A randomized controlled safety/efficacy trial of therapeutic vaccination in HIV-infected individuals who initiated antiretroviral therapy early in infection

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Despite substantial clinical benefits, complete eradication of HIV has not been possible using antiretroviral therapy (ART) alone. Strategies that can either eliminate persistent viral reservoirs or boost host immunity to prevent rebound of virus from these reservoirs after discontinuation of ART are needed; one possibility is therapeutic vaccination. We report the results of a randomized, placebo-controlled trial of a therapeutic vaccine regimen in patients in whom ART was initiated during the early stage of HIV infection and whose immune system was anticipated to be relatively intact. The objectives of our study were to determine whether the vaccine was safe and could induce an immune response that would maintain suppression of plasma viremia after discontinuation of ART. Vaccinations were well tolerated with no serious adverse events but produced only modest augmentation of existing HIV-specific CD4+ T cell responses, with little augmentation of CD8+ T cell responses. Compared with placebo, the vaccination regimen had no significant effect on the kinetics or magnitude of viral rebound after interruption of ART and no impact on the size of the HIV reservoir in the CD4+ T cell compartment. Notably, 26% of subjects in the placebo arm exhibited sustained suppression of viremia (<400 copies/ml) after treatment interruption, a rate of spontaneous suppression higher than previously reported. Our findings regarding the degree and kinetics of plasma viral rebound after ART interruption have potentially important implications for the design of future trials testing interventions aimed at achieving ART-free control of HIV infection.

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 of infected individuals (1). However, even with prolonged treatment, complete eradication of HIV has not been possible using ART alone, and the vast majority of HIV-infected individuals must remain on lifelong therapy. Persistence of HIV reservoirs carrying replication-competent virus despite suppression of plasma viremia with ART is considered to be the major obstacle to the eradication of HIV (2). Despite the success of ART in suppressing HIV replication and plasma viremia, the burden of taking daily medication for life, long-term toxicity of ART, and the potential for resistance to antiretroviral drugs necessitates a continued search for effective alternatives for achieving durable control of HIV replication in infected individuals. One strategy for achieving sustained suppression of HIV replication in the absence of ART is therapeutic vaccination.

The goal of therapeutic vaccination is to augment virus-specific immune responses using a controlled exposure to HIV antigens. A large number of phase 1/2 therapeutic HIV-1 vaccine trials have been conducted since the advent of effective ART (3). The vaccine regimens tested in these trials were found to be largely safe and inducing varying degrees of CD4+ and CD8+ T cell–mediated anti-HIV responses. However, in placebo-controlled studies that included interruption of ART to assess antiviral efficacy, no vaccine has been successful at achieving durable, clinically relevant suppression of HIV viremia in the absence of ART (4–12). Despite these negative results, therapeutic vaccination has the potential to become an effective immune-based therapy for HIV infection if improved vaccination strategies can be developed and implemented in selective categories of HIV-infected individuals.

Here, we report the results of a randomized, controlled trial of a therapeutic vaccine regimen in an HIV-infected population who began ART early in the course of infection. The objectives of our study were to determine whether the vaccine regimen was safe and could maintain suppression of HIV viremia after discontinuation of ART. We used a vaccine strategy that involved priming of the immune response with a plasmid DNA (pDNA) vaccine containing genes encoding multiple HIV proteins (clade B gag/pol and nef/tat/vif and env), followed by boosting with an attenuated live viral vector containing the HIV gag gene. This vaccination regimen has several features designed to maximize immunogenicity. First, the HIV-multiantigen DNA vaccine (HIV-MAG pDNA) was administered in combination with a DNA plasmid encoding the human interleukin (IL)–12 p35 and p40 proteins (IL-12 pDNA) to enhance the immunogenicity of the pDNA vaccine (13). Second, the HIV-MAG pDNA/IL-12 pDNA vaccines were administered using an in vivo electroporation delivery system designed to enhance the cellular uptake of pDNA (14). Preclinical studies in nonhuman primates provide compelling evidence that in vivo electroporation enhances the potency of the HIV pDNA vaccination (13, 15). Third, the booster
component of the vaccine uses an attenuated recombinant vesicular stomatitis virus (rVSVN4CT1) vector encoding HIV gag. Wild-type VSV rarely causes infections in humans; thus, the use of an attenuated rVSVN4CT1 vector minimized any interference of pre-existing immune responses in study subjects. Finally, we limited enrollment to subjects who began ART during the acute or early phase of HIV infection. Compared to individuals who initiated ART during the chronic phase of HIV infection, those treated during early infection may be more likely to respond to therapeutic vaccination because of relatively preserved immune function and a smaller reservoir of persistently infected CD4+ T cell (9, 16).

