

HIV

An open-label phase 1 clinical trial of the anti- $\alpha_4\beta_7$ monoclonal antibody vedolizumab in HIV-infected individuals

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Despite the substantial clinical benefits of antiretroviral therapy (ART), complete eradication of HIV has not been possible. The gastrointestinal tract and associated lymphoid tissues may play an important role in the pathogenesis of HIV infection. The integrin $\alpha_4\beta_7$ facilitates homing of T lymphocytes to the gut by binding to the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on venules in gut-associated lymphoid tissue. CD4⁺ T cells with increased expression of $\alpha_4\beta_7$ are susceptible to HIV infection and may be key players in subsequent virus dissemination. Data from nonhuman primate models infected with simian immunodeficiency virus (SIV) have suggested that blockade of the $\alpha_4\beta_7$ /MAdCAM-1 interaction may be effective at preventing SIV infection and may have beneficial effects in animals with established viral infection. To explore whether these findings could be reproduced in HIV-infected individuals after interruption of ART, we conducted an open-label phase 1 clinical trial of vedolizumab, a monoclonal antibody against $\alpha_4\beta_7$ integrin. Vedolizumab infusions in 20 HIV-infected individuals were well tolerated with no serious adverse events related to the study drug. After interruption of ART, the median time to meeting protocol criteria to restart therapy was 13 weeks. The median duration of plasma viremia of <400 copies/ml was 5.4 weeks. Only a single subject in the trial experienced prolonged suppression of plasma viremia after interruption of ART. These results suggest that blockade of $\alpha_4\beta_7$ may not be an effective strategy for inducing virological remission in HIV-infected individuals after ART interruption.

INTRODUCTION

Over the past two decades, advances in the treatment of HIV infection with antiretroviral therapy (ART) have led to marked improvements in clinical outcomes for HIV-infected individuals (1). However, eradication of the virus has not been possible with ART alone due to the persistence of viral reservoirs in the peripheral blood and other body compartments of HIV-infected individuals (2). Despite the success of ART in suppressing HIV replication and plasma viremia, the burden of taking daily medication for life, the long-term toxicity of ART, and the potential for emerging resistance to antiretroviral drugs necessitate a continued search for effective alternatives in the absence of ART for achieving durable control of viral replication in HIV-infected individuals.

The gastrointestinal tract and associated lymphoid tissues appear to play an important role in the pathogenesis of HIV infection (3, 4). During acute HIV and simian immunodeficiency virus (SIV) infection, there is rapid depletion of CD4⁺ T cells from gut-associated lymphoid tissues, damage to the gut epithelium, and formation of viral reservoirs in the CD4⁺ T cell compartment (4, 5). These changes persist into the chronic phase of HIV infection and are not fully reversed by clinically effective ART (6).

Homing of effector T lymphocytes to gut-associated lymphoid tissues is facilitated by the integrin $\alpha_4\beta_7$, which is expressed on the surface of a subset of T lymphocytes. Integrin $\alpha_4\beta_7$ enables homing of CD4⁺ T lymphocytes to the gut by binding to mucosal addressin cell adhesion molecule-1, an adhesion molecule preferentially expressed on venules in gut-associated lymphoid tissues (7). In addition to preferentially migrating to gut-associated lymphoid tissues, $\alpha_4\beta_7$ ⁺ CD4⁺ T cells are highly susceptible to productive HIV infection in vitro (8). Furthermore, it has been shown that HIV gp120 can bind to $\alpha_4\beta_7$ integrin expressed on CD4⁺ T cells leading to rapid activation of lymphocyte function-associated antigen 1, the integrin involved in the establishment of virological “synapses” and in facilitating cell-to-cell transmission of infection (9). Thus, $\alpha_4\beta_7$ ⁺ CD4⁺ T cells are in a prime position to disseminate HIV between gut-associated lymphoid tissues and the periphery and represent a potential target for both prevention and treatment of HIV infection.

The therapeutic efficacy of anti- $\alpha_4\beta_7$ monoclonal antibody (mAb) in preventing transmission of SIV has been evaluated in a nonhuman primate model. Initial results demonstrated that administration of anti- $\alpha_4\beta_7$ mAb before and during acute infection provided protection from transmission in some rhesus macaques after repeated low-dose intravaginal challenge with SIV (10). In those anti- $\alpha_4\beta_7$ mAb-treated animals that did become infected, gut-associated lymphoid tissues were partially protected from SIV infection, and CD4⁺ T cell numbers were maintained in both peripheral blood and gut-associated lymphoid tissues compared with those animals that became infected but did not receive anti- $\alpha_4\beta_7$ mAb infusions. Further evidence supporting a role of $\alpha_4\beta_7$ in the pathogenesis of HIV infection comes from a recent report, demonstrating that the frequency of $\alpha_4\beta_7$ ⁺ CD4⁺ T cells in

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peripheral blood was independently associated with increased rates of HIV acquisition in a cohort of South African women at high risk for infection (11). Higher preinfection numbers of $\alpha_4\beta_7^+$ CD4⁺ T cells also predicted greater plasma viremia and an increased rate of CD4⁺ T cell decline after infection in this cohort (11). Together, these findings suggest that antibodies directed against $\alpha_4\beta_7$ integrin might have potential as an immune-based therapy for HIV infection.

