihood of viral resistance mutation. To be practical, such mAbs must competitively bind to CD4 with higher affinity than HIV glycoprotein 120 (gp120), inhibit viral entry as well as cell-to-cell spread, and be noncytotoxic and nonimmunosuppressive.

UB-421 is an Fc-aglycosylated, non–T-cell–depleting and CD4-specific humanized IgG1 derived from the parent murine B4, which binds to discontinuous, conformational epitopes on the HIV-receptor complex, including CD4 domain 1, and competitively blocks HIV entry.[12] (For details, see Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Both the murine and humanized mAbs bind to CD4+ T cells with higher affinity than HIV gp120. Furthermore, B4 exhibits potent activity to inhibit HIV entry independent of viral strains and coreceptor tropism and has been shown to arrest viral spread in chimpanzees before and after exposure to chimp-adapted dual-tropic primary isolate HIV_{DH-12}.[12]

Because the bNAbs that directly target HIV can incur rapid selection of resistant HIV, leading to viral rebound in the absence of ART,[9] we hypothesize that UB-421, which targets host CD4, will provide virologic suppression through a different mechanism that may be less susceptible to viral escape. UB-421 has been shown to inhibit viral entry in a phase 1 study (ClinicalTrials.gov number, NCT01140126) and a phase 2a study (NCT01668043), both involving HIV-infected persons who had not previously received treatment.

In the present phase 2 study involving ART-stabilized HIV-infected persons, we assess whether UB-421 ART-substitution monotherapy can prevent plasma viremia and reversibly reduce the population of regulatory T cells (Tregs), while maintaining CD4+ T-cell counts.[13]

METHODS

STUDY DESIGN

We conducted an open-label, nonrandomized, phase 2 study in Taiwan involving HIV-infected persons who received UB-421 monotherapy during analytic treatment interruption. The study involved two cohorts (Fig. 1); participants received eight intravenous infusions of UB-421, at a dose of either 10 mg per kilogram of body weight every week (Cohort 1) or 25 mg per kilogram every 2 weeks (Cohort 2). The ART regimens that the study participants were receiving at baseline are shown in Table S1 in the Supplementary Appendix. The first dose of UB-421 was administered 1 week before analytic treatment interruption. Participants were prophylactically premedicated with an antihistamine (5 mg of chlorpheniramine) and a corticosteroid (100 mg of hydrocortisone) before each dose. On completion of the eight doses of UB-421, the study participants resumed ART and were followed for 8 additional weeks.

The study was conducted according to the principles of Good Clinical Practice. All the authors vouch for the accuracy and completeness of the data and analyses presented and for the fidelity of the study to the protocol, available at NEJM.org. The protocol was approved by the ethics committee at each site, and all the participants provided written informed consent. Details of the study design are provided in the protocol and statistical analysis plan (available at NEJM.org).

STUDY OBJECTIVES

The study objectives included the safety, adverse-event profile, and efficacy of UB-421 monother-

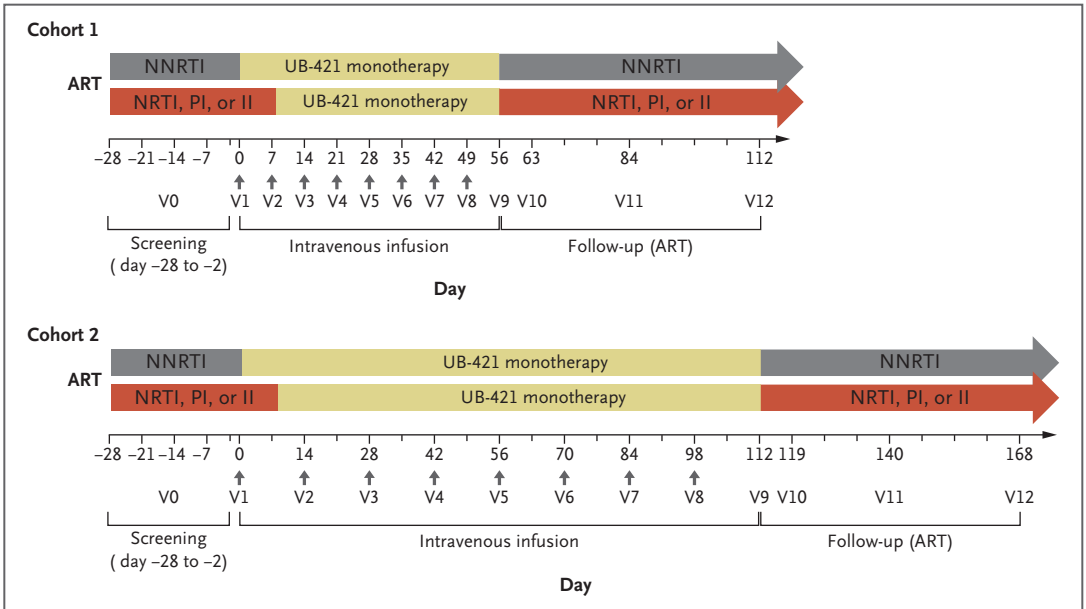

Figure 1. Study Design.