RESULTS
Study participants
Between September 2013 and February 2015, a total of 31 HIV-infected subjects from the United States and Canada were enrolled and randomized. One subject withdrew from the study before completing the vaccine phase and was not included in the primary or secondary endpoint analysis. A total of 30 subjects completed the vaccine phase of the study (Fig. 1A). The duration of ART before study enrollment showed a trend toward a longer period of treatment in the placebo group (P = 0.058). Otherwise, the two study arms were balanced at baseline (Table 1).

Safety
HIV-MAG pDNA, IL-12 pDNA vaccine, or placebo priming injections were administered at study weeks 0, 4, 12, and 36. The rVSVN4CT1gag booster vaccine or placebo injections were administered at study weeks 24 and 48 (Fig. 1B and Materials and Methods). The vaccinations were well tolerated, with the most common adverse events (AEs) being transient (<48 hours) grade 1 pain or tenderness at the injection site and mild-to-moderate (grade 1 or 2) transient reactogenicity symptoms (fatigue, myalgia, and arthralgia; table S1). No subject discontinued participation in the study because of vaccine-related AE. There was no grade 3 or higher AE including serious AEs (SAEs) that were judged possibly, probably, or definitely related to the study injections.

Antiretroviral treatment interruption
Figure 2A shows the level of longitudinal plasma viremia of each study participant in the vaccine (n = 14) and placebo (n = 15) groups during the analytical treatment interruption (ATI) phase. One subject in the placebo arm was lost to follow-up before entering the treatment interruption phase and did not contribute to the virologic endpoint analysis. Thus, 29 subjects discontinued ART at study week 56 and contributed to the virologic endpoint analysis. The efficacy endpoint of plasma viral load at the end of ART interruption was very similar in the two study arms (Fig. 2, A and B). The median plasma viral loads were 4932 and 3170 copies/ml in the vaccine and placebo arms, respectively (P = 0.406). Five subjects in the vaccine arm and one subject in the placebo arm met protocol criteria to restart ART before the protocol-defined end of the ART interruption phase at study week 72, which was 16 weeks after the interruption of ART (P = 0.042; Fig 2C). One additional subject in the placebo arm became concerned about his viral load (15,442 copies/ml), broke protocol, and restarted ART before the scheduled end of the treatment interruption phase. There was no evidence for differences between the vaccine and placebo arms in post hoc analyses that included time from cessation of ART to viral loads of ≥40 copies/ml (median, 28 versus 28 days; P = 0.644; Fig. 2C) and ≥400 copies/ml (median, 28 versus 30 days; P = 0.402; Fig. 2C) or the proportion of protocol visits with viral load of <400 copies/ml (P = 0.894; Fig. 2D). Figure 2E depicts the pattern of CD4+ T cell counts across three time points: baseline, end of the treatment interruption, and end of study. This pattern was significantly different between the two arms (P = 0.019), with the difference driven by a greater decrease in CD4+ T cell count from the baseline to the end of the treatment interruption in the vaccine arm. The difference in CD4+ T cell counts between study arms was no longer apparent at the end-of-study time point, likely reflecting the effect of restarting ART.

One subject (number 31) in the vaccine arm and three subjects (numbers 04, 17, and 30) in the placebo arm exhibited suppression of viremia to <40 copies/ml at the end of the 16-week ART interruption phase (Fig. 2B and fig. S1). In addition, two subjects (number 10 in the vaccine arm and number 9 in the placebo arm) had viral loads of <400 copies/ml at the...
end of the ART interruption phase. Thus, 4 of 15 (26%) subjects in the placebo arm and 2 of 14 (14%) in the vaccine arm exhibited suppression of viremia to <400 copies/ml at the end of the 16-week ART interruption phase (Fig. 2B). All of these subjects had an initial rebound viremia before exhibiting spontaneous resuppression of viremia (Fig. 2B and fig. S1). Subjects 4 and 30 in the placebo arm maintained the suppression of viremia to <40 copies/ml in the absence of ART until the end of the study (10 months after stopping ART; fig. S1B). The remaining two subjects who exhibited suppression to <40 copies/ml through the end of the treatment interruption phase [number 31 (vaccine) and number 17 (placebo)] subsequently had return of viremia before their final study visit (fig. S1). One subject (number 17) in the placebo arm who exhibited post-ART suppression had one human leukocyte antigen (HLA)–B57 allele; no study participant had the HLA-B27 allele.