To further delineate the role of $\alpha_4\beta_7$ in established SIV infection, a preclinical study in rhesus macaques was undertaken in which infected animals were treated with ART and a limited course of either a simianized anti- $\alpha_4\beta_7$ mAb or an isotype control (12). Three of the animals in the anti- $\alpha_4\beta_7$ mAb treatment group developed antibodies against the simianized anti- $\alpha_4\beta_7$ mAb and were not included in the data analysis. All of the eight remaining animals in the anti- $\alpha_4\beta_7$ treatment group showed suppressed plasma viremia for more than 9 months after cessation of ART and antibody infusions, whereas plasma viremia promptly rebounded to pre-ART levels in all animals treated with an isotype control antibody (12). This study suggested that a limited course of treatment with anti- $\alpha_4\beta_7$ mAb and ART allowed SIV-infected macaques to control plasma viremia for a prolonged period of time after cessation of ART and without additional mAb infusions.

Vedolizumab is a humanized anti- $\alpha_4\beta_7$ mAb that is effective in the treatment of inflammatory bowel disease (13, 14) and contains the same complementarity-determining regions as the simianized mAb used in the macaque studies (15, 16). To explore whether the sustained suppression of plasma viremia after ART interruption reported in the nonhuman primate study could be reproduced in humans, we conducted an open-label phase 1 clinical trial of vedolizumab in 20 individuals with established HIV-1 infection whose viremia was successfully suppressed with ART.

RESULTS

Study participants and safety analyses

This study was an open-label, single-arm phase 1 clinical trial to assess the safety and virological effects of vedolizumab (Entyvio) in subjects with HIV infection undergoing interruption of ART (Fig. 1). Between September 2016 and October 2017, a total of 20 HIV-infected subjects were enrolled in the study and received at least one dose of vedolizumab. One subject was withdrawn from the study after his first antibody infusion for noncompliance with ART. This subject's plasma viremia immediately before his first infusion was 20,026 copies of HIV RNA/ml. The subject subsequently admitted to stopping his ART several weeks after his protocol screening visit. He was removed from the study and was not included in the primary or secondary end point analysis. Clinical features of the remaining

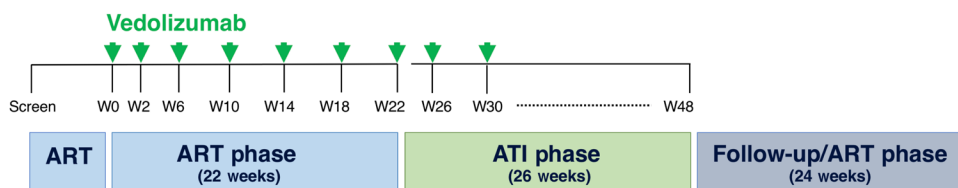


Fig. 1. Clinical trial design. Nineteen study participants received intravenous infusions of vedolizumab (300 mg) for 30 min at weeks 0, 2, 6, 10, 14, 18, 22, 26, and 30. Antiretroviral therapy (ART) was discontinued after the infusion at week 22. During the analytical treatment interruption phase (ATI), CD4⁺ T cell counts and plasma viremia were monitored every 2 weeks. The green arrows indicate time of vedolizumab administration in weeks.

19 subjects are shown in Table 1. Three subjects (03, 10, and 13) had started ART within 90 days of being diagnosed with early/acute HIV infection (17). For the remaining subjects, the time between acquisition of HIV infection and start of ART was unknown.

Study participants received 4 to 9 (median, 9) vedolizumab infusions, and no adverse events occurred during the infusion or immediate postinfusion period. No subject discontinued participation in the study due to a vedolizumab-related adverse event. There were no serious adverse events related to vedolizumab treatment. There was a single grade 3 adverse event judged as possibly related to vedolizumab. Subject 19 developed acute gastroenteritis 19 days after his eighth vedolizumab infusion. For the first 24 hours of the illness, diarrhea was grade 3 (>10 stools/day). He was empirically treated with ciprofloxacin for presumed infectious diarrhea, and his illness was resolved within 7 days. A complete listing of adverse events is shown in table S1.

Interruption of ART after vedolizumab infusions

Virological efficacy of the vedolizumab infusions was determined by conducting an analytical treatment interruption in which ART was discontinued in all participants at study week 22 (Fig. 1). Participants were then monitored closely for return of plasma viremia after interruption of ART. One subject withdrew from the study for personal reasons after receiving four infusions of vedolizumab but before entering the analytical treatment interruption phase of the study. This subject was not included in the virological end point analysis. Thus, 18 subjects discontinued ART at study week 22 per protocol and were included in the virological end point analysis. Thirteen of these subjects (72%) met criteria for restarting ART before week 26 of the analytical treatment interruption phase. Eleven subjects met virological criteria (>1000 copies of HIV RNA/ml for >4 weeks), and two subjects experienced a >30% decline in CD4⁺ T cell count in the setting of high plasma viremia (143,439 and 1,033,158 copies of HIV RNA/ml; Fig. 2A). The median time from stopping ART to meeting restart criteria for these 13 subjects was 11 weeks with an interquartile range (IQR) of 7.7 to 17.7. The median plasma viremia at the time of restarting ART was 8094 copies of HIV RNA/ml (IQR, 3274 to 46,390). All three subjects (03, 10, and 13) who had started ART within 90 days of being diagnosed with early/acute HIV infection met ART restart criteria before week 26 of the analytical treatment interruption phase (Fig. 2A).