Participants in the phase 2 study were recruited from three tertiary hospitals designated as AIDS-referring centers in Taiwan. The qualified study participants were antiretroviral therapy (ART)–stabilized, having undetectable plasma viremia (<20 copies of human immunodeficiency virus [HIV] RNA per milliliter) and a CD4+ T-cell count of more than 350 cells per microliter at the screening visit. A total of 29 qualified participants were assigned to receive eight intravenous infusions of UB-421, at a dose of 10 mg per kilogram of body weight every week (Cohort 1) or 25 mg per kilogram every 2 weeks (Cohort 2). The primary objective of the study was to evaluate the effectiveness of UB-421 monotherapy in maintaining virologic suppression without rebound during analytic treatment interruption. ART was reinitiated after the completion of eight doses of UB-421 or on the occurrence of viral rebound, the development of unacceptable adverse reactions, or nonadherence to the UB-421 therapy. A total of 27 participants received all eight doses of UB-421; 2 participants resumed ART before the completion of scheduled UB-421 treatment. After ART reinitiation, participants were followed for 8 additional weeks. Viral rebound was defined as a viral load of at least 400 RNA copies per milliliter at two consecutive visits. A total of 5 study participants who completed the eight required doses chose not to reinitiate ART; however, on plasma viral rebound, they subsequently resumed ART. II denotes integrase inhibitor, NNRTI nonnucleoside reverse-transcriptase inhibitor, NRTI nucleoside reverse-transcriptase inhibitor, and PI protease inhibitor.

apy in maintaining viral suppression after analytic treatment interruption. The primary efficacy end point was the time to viral rebound after the first UB-421 infusion; viral rebound was defined as plasma HIV RNA levels of at least 400 copies per milliliter at two consecutive visits. The secondary end points were the cumulative proportion of participants who discontinued UB-421 monotherapy owing to viral rebound or unacceptable adverse events; the change in plasma viremia from baseline to viral rebound; the pharmacokinetic characteristics of serum UB-421; the frequency of grade 3 or 4 adverse events, as defined by the Division of AIDS adverse-events scale; the changes in CD4+ and CD8+ T-cell counts from baseline; and the serum levels of anti–UB-421 antibody.

STATISTICAL ANALYSIS

Because the phase 2 study of ART substitution with UB-421 monotherapy in two dose cohorts was explorative (proof of concept) in nature, the sample size was not based on formal power estimation, nor was the goal to detect statistical differences between the two cohorts. Participants were assigned to either dose cohort after discussion between the principal investigator of each study site and participants regarding the duration of the study, dose schedule, and infusion volumes. Participants with a higher body weight (>80 kg) were more likely to be assigned to the cohort receiving 10 mg per kilogram every week so that the infusion volumes would not be too large for each treatment visit. Thus, the between-cohort antiviral efficacy or safety was not com-

pared. When within-participant laboratory results were compared between study visits, the Fisher's exact test for categorical variables and the Wilcoxon signed-rank test or Wilcoxon rank-sum test for continuous variables were used for analysis. A two-sided P value of less than 0.05 was considered to indicate statistical significance, unless stated otherwise.

RESULTS

STUDY PARTICIPANTS

A total of 29 participants were enrolled, 14 in Cohort 1 and 15 in Cohort 2 (Fig. 1 and Table 1). All the participants had undetectable plasma viremia (<20 copies of HIV RNA per milliliter) and a CD4+ T-cell count of more than 350 cells per microliter at the screening visit. The median age of the participants was 35 years in Cohort 1 and 31 years in Cohort 2. The median duration of HIV infection was 5.7 years in Cohort 1 and 5.8 years in Cohort 2. At baseline, the median duration from ART initiation to study entry was 4.8 years in Cohort 1 and 5.2 years in Cohort 2.

SAFETY AND ADVERSE-EVENT PROFILE

A total of 27 of the 29 participants received all eight doses of UB-421 (Table 1); 2 participants in Cohort 2 did not complete treatment with UB-421 (1 was lost to follow-up after the first dose and 1 stopped after the second dose owing to a rash of grade 2). A total of 3 participants in Cohort 1 (21%) and 5 participants in Cohort 2 (33%) had adverse events of grade 2 or higher (Table 1, and Table S4 in the Supplementary Appendix). Grade 4 elevations in liver-enzyme levels, possibly associated with consumption of green-tea extract,[14] were observed in 1 participant (Participant 2-1-04 in Table S5 in the Supplementary Appendix). No deaths occurred in the 29 participants.

Of all investigator-assessed possible or probable drug-related adverse events, the most common was mild transitory rash (in 15 participants [52%]) (Table S5 in the Supplementary Appendix); 12 participants (41%) had a rash of grade 1, and 3 participants (10%) had a rash of grade 2 (Table 1). There was one serious adverse event observed during the study: a grade 1 inguinal hernia in a Cohort 1 participant. Neither postinfusion clinical findings nor significant changes in serum cytokine levels (interleukin-1β, -2, -4, -6, and -10; tumor necrosis factor α; and interferon-γ) were observed (Fig. S2 in the Supplementary Appendix). No antidrug antibody was detected.

The CD4+ T-cell counts, measured with a noninterfering antibody that recognizes CD4 domain 2, remained stable, whereas a moderate increase in the CD8+ T-cell counts was observed in both cohorts (Fig. 2). The stable CD4+ T-cell counts were confirmed with the use of a standard diagnostic anti–CD4 domain 1 antibody at the study sites before the first UB-421 infusion (day 0 or week 1) and at the end of the study (visit 12, at week 17 for Cohort 1 and week 25 for Cohort 2) when serum UB-421 was no longer detected (Fig. 2). Similarly, lymphocyte phenotyping showed a slight decrease in the percent of CD4+ T cells and an increase in CD8+ T cells, whereas levels of CD3+ T cells and total lymphocytes remained unchanged (Fig. S3 in the Supplementary Appendix). Further subset analyses of CD4+ and CD8+ T cells (Fig. S4 in the Supplementary Appendix) revealed minor changes without consistent population shift among naive, central memory, effector memory, or effector T cells.

PHARMACOKINETICS, CD4-RECEPTOR OCCUPANCY, AND SUSTAINED VIRAL SUPPRESSION

Before the present phase 2 study, the intrinsic antiretroviral potency of UB-421 as a single agent had been defined in asymptomatic, HIV-infected, previously untreated persons who received a single intravenous infusion of UB-421 at a dose of 1 to 25 mg per kilogram (phase 1 study), and eight infusions at a dose of 10 mg per kilogram every week or four infusions at a dose of 25 mg per kilogram every 2 weeks (phase 2a study). The mean maximum viral-load reduction was 1.6 $\log_{10}$ by a single dose of UB-421 (Fig. S5 and Table S2 in the Supplementary Appendix) and 2.7 $\log_{10}$ by repeat doses (Fig. S6 and Table S3 in the Supplementary Appendix). In both clinical studies, a 100% response rate of at least 1.0 $\log_{10}$ viral-load reduction was observed for the dose levels at 10 and 25 mg per kilogram. The observed antiviral activity (Figs. S5 and S6 in the Supplementary Appendix) was corroborated in this phase 2 study, in which all the participants had plasma viremia of less than 20 copies per milliliter (94.5% of measurements at study visits 2 through 9) and in which eight study partici-

Table 1. Baseline Characteristics of Study Participants and Adverse Events That Developed during UB-421 Monotherapy.*

Variable	Cohort 1 (N=14)	Cohort 2 (N=15)
Baseline characteristics		
Asian race — no. (%)†	14 (100)	15 (100)
Male sex — no. (%)	14 (100)	15 (100)
Median age (range) — yr	35 (25–47)	31 (21–56)
Median weight (range) — kg	70.3 (55–97)	62.3 (46–74)
Median height (range) — cm	175 (168–183)	169 (159–178)
Median duration of HIV infection (range) — yr	5.7 (2.9–17.7)	5.8 (1.3–15.7)
Median duration of ART (range) — yr	4.8 (1.7–16.3)	5.2 (1.3–10.9)
Plasma viremia <20 copies of HIV RNA/ml — no. (%)	14 (100)	15 (100)
Median red-cell count (range) — $\times 10^{-6}/mm^3$	4.1 (3.6–5.0)	4.3 (3.4–5.3)
Median CD4+ T-cell count (range) — cells/mm^3	653 (370–951)	640 (394–1087)
Median CD8+ T-cell count (range) — cells/mm^3	721 (392–1145)	831 (379–1511)
Adverse event of grade 2 or higher — no./total no. (%)‡		
Rash	1/14 (7)	2/14 (14)§
Eosinophilia	1/14 (7)	2/14 (14)§
Bilirubin elevation	1/14 (7)¶	0/14
Alkaline phosphatase elevation	1/14 (7)¶	0/14
γ-Glutamyltransferase elevation	1/14 (7)¶	0/14
Alanine aminotransferase elevation	1/14 (7)¶	1/14 (7)§
Aspartate aminotransferase elevation	1/14 (7)¶	2/14 (14)§