Five subjects (02, 06, 08, 18, and 20) reached week 26 of the analytical treatment interruption period without meeting criteria to restart ART (Fig. 2A). Medical records for subject 08 documented a low-plasma viremia (1843 copies of HIV RNA/ml) before first initiating ART (28 months before study entry). Documentation of pre-ART plasma viremia was not available for the other four subjects. Per protocol, subjects with detectable plasma viremia (>40 copies of HIV RNA/ml) at week 26 of the analytical treatment interruption phase were instructed to restart ART. Three of these five subjects restarted ART between weeks 26 and 30 of the analytical treatment interruption phase due to persistently detectable plasma viremia. Subject 20 chose not to restart ART at week 26 and withdrew from the study (plasma viremia at time of withdrawal was 5790 copies of

Table 1. Baseline characteristics of study participants. NNRTI, non-nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; INSTI, integrase strand transfer inhibitor.

Characteristics	Vedolizumab (n = 19)	Historical controls* (n = 16)	P value**
Sex, number (%)			0.49
Male	17 (89)	16 (100)	
Female	2 (11)		
Age, years			0.55
Median (interquartile range)	42 (32, 52)	42 (32, 48)	
Range	29–59	24–65	
Race or ethnic group, number (%)			0.51
African-American	4 (21)	2 (12.5)	
Caucasian	12 (63)	10 (62.5)	
Hispanic	3 (16)	2 (12.5)	
Asian	0	2 (12.5)	
Antiretroviral regimen, number (%)			0.45
NNRTI	4 (21)	7 (44)	
PI	2 (11)	2 (12)	
PI/INSTI	1 (5)	0	
INSTI	12 (63)	7 (44)	
Duration of HIV suppression on ART at study entry			0.15
Years, median (interquartile range)	8 (5, 12)	4.8 (2.7, 10.3)	
Range	2.1–21	1.1–13	
CD4 ⁺ T cell count			0.24
Cells/mm ³ at study entry, median (interquartile range)	870 (741, 945)	758 (596, 1165)	
Range	597–1197	501–2162	

*Historical participants in the placebo arm of a therapeutic vaccine trial (18). Fisher's exact test (sex, race/ethnic group, and antiretroviral drug regimen).

**Wilcoxon rank sum test (age, ART duration, and CD4⁺ T cell count) and

HIV RNA/ml). The remaining subject (02) maintained sustained suppression of plasma viremia of <40 copies of HIV RNA/ml until 46 weeks after stopping ART. This subject subsequently developed persistently detectable plasma viremia necessitating reinstitution of ART.

In a post hoc analysis, time from cessation of ART to meeting restart criteria and time to first plasma viremia of >400 copies of HIV RNA/ml for the 18 vedolizumab-treated subjects were compared to a group of historical controls who underwent analytical treatment interruption with identical virological monitoring while participating in the placebo arm of a therapeutic vaccine study reported by us that enrolled subjects who began ART during early infection (18). There was no evidence for a significant effect of vedolizumab on either time to restart ART or time to first plasma viremia of >400 copies of HIV RNA/ml when compared to these historical controls (Fig. 2, B and C).

Effect of vedolizumab on T cell populations

Figure 3A and fig. S1 depict the pattern of CD4⁺ T cell counts across four time points: baseline, study week 10 (after three infusions of vedolizumab), study week 22 (after six vedolizumab infusions before analytical treatment interruption), and end-of-study follow-up (after

reinitiation of ART). The median CD4⁺ T cell count at week 10 was higher than that at the baseline ($P = 0.009$). However, this difference was no longer significant at week 22. In addition, there was no significant difference in the CD4⁺ T cell counts at baseline and after analytical treatment interruption (last study time point).

Baseline CD4⁺ T cell counts and percentage of CD8⁺ T cells co-expressing CD38 and HLA-DR (CD38⁺HLA-DR⁺CD8⁺) were compared between the 5 participants who did not meet ART restart criteria before week 26 of analytical treatment interruption and the 13 participants who met restart criteria before this time point (table S2). There were no significant differences between these two groups in baseline CD4⁺ T cell counts ($P = 0.44$) or percentage of CD38⁺HLA-DR⁺CD8⁺ T cells at baseline ($P = 0.12$) before starting analytical treatment interruption ($P = 0.66$) or at the time of restarting ART ($P = 0.18$).

The percentage of CD4⁺ T cells expressing β_7 was determined throughout the study period. A significant down-regulation of β_7 expression on CD4⁺ T cells was observed after the first infusion of vedolizumab compared to baseline ($P < 0.0001$; Fig. 3B). The down-regulation of β_7 by CD4⁺ T cells persisted through week 22; however, it returned to baseline within a median of 29 weeks after the last vedolizumab infusion ($P = 0.93$).