* The phase 2 study began on July 1, 2015, and ended on July 12, 2016. Of 36 human immunodeficiency virus (HIV)–infected Asian participants screened, 29 were qualified for enrollment to receive eight intravenous infusions of UB-421, at a dose of 10 mg per kilogram of body weight every week (Cohort 1) or 25 mg per kilogram every 2 weeks (Cohort 2). Although participants in Cohort 2 had lower body weight and height than those in Cohort 1 ($P=0.02$ and $P=0.001$, respectively, by Wilcoxon rank-sum test) at baseline, no significant differences were detected for body-mass index, age, CD4+ or CD8+ T-cell counts, duration of HIV infection, types and durations of antiretroviral therapy (ART), results of physical examination, and vital signs (all $P>0.20$) between the two cohorts. Of 15 enrollees in Cohort 2, 1 was lost to follow-up after the first dose and 1 withdrew from the study after the second dose owing to a rash (grade 2). A total of 27 enrollees (14 in Cohort 1 and 13 in Cohort 2) completed all eight doses.

† Race was reported by the participants.

‡ The participant in Cohort 2 who was lost to follow-up after the first dose was excluded from the analysis. All the adverse events that emerged during treatment were grade 2 except for the elevated levels of γ-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase, which were grade 4, in 1 participant who had consumed green-tea extract and other herbal supplements, as described below. In total, 3 participants in Cohort 1 and 5 participants in Cohort 2 had adverse events of grade 2 or higher; among them, 3 participants had more than one type of adverse event, as detailed below. For more on adverse events, see Tables S4 through S6 in the Supplementary Appendix.

§ One participant had rash and eosinophilia; another participant had elevated levels of alanine aminotransferase and aspartate aminotransferase.

¶ The five abnormalities in liver-function tests were observed on the last treatment-visit day in the same participant in Cohort 1 who had consumed a multiherbal supplement containing high levels of green-tea extract.[14]

pants (28%) had viral blips (21 to 142 copies per milliliter) during analytic treatment interruption (Table 2) that did not result in additional treatment. Both doses appeared to be equally effective in preventing plasma viral rebound during the UB-421 treatment.

Concurrent pharmacokinetic and pharmacodynamic analyses (Fig. 3) revealed that the level of serum UB-421 during analytic treatment interruption (Fig. 3A) was sufficient for complete occupancy of surface CD4 molecules on CD4+ T cells (Fig. 3B), a finding consistent with the absence of plasma viral rebound during the treatment (Fig. 3C). Sustained viral suppression

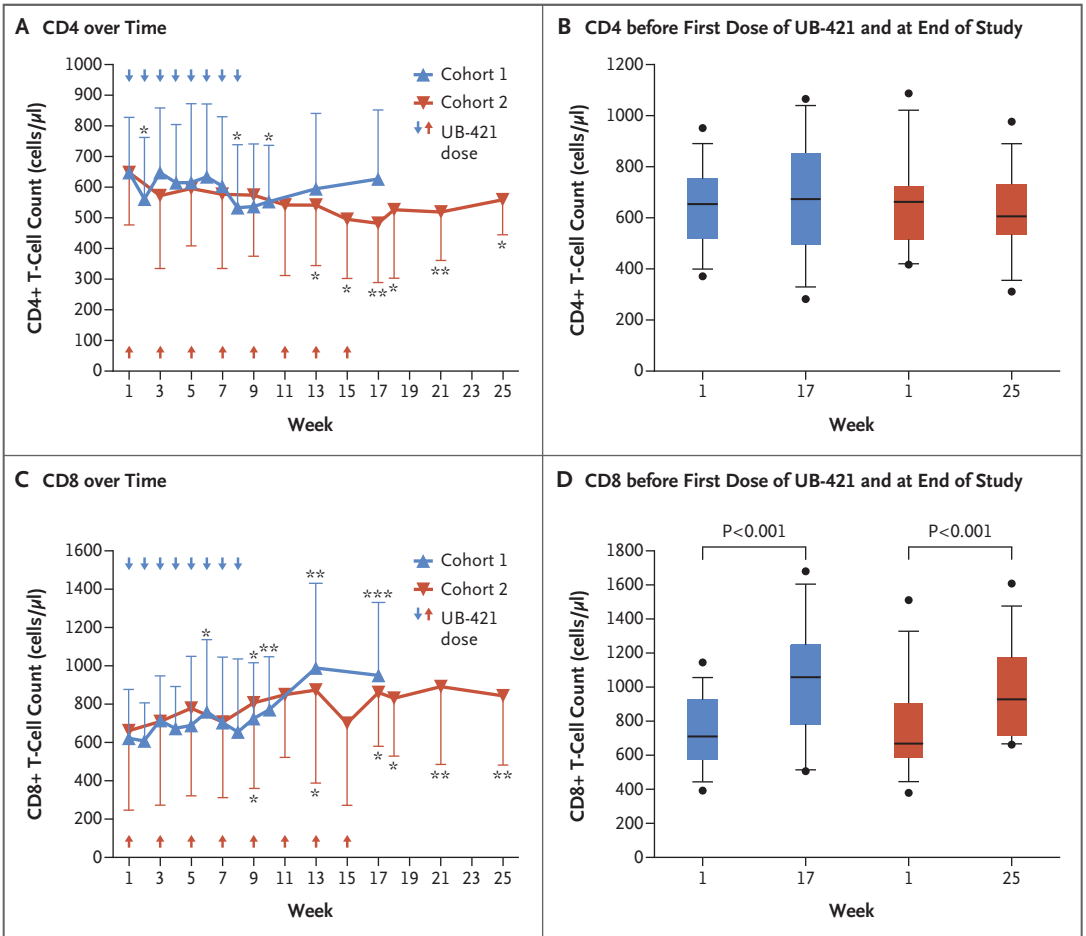

Figure 2. Effects of UB-421 Monotherapy on CD4+ and CD8+ T-Cell Counts during Analytic Treatment Interruption and after Reinitiation of ART.

Data are for 14 participants in Cohort 1 and 13 participants in Cohort 2. Panel A shows the change in CD4+ T-cell counts over time. The counts, which were analyzed with a noninterfering anti–CD4 domain 2 antibody (clone M-T441, Ancell), remained stable in both Cohort 1 (weeks 1 to 17) and Cohort 2 (weeks 1 to 25). Panel B shows CD4+ T-cell counts before the first dose of UB-421 and at the end of the study, when serum UB-421 was no longer detected. The counts, which were measured with an anti–CD4 domain 1 antibody, did not change significantly in either cohort. Panel C shows the change in CD8+ T-cell counts over time. The counts, which were measured by flow cytometry with an anti-CD8 antibody, increased in both cohorts. Panel D shows the CD8+ T-cell counts before the first dose of UB-421 and at the end of the study. The counts, which were determined by standard diagnostic methods at each study site hospital, showed a significant increase in both cohorts. In Panels A and C, one asterisk denotes $P<0.05$, two asterisks $P<0.01$, and three asterisks $P<0.001$; the vertical bars indicate standard deviations. In the box-and-whisker plots in Panels B and D, the boxes show the 25th, 50th (median), and 75th percentiles, and the whiskers show the 10th and 90th percentiles. Data points outside the 10th or 90th percentiles are shown as solid circles.

(Fig. 3C and Table 2) was achieved with the serum UB-421 concentrations obtained (Fig. 3A). The mean C_{trough} levels of serum UB-421 increased from 22.9 μg per milliliter on day 7 to 50.5 μg per milliliter on day 49 in Cohort 1 and from 24.3 μg per milliliter on day 14 to 38.7 μg per milliliter on day 98 in Cohort 2 (Table S7 in the Supplementary Appendix). These values were above the estimated level of 10 μg per milliliter required for full receptor occupancy.

EFFECT ON REGULATORY CD4+ T CELLS

It has been shown that Tregs harbor higher levels of HIV proviral DNA than nonregulatory T cells.[15] We investigated whether UB-421 might modulate the level of Treg (CD25+FoxP3+)CD4+ T cells.