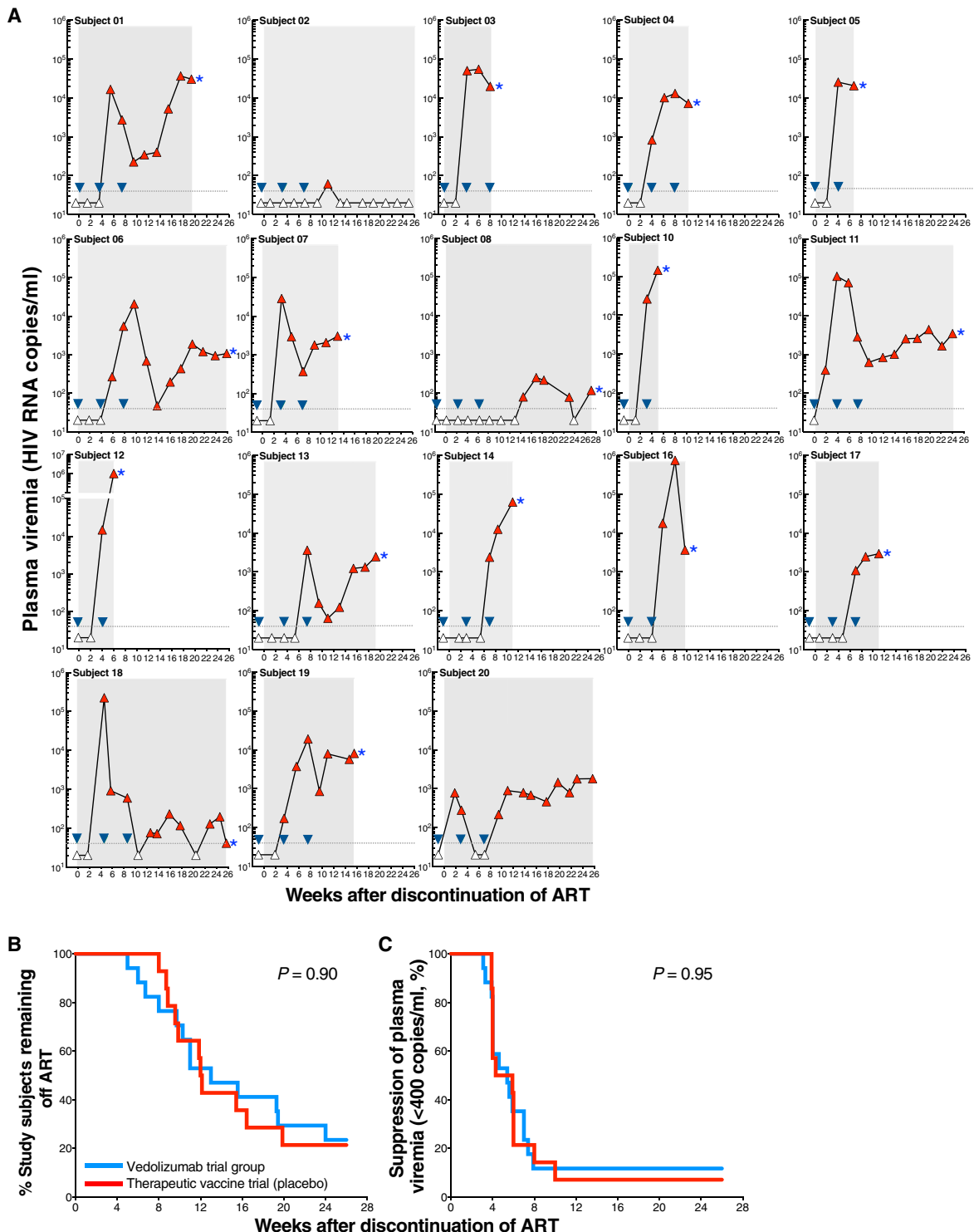


Fig. 2. Effect of vedolizumab administration on plasma viremia after discontinuation of antiretroviral therapy. (A) Longitudinal plasma viremia of study participants during the ATI phase is shown. The gray dotted horizontal line indicates the limit of detection of the assay (40 HIV RNA copies/ml). The white triangles indicate undetectable plasma viremia. The gray shaded areas indicate duration of ATI. The blue asterisks indicate the time point at which ART was reinitiated. (B) Kaplan-Meier analysis of the percentage of study participants ($n = 18$) remaining off ART during the treatment interruption phase of the study compared to historical participants ($n = 15$) in the placebo arm of a therapeutic vaccine trial (18). The x axis indicates the time in weeks since stopping ART. P value was calculated by log-rank test. (C) Kaplan-Meier analysis of suppression of plasma viremia after cessation of ART. Duration of plasma viremia under 400 HIV RNA copies/ml after discontinuation of ART was compared between the vedolizumab study participants ($n = 18$) and historical controls from the placebo arm of a therapeutic vaccine trial ($n = 15$) (18). The graph depicts the percentage of participants in both groups with plasma viremia of <400 HIV RNA copies/ml (y axis) as a function of time after discontinuation of ART (x axis). P value was calculated by log-rank test.

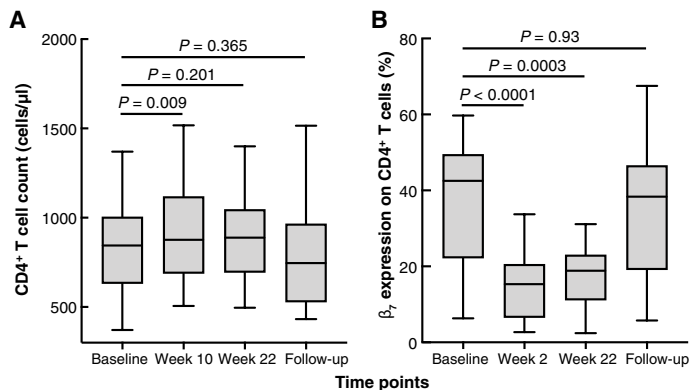


Fig. 3. Effect of vedolizumab administration on CD4⁺ T cell counts and β₇ expression. (A) Shown are CD4⁺ T cell counts of study participants who received vedolizumab infusions over a 22-week period. (B) Shown is β₇ expression on peripheral blood CD4⁺ T cells from study participants. Blood samples taken at baseline and at weeks 10 and 22 of vedolizumab infusions were obtained, while the study participants were receiving ART. Blood samples taken at follow-up time points were obtained after the study participants reinitiated ART at the end of the analytical treatment interruption phase. *P* values were computed using the Wilcoxon signed-rank test.

Vedolizumab pharmacokinetics and immunogenicity

Serum concentrations of vedolizumab measured before each monthly infusion (Fig. 4) were similar to those reported in phase 3 trials of patients with inflammatory bowel disease. The median trough vedolizumab concentration at steady state in our study was 42.3 μg/ml compared to 34.8 and 38.3 μg/ml in patients with ulcerative colitis (13) and Crohn's disease (14), respectively, who received vedolizumab infusions every 4 weeks (Fig. 4). Serum was analyzed for anti-vedolizumab antibodies for each subject at weeks 0 (before first vedolizumab infusion), 6, 14, and 22 or 30. No subject had detectable anti-vedolizumab antibodies at baseline or at any of the postinfusion time points.

Effect of vedolizumab on the HIV reservoir

To examine the effect of vedolizumab on the size of the viral reservoir, the frequency of peripheral blood CD4⁺ T cells carrying HIV DNA and cell-associated HIV RNA was measured throughout the infusion period up to the point of discontinuation of ART. There were no significant changes in the frequency of peripheral blood CD4⁺ T cells carrying HIV proviral DNA or cell-associated HIV RNA (Fig. 5, A and B, respectively).

DISCUSSION

Here, we report the results of an open-label phase 1 clinical trial examining the safety and viral suppressive activities of vedolizumab in HIV-infected individuals who had been receiving clinically effective ART. We demonstrated that administration of monthly vedolizumab infusions was well tolerated with no safety concerns identified. Treatment with vedolizumab resulted in down-regulation of surface α₄β₇ expression on CD4⁺ T lymphocytes that was observed after the first infusion and persisted for a median of 29 weeks after the final infusion. This finding is consistent with both in vitro observations that vedolizumab results in rapid internalization of α₄β₇ on lymphocytes, followed by reexpression of the integrin after the vedolizumab is removed (16), and in vivo pharmacodynamic studies of vedolizumab in patients with inflammatory bowel disease (19).

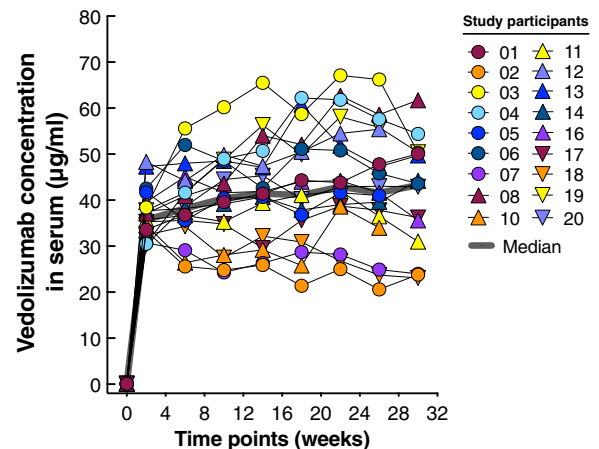


Fig. 4. Pharmacokinetics of vedolizumab in study participants. Blood samples from HIV-infected patients on ART were obtained at baseline and at the indicated time points during vedolizumab treatment. Serum concentrations of vedolizumab were determined using a validated sandwich enzyme-linked immunosorbent assay. Thick gray line represents the median value for vedolizumab serum concentrations for the group of study participants.

We did not reproduce, however, the marked antiviral effects observed in the preclinical nonhuman primate study in which a limited course of treatment with ART and anti-α₄β₇ mAb resulted in sustained suppression of SIV plasma viremia in all animals that persisted long after all treatment was discontinued (12). Although five subjects in our phase 1 clinical trial remained off of ART for 26 weeks without meeting virological criteria for restarting antiretroviral drugs (>1000 copies of HIV RNA/ml for >4 weeks), only one subject exhibited sustained suppression of plasma viremia (<40 HIV RNA copies/ml) comparable to what was observed in the nonhuman primate study. It is plausible that treatment with vedolizumab played a role in this subject's prolonged control of plasma viremia. However, prolonged control of plasma viremia after interruption of ART has been reported to occur in up to 4% of HIV-infected individuals who initiated ART during the chronic stage of infection and who received no additional intervention (20). It is also plausible that a number of immunological and virological factors may influence the kinetics of plasma viral rebound after analytical treatment interruption. Thus, it is not possible to determine what role, if any, vedolizumab played in this subject's prolonged control of HIV replication after ART interruption.

There are several possible explanations as to why our vedolizumab trial in humans could not reproduce the viral suppression reported for anti-α₄β₇ treatment in SIV-infected macaques (12). In that study, animals were infected with an attenuated SIV_{mac239} with a stop codon engineered in the *nef* gene (12). Furthermore, ART was initiated in all animals in the early phase of chronic infection shortly after reaching steady-state plasma viremia. This may have allowed time for the generation of an antiviral immune response that would not have been possible if a wild-type virus was used or if initiation of ART had been delayed. In contrast, our subjects were infected with HIV, and we were unable to determine when ART was initiated in relation to primary infection for most of our participants. However, it is likely that most subjects in our study began therapy in the chronic stage of HIV infection. Thus, use of an attenuated SIV_{mac239} strain and timing of ART initiation relative to time of infection with SIV may have contributed to