Table 2. Plasma Viremia before and after UB-421 Monotherapy as ART Substitution during Analytic Treatment Interruption.*

Cohort and Participant No.	Visit 0, Day S	Visit 1, Day 0	Visit 2, Day 7	Visit 3, Day 14	Visit 4, Day 21	Visit 5, Day 28	Visit 6, Day 35	Visit 7, Day 42	Visit 8, Day 49	Visit 9, Day 56	Visit 10, Day 63	Visit 11, Day 84	Visit 12, Day 112
						copies of HIV RNA/milliliter							
Cohort 1													
1-1-01	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-1-02	<20	251	25	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-1-03	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-1-04	<20	<20	49	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-1-05	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2-1-01	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	230,000†	1920‡
2-1-02	<20	38	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2-1-03	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	12,000†‡
2-1-04	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	127,000†	453‡
2-1-05	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
3-1-01	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
3-1-02	<20	<20	<20	<20	<20	<20	27	88	<20	<20	<20	<20	<20
3-1-03	<20	<20	<20	<20	<20	<20	<20	<20	<20	51	<20	<20	48
3-1-04	<20	31	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	Visit 0, Day S	**Visit 1, Day 0**	**Visit 2, Day 14**	**Visit 3, Day 28**	**Visit 4, Day 42**	**Visit 5, Day 56**	**Visit 6, Day 70**	**Visit 7, Day 84**	**Visit 8, Day 98**	**Visit 9, Day 112**	**Visit 10, Day 119**	**Visit 11, Day 140**	**Visit 12, Day 168**
Cohort 2													
1-2-01	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-2-02	<20	<20	<20	47	142	21	<20	<20	<20	<20	<20	<20	<20
1-2-03	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-2-04	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
1-2-05	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2-2-01	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	573,000†	1180‡
2-2-02	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	30,900†	178‡
2-2-03	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
2-2-04	<20	<20	<20	<20	<20	121	<20	<20	<20	<20	<20	<20	<20
2-2-05	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	329	<20
3-2-01	<20	<20	<20	101	<20	<20	<20	<20	<20	<20	<20	<20	<20
3-2-02	<20	<20	WC										
3-2-03	<20	<20	<20	<20	WC								
3-2-04	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	43	29
3-2-05	<20	<20	<20	<20	40	<20	<20	<20	36	<20	<20	<20	<20

* Undetectable plasma viremia was defined as less than 20 copies of HIV RNA per milliliter on day S (screening period between –28 to –2). WC denotes withdrawal of participant with consent.

† Three participants in Cohort 1 and two participants in Cohort 2 chose not to resume the scheduled ART after the last dose of UB-421. The time to plasma viral rebound in these participants was 35 days for two participants and 63 days for one participant in Cohort 1 and 42 days for two participants in Cohort 2. Their plasma viremia subsequently returned to an undetectable level after reinitiation of ART.

‡ Shown are the five participants who restarted ART after viral rebound of more than 1000 copies per milliliter rather than directly after the last dose of UB-421.