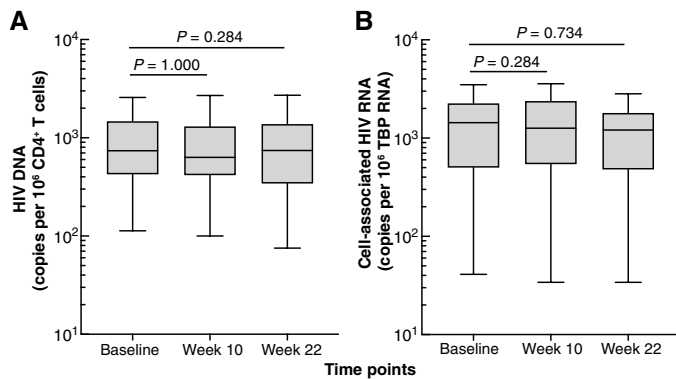


Fig. 5. Effect of vedolizumab on the HIV reservoir during antiretroviral drug treatment. (A) HIV DNA in peripheral blood CD4⁺ T cells from study participants was measured before discontinuation of ART. The frequency of CD4⁺ T cells carrying HIV DNA was determined by droplet digital PCR. (B) Cell-associated HIV RNA in peripheral blood CD4⁺ T cells from study participants was measured before discontinuation of ART. Cell-associated HIV RNA copy number was determined by droplet digital PCR and was normalized per 1×10^6 TATA-box binding protein (TBP) RNA. *P* values were computed using the Wilcoxon signed-rank test.

differing results between the preclinical nonhuman primate study and our trial. All three subjects in our trial who began ART during acute/early HIV infection experienced sustained rebound of plasma viremia after treatment interruption and required reinstatement of ART before week 26 of the analytical treatment interruption period.

Although vedolizumab and the simianized anti- $\alpha_4\beta_7$ mAb are both derived from the ACT-1 mAb clone and contain the same complementarity-determining regions, they differ in the Fc region. Vedolizumab was engineered to contain point mutations in the Fc γ binding motif that reduced binding to Fc γ receptors. These mutations were introduced to ensure that vedolizumab did not cause *in vivo* depletion of $\alpha_4\beta_7^+$ CD4⁺ T cells through Fc receptor-mediated effector functions (16, 19). In contrast, the simianized anti- $\alpha_4\beta_7$ mAb has a wild-type simian Fc region. Despite containing no Fc mutations, the simianized anti- $\alpha_4\beta_7$ mAb, similar to vedolizumab, does not appear to cause depletion of $\alpha_4\beta_7^+$ CD4⁺ T cells *in vivo* (15, 21). However, it is still conceivable that the wild-type simian Fc region of the anti- $\alpha_4\beta_7$ mAb played a direct or indirect role in the induction of an antiviral immune response in the nonhuman primate study that was not seen in our human study.

Given that our current study involved interruption of ART in humans, safety considerations necessitated our having predefined criteria for restarting therapy. Although six anti- $\alpha_4\beta_7$ -treated animals in the nonhuman primate study exhibited transient low-level “blips” in plasma viremia before achieving sustained virological suppression, all six of these animals regained control of viremia within 4 weeks (12). Thus, none of the animals exhibiting intermittent rebounds of plasma viremia would have met our protocol criteria to restart ART. Nonetheless, we cannot rule out the possibility that one or more of the subjects in our trial may have regained control of plasma viremia had not the protocol required that we restart ART.

Treatment with vedolizumab had no measurable impact on the size of the HIV reservoir as measured by HIV DNA and cell-associated HIV RNA in peripheral blood CD4⁺ T cells. However, we cannot exclude the possibility that vedolizumab infusions led to a measurable change in the HIV reservoir that could only be detected by other assays specific for replication-competent virus.

Such an effect, if present, would be unlikely to have clinical significance, given that vedolizumab did not prevent plasma viral rebound nor lead to sustained suppression of plasma viremia in the absence of ART.

Last, our study was designed to detect a large and obvious positive effect of vedolizumab on plasma viral rebound comparable to what was reported in SIV-infected macaques treated with anti- $\alpha_4\beta_7$ mAb (12). Because of the small size of our study and the lack of a control group, a smaller effect of vedolizumab on plasma viral rebound in humans cannot be excluded by our results.

Recently, three independent laboratories conducted studies in SIV-infected rhesus macaques (22–24) that attempted to reproduce and extend the results reported in the initial animal study involving anti- $\alpha_4\beta_7$ mAb treatment (12). All of these studies used the same anti- $\alpha_4\beta_7$ mAb as the original study. Two of the studies (23, 24) used the same attenuated SIVmac239 viral strain with a 172 stop codon in the *nef* gene as the original report (12); one study used a wild-type SIVmac251 strain (22). None of these three studies demonstrated a beneficial effect of anti- $\alpha_4\beta_7$ mAb treatment on plasma viral rebound after interruption of ART. In addition, anti- $\alpha_4\beta_7$ mAb treatment had no impact on SIV proviral DNA in lymph nodes or gut tissue (22–24).

The present study was undertaken to determine whether the marked antiviral effect reported in an initial study of anti- $\alpha_4\beta_7$ mAb treatment of SIV-infected rhesus macaques (12) could be reproduced in HIV-infected humans. Despite using a similar study design and treatment regimen, we were unable to convincingly demonstrate a durable suppressive effect of vedolizumab treatment on rebound of plasma viremia after interruption of ART. Our clinical trial results are consistent with those reported in three studies of anti- $\alpha_4\beta_7$ mAb treatment in SIV-infected macaques (22–24). Together, these results suggest that mAb treatment targeting the $\alpha_4\beta_7$ integrin is unlikely to be an effective strategy for inducing sustained ART-free control of HIV infection.

MATERIALS AND METHODS

Study design

This was an open-label, single-arm phase 1 clinical trial to assess the safety and virological effect of vedolizumab (Entyvio) in subjects with HIV infection undergoing analytical treatment interruption (Fig. 1). The vedolizumab was supplied by Takeda Pharmaceuticals International. The study was conducted at the National Institutes of Health Clinical Research Center in Bethesda, Maryland (ClinicalTrials.gov no. NCT02788175). HIV-infected subjects were eligible if they were 18 to 65 years of age and in general good health. Participants were required to have a CD4⁺ T cell count of >450 cells/mm³ and be on a stable ART regimen with documented suppression of plasma viremia below the limits of assay detection for at least 2 years before enrollment. Patients with hepatitis B virus or hepatitis C virus coinfection were excluded as were participants with a history of nadir CD4⁺ T cell count of <200 cells/ μ l or an AIDS-defining condition.

The clinical protocol was approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health. All subjects provided written informed consent. The study team conducted weekly review of all adverse events. After reviewing reported adverse events, the study team assessed the relationship of the adverse events to the vedolizumab infusions.

Treatment phase (study weeks 0 to 30)

Eligible subjects received intravenous infusions of vedolizumab (300 mg) at weeks 0, 2, 6, 10, 14, 18, 22, 26, and 30 (Fig. 1).

Analytical treatment interruption phase

At study week 22, all subjects discontinued ART. Individuals taking non-nucleoside reverse transcriptase inhibitors were switched to a protease inhibitor or integrase inhibitor-based regimen for 2 weeks before the discontinuation of ART to ensure that the washout period of antiretroviral agents was roughly equal. During the treatment interruption phase, subjects were closely monitored every 2 weeks by measuring CD4⁺ T cell counts and plasma viremia. Criteria to restart ART during the treatment interruption phase were a sustained (>4 weeks) plasma viremia of >1000 copies of HIV RNA/ml, a confirmed >30% decline in CD4⁺ T cell count, an absolute CD4⁺ T cell count of <350 cells/mm³, or the development of acute retroviral syndrome. Participants who reached study week 48 (26 weeks after stopping ART) without meeting restart criteria were advised to restart treatment if they had detectable (>40 copies/ml) plasma viremia.

Study end points

The primary predefined safety end point was the rate of occurrence of grade 3 or higher adverse events, including severe adverse events, which were at least possibly related to the vedolizumab infusions. Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.0, November 2014. The predefined virological end point was the number of subjects who experienced rebound of plasma viremia after analytical treatment interruption and met criteria to restart ART before week 48 (26 weeks after stopping ART).

Quantitation of HIV DNA

The level of CD4⁺ T cells carrying HIV DNA was determined by droplet digital PCR. Genomic DNA was isolated from enriched CD4⁺ T cells and subjected to restriction digestion (MscI, New England BioLabs), followed by droplet digital PCR (Bio-Rad Laboratories) according to the manufacturer's specifications. PCR primers and probe used for amplification of HIV DNA (HXB2, K03455.1) were the following: 5' primer, 5'-GRAACCCACTGCTTAAGCCTCAA-3' (506 to 528 nt); 3' primer, 5'-TGTTTCGGGCGCCACTGCTAGAGA-3' (648 to 626 nt); and the fluorescent probe, 5'-6FAM-AGTAGTGTGTGCCCGTCTGTT-IABkFQ-3' (552 to 572 nt). PCR primers and probe used for amplification of housekeeping gene Ribonuclease P/MRP subunit p30 (RPP30, NM_001104546.1) were the following: 5' primer, 5'-GATTTGGACCTGCGAGCG-3' (nt, 51 to 68); 3' primer, 5'-GC-GGCTGTCTCCACAAGT-3' (112 to 95 nt); and the fluorescent probe, 5'-HEX-TTCTGACCTGAAGGCTCTGCGC-IABkFQ-3' (71 to 92 nt).

HIV DNA copy numbers were normalized per 1×10^6 CD4⁺ T cells.

Quantitation of cell-associated HIV RNA

RT-PCR was carried out to determine the level of CD4⁺ T cells carrying cell-associated HIV RNA. Total RNA was isolated from enriched CD4⁺ T cells (RNeasy Mini Kit, QIAGEN) and subjected to cDNA synthesis using qScript XLT cDNA SuperMix (Quanta BioSciences) as per the manufacturer's specifications. cDNA was subjected to droplet digital PCR (Bio-Rad Laboratories) using HIV-specific primers (HXB2, K03455.1): 5' primer, 5'-TCTCTAGCAGTGGCGCCCGAACA-3' (626 to 648 nt); 3' primer, 5'-TCTCCTTCTAGCCTCCGCTAGTC-3' (786 to 764 nt); and the fluorescent probe, 5'-6FAM-CAAGCCGAGTC-CTGCGTTCGAGAG-IABkFQ-3' (705 to 683 nt); and TATA-box binding protein (TBP; housekeeping gene, NM_003194.4)-specific primers: 5' primer, 5'-CACGAACCACGGCACTGATT-3' (899 to 918 nt); 3' primer, 5'-TTTTCTTGCTGCCAGTCTGGAC-3' (987 to

966 nt); and the fluorescent probe 5'-HEX-TGTGCACAGGAGC-CAAGAGTGAAGA/3-IABkFQ-3' (938 to 962 nt).

HIV RNA copy numbers were normalized per 1×10^6 copies of TBP.

Flow cytometry

Cryopreserved peripheral blood mononuclear cells were thawed, washed, and stained with the following fluorophore-conjugated antibodies to determine the level of β_7 expression on CD4⁺ T cells: CD3-APC-H7 (clone SK7, no. 560176, BD Biosciences), CD4-PerCP (clone SK3, no. 347324, BD Biosciences), β_7 -PE (clone FIB504, no. 555945, BD Biosciences), CD45RA-FITC (clone L48, no. 347723, BD Biosciences), and CD8-BV421 (clone RPA-T8, no. 562428, BD Biosciences). Data were acquired by BD FACSLyric cytometer using BD FACSuite clinical software (BD Biosciences) and analyzed using FlowJo version 10.1r5.