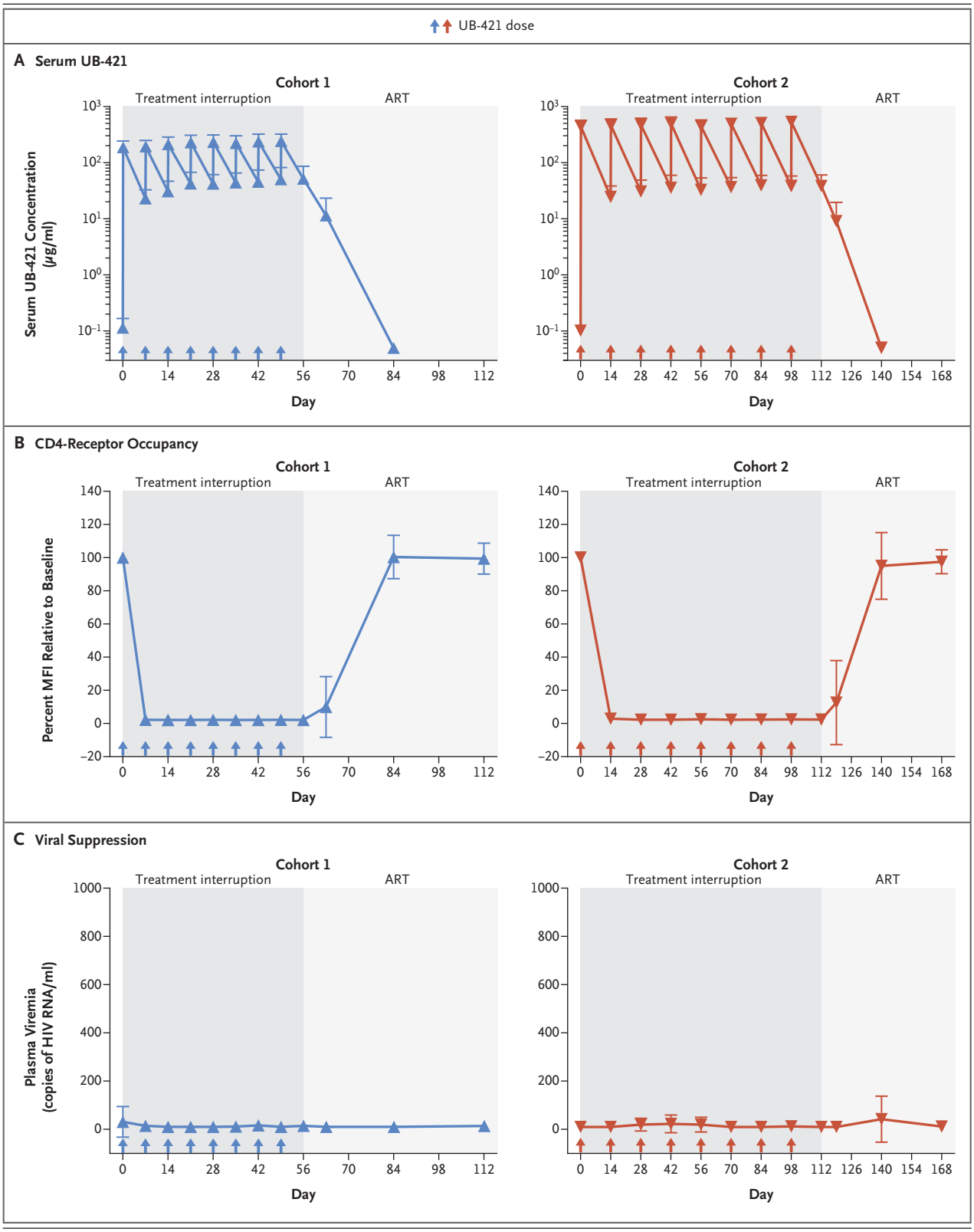

Figure 3 (facing page). Serum UB-421 Concentration, CD4-Receptor Occupancy, and Viral Suppression with UB-421 Monotherapy during Analytic Treatment Interruption and after Reinitiation of ART.

Data are for 14 participants in Cohort 1 and 13 participants in Cohort 2. Panel A shows the time course of serum UB-421 concentrations. The troughs and peaks were measured by a validated enzyme-linked immunosorbent assay. The mean trough and peak concentrations at each visit are depicted here (also see Table S7 in the Supplementary Appendix); throughout the figure, triangles indicate mean values, and the vertical bars indicate standard deviations. Panel B shows the time course of free CD4 receptors left after UB-421 dose administration. The CD4 receptors on T cells were rapidly occupied (coated) by UB-421, leaving no free binding sites for the UB-421–Alexa488 probe, expressed as 0% mean fluorescence intensity (MFI); at the end of analytic treatment interruption, when UB-421 therapy was terminated and ART reinitiated, the free binding sites (unbound CD4 receptors) were once again available. Panel C shows the time course of plasma viremia after UB-421 dose administration. The viremia remained suppressed by UB-421 during analytic treatment interruption and after ART was reinitiated.

The percentage of CD4+ Tregs in all the participants declined on administration of UB-421. The mean levels of reduction ranged from 31 to 56% from baseline during the treatment phase and then returned to baseline levels (Fig. S7 and Table S9 in the Supplementary Appendix). Further flow-cytometric analysis showed that the Tregs lacked $CD127^{neg\text{-}low}$ expression[16] and that the $CD4+CD25+CD127^{neg\text{-}low}FoxP3+$ Treg population was transiently lower in both cohorts at the end of UB-421 treatment than at baseline (Fig. S8 in the Supplementary Appendix).

DISCUSSION

In this study, UB-421 monotherapy maintained suppression of plasma viremia (<20 copies per milliliter) in the absence of ART for up to 8 weeks in participants receiving 10 mg per kilogram every week and for up to 16 weeks in participants receiving 25 mg per kilogram every 2 weeks. Occasional low-level viral blips, which did not require specific treatment, were detected in eight participants (28%). No evidence of HIV resistance to UB-421 was observed, but this small study of short duration has limited power to detect these changes. Future clinical studies with continued monitoring for drug-resistant strains during long-term administration of UB-421 are needed to properly assess this concern.

A durable virologic control in the absence of ART, such as observed with the UB-421 monotherapy, has not been achievable with anti–HIV gp120 bNAbs as a single agent[9-11] owing to rapid viral rebound and the emergence of resistant mutations, which has prevented even the most potent of these antibodies from achieving ideal efficacy.[17] Ex vivo analyses that were performed on samples from a clinical study involving VRC01, in which UB-421 was used as a positive control for assessing the presence or emergence of resistance mutants,[9] have shown that none of the bNAbs tested, including VRC01, 3BNC117, 10-1074, and PGT121, had a viral inhibition capability similar to that of UB-421 (Fig. S9 in the Supplementary Appendix).

UB-421 has previously been shown to exhibit potency against all HIV isolates that were tested.[12,13] An earlier PhenoSense drug-resistance assay that was designed to examine more than 850 HIV isolates, including multidrug-resistant strains, has shown 100% inhibition by the parent murine B4 and the bioequivalent UB-421 antibody, independent of viral strains and coreceptor tropism. Thus, UB-421 would be expected to provide a comprehensive coverage of viruses.

UB-421 exhibits a CD4 domain 1–binding affinity (K_d, approximately 5.6×10^{-11} M) that is approximately 50 to 100 times as strong as that of HIV gp120 (Fig. S10 and Table S8 in the Supplementary Appendix). In addition, the binding affinity showed no variability among four studied ethnic populations (Fig. S11B in the Supplementary Appendix). This may reflect equal effectiveness of UB-421 in the treatment of HIV infections across various ethnic populations, despite differences of up to 50% in the CD4-receptor density (Fig. S11A in the Supplementary Appendix).

It has been shown that PRO-140, an anti-CCR5 mAb, suppressed HIV in 23 of 41 participants in an ART-substitution study[18] and reduced plasma viremia (by 1.0 to 1.7 $\log_{10}$) in persons who had not previously received treatment.[19] In addition, previous studies have shown that an anti–CD4 domain 2 mAb, ibalizumab, reduced plasma viremia by 1.0 $\log_{10}$ in infected per-

sons,[20,21] although subsequent rebounding HIV developed resistant mutations to the V5 region.[22] In a trial involving infected persons with multidrug-resistant HIV, ibalizumab in combination with ART achieved a reduction of plasma viremia by 1.6 $\log_{10}$ and virologic control in 17 of 40 participants.[23]

In addition to blocking viral entry, UB-421 may function in part by reducing the CD4+ Treg population. We found significant reduction of CD4+ Tregs (31 to 56%) (Fig. S7 and Table S9 in the Supplementary Appendix) along with a concomitant and reversible decrease in the expression of both cytotoxic T-lymphocyte–associated antigen 4 and programmed death 1 (Fig. S12 in the Supplementary Appendix) after administration of UB-421. Although a significant reduction of CD4+ Tregs could theoretically lead to enhanced HIV-specific cytotoxic T-lymphocyte responses, the clinical significance of such a modulation is unclear, and the role of Tregs[24,25] associated with UB-421 administration warrants further investigation.

Although UB-421 binds to CD4 on T cells in a region partially overlapping the binding site for major histocompatibility complex class II,[12] immune competency was not inhibited in either ART-stabilized persons (Fig. 2) or HIV-infected persons who had not previously received ART (Fig. S13 in the Supplementary Appendix). The fact that there were no significant changes in the ex vivo antigen-stimulated T-cell proliferation responses in peripheral-blood mononuclear cells of HIV-infected participants, both those who had not previously received ART and those who were ART-stabilized (Figs. S14 and S15 in the Supplementary Appendix), suggests the absence of any consistent, irreversible immunosuppression. Although neither deleterious immunologic perturbations nor serious side effects were seen in the study participants after repeated administration of anti-CD4 antibody, the determination of the longer-term safety of UB-421 will require clinical studies involving larger cohorts of HIV-infected persons and extended periods of monitoring.

In this small study, UB-421 monotherapy maintained viral suppression for up to 16 weeks without viral rebound in the absence of ART. Targeting a host viral receptor (CD4) complements direct antiviral strategies. Future studies are warranted to determine the longer-term safety and efficacy of UB-421 in HIV-infected persons.

Supported mostly by private funding from United Biomedical, United Biomedical Asia, and United BioPharma, and in part by a grant (104-EC-17-A-20-I5-0007) from the Ministry of Economic Administration, Taiwan, and by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the co-principal investigators — Yu-Ting Tseng, Chih-Chen Chou, Wei-Ru Lin, Po-Liang Lu, Tun-Chieh Chen, and Chung-Hao Huang — who participated in this clinical study, Ling Chien and Wing Chuang for medical-affairs support, and Hok-Hui Teng, I-An Lai, and Liz Chen for technical support.

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