Pharmacokinetics and immunogenicity

Serum concentrations of vedolizumab were determined using a validated sandwich enzyme-linked immunosorbent assay. In this assay, a mouse anti-vedolizumab idiotype antibody was immobilized on microtiter plates to capture vedolizumab. After blocking the wells, the serum samples were added, and bound vedolizumab detected with F(ab')₂ mouse anti-human IgG was conjugated to horseradish peroxidase, followed by a colorimetric substrate. The lower limit of quantification of the assay was 0.2 μ g/ml in undiluted serum, the upper limit of quantification was 8 μ g/ml, and the minimum required dilution was 100 (1% serum).

The presence of antidrug antibodies (ADAs) was determined using a validated, bridging ECL assay following the MSD standard acid dissociation protocol. In this protocol, the sample is treated with acid to dissociate ADA complexed to drug and then neutralized in the presence of excess SUFO-TAG-labeled and biotinylated drug. The captured ADA/drug complexes are detected by MSD SECTOR instrument (Meso Scale Diagnostics).

Statistical methods

The log-rank test was used to compare vedolizumab-treated patients to historical controls with respect to time to meeting ART restart criteria and time to reaching a plasma viremia of ≥ 400 HIV RNA copies/ml. Changes in CD4⁺ T cell counts, percentage of CD38⁺HLA-DR⁺CD8⁺ T cells, and β_7 expression from baseline to various time points were evaluated using a Wilcoxon signed-rank test, as were changes from baseline in the frequency of CD4⁺ T cells carrying HIV DNA and cell-associated HIV RNA.

SUPPLEMENTARY MATERIALS

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Fig. S1. Effect of vedolizumab administration on immunological and virological parameters.

Table S1. List of adverse events.

Table S2. Comparison of baseline CD4⁺ T cell counts and percentages of CD38⁺HLA-DR⁺CD8⁺ T cell between participants who met ART restart criteria before or after week 26 of analytical treatment interruption.

Study protocol

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Acknowledgments: We thank the study volunteers for participation in this study, the National Institute of Allergy and Infectious Diseases HIV Outpatient Clinic staff for assistance in the execution of this clinical trial, and A. Nath and B. Smith for performing baseline neurological examinations on study participants. We are grateful to Takeda Pharmaceuticals Company Limited for supplying the vedolizumab (Entyvio) and for performing pharmacokinetic and ADA assays. **Funding:** This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH. **Author contributions:** M.C.S., J.R.M., M.S., H.C.L., T.-W.C., and A.S.F. conceived the clinical trial. M.C.S. and C.S. supervised the clinical trial. K.E.C., V.S., M.D.Z., J.S.J., J.B., E.D.H., and T.-W.C. obtained biological samples. K.E.C., V.S., M.D.Z., J.S.J., J.B., E.D.H., S.M., and T.-W.C. carried out biological assays. M.C.S., M.A.P., J.R.M., S.M., H.C.L., T.-W.C., and A.S.F. analyzed clinical and research data. M.C.S., T.-W.C., and A.S.F. wrote the paper. **Competing interests:** M.S. was a full-time employee of Takeda Pharmaceuticals Company Limited during the conduct of the trial and is currently a full-time employee at Ironwood Pharmaceuticals. J.R.M. is a full-time employee of Takeda Pharmaceuticals Company Limited. A.S.F. is a co-inventor on U.S. patent nos. 9,193,790, 9,441,041 and 9,896,509 entitled "Use of antagonists of the interaction between HIV gp120 and $\alpha_4\beta_7$ integrin" held by the National Institute of Allergy and Infectious Disease. These patents cover the therapeutic use of antagonists of the interaction between HIV gp120 and $\alpha_4\beta_7$ integrin. All other authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study are in the paper or the Supplementary Materials. Deidentified human data generated from this trial can be shared with approved outside collaborators under a materials transfer agreement; requests should be sent to T.-W.C., twchun@niaid.nih.gov.

Submitted 15 March 2019
Accepted 9 August 2019
Published First Release 5 September 2019
Published 11 September 2019
10.1126/scitranslmed.aax3447

Citation: M. C. Sneller, K. E. Clarridge, C. Seamon, V. Shi, M. D. Zorawski, J. S. Justement, J. Blazkova, E. D. Huiting, M. A. Proschan, J. R. Mora, M. Shetzline, S. Moir, H. C. Lane, T.-W. Chun, A. S. Fauci, An open-label phase 1 clinical trial of the anti- $\alpha_4\beta_7$ monoclonal antibody vedolizumab in HIV-infected individuals. *Sci. Transl. Med.* **11**, eaax3447 (2019).

An open-label phase 1 clinical trial of the anti- $\alpha_4\beta_7$ monoclonal antibody vedolizumab in HIV-infected individuals

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Sci Transl Med 11, eaax3447.
First published 5 September 2019
DOI: 10.1126/scitranslmed.aax3447

Targeting $\alpha_4\beta_7$ in HIV

Nonhuman primate models can be useful in the development of new approaches to treating HIV infection. Sneller *et al.* conducted a human therapeutic trial based on promising results of a single nonhuman primate study in which treatment with an anti-integrin monoclonal antibody led to sustained suppression of SIV plasma viremia. The human clinical trial did not reproduce the positive effects reported in the animal study. These results illustrate that embarking on human therapeutic trials based on findings from a single animal study should be undertaken cautiously and only after careful consideration of factors, such as reproducibility of the findings in animals and the potential risks associated with the experimental intervention.

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