There were no significant AEs related to ART interruption. No subject developed manifestations of acute retroviral syndrome or serious infections during the treatment interruption phase. No subject developed new drug resistance mutations, and all subjects restarting ART resuppressed viremia to <40 copies/ml.

Immunogenicity

Immunologic analyses were performed on 26 participants, which had specimens available at multiple time points. When evaluating the results by study arm (placebo versus vaccine recipients) as a whole, we observed greater increases from baseline measurements for the vaccine group in CD3+CD4+ T cell responses to several HIV-1 peptide pools, as measured by flow cytometry–based intracellular cytokine staining (ICS) for antigen-specific expression of interferon-γ (IFN-γ) and/or IL-2 (Table 2 and fig. S3). We found increases in IFN-γ– and/or IL-2–expressing CD3+CD4+ T cells to the Gag 1 peptide pool in 7 of 13 (54%) of vaccine recipients compared to in 0 of 13 (0%) of placebo recipients compared to baseline. Similarly, we found increases in IFN-γ– and/or IL-2–expressing CD3+CD4+ T cells to the Env 1 peptide pool in 4 of 12 (33%) of vaccine recipients compared to in 0 of 13 of placebo recipients. The vaccine was less effective at inducing increases in

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**Table 1. Baseline characteristics of participants.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 16)</th>
<th>Vaccine (n = 14)</th>
<th>Total (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (100)</td>
<td>14 (100)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>42 (32, 48)</td>
<td>40 (44, 26)</td>
<td>41.5 (46, 31)</td>
</tr>
<tr>
<td>Range</td>
<td>24–65</td>
<td>21–60</td>
<td>21–65</td>
</tr>
<tr>
<td>Inclusion criteria met*, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute infection</td>
<td>6 (38)</td>
<td>3 (21)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Early infection</td>
<td>10 (62)</td>
<td>11 (78)</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Reported seroconversion illness, no. (%)</td>
<td>9 (56)</td>
<td>9 (64)</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Time between HIV diagnosis and start of ART, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>25 (8, 53)</td>
<td>28 (3, 73)</td>
<td>28 (18, 46)</td>
</tr>
<tr>
<td>Range</td>
<td>0–88</td>
<td>0–88</td>
<td>0–88</td>
</tr>
<tr>
<td>Duration of HIV suppression on ART at study entry, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>4.8† (2.7, 10.3)</td>
<td>2.1 (1.7, 3.2)</td>
<td>3 (1.8, 6.6)</td>
</tr>
<tr>
<td>Range</td>
<td>1.1–13</td>
<td>1.2–19</td>
<td>1.1–19</td>
</tr>
<tr>
<td>CD4+ count, cells/mm³ at study entry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range)</td>
<td>758 (596, 1165)</td>
<td>726 (610, 942)</td>
<td>749 (604, 942)</td>
</tr>
<tr>
<td>Range</td>
<td>501–2162</td>
<td>363–1017</td>
<td>363–2162</td>
</tr>
<tr>
<td>Viral clade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>A/G</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*See Materials and Methods for acute and early infection inclusion criteria. †P = 0.0526.
IFN-γ- and/or IL-2–expressing CD3+CD8+ T cells in response to individual HIV-1 antigens or boosting T cell responses to Pol or Nef antigens (fig. S3). However, 6 of 13 (46%) of the study participants in the vaccine group had CD3+CD4+ T cell tumor necrosis factor–α (TNF-α) responses against Gag peptide pools 1 and 2. In addition, 6 of 13 (46%) of the study participants in the vaccine group had CD3+CD8+ T cell TNF-α responses against Gag peptide pool 1 (fig. S3). In contrast, few, if any, CD154 responses were increased by vaccination (fig. S3). Although there was no appreciable IL-17 cytokine production detected, there were a couple IL-4 responses detected among CD3+CD8+ T cells in the vaccine group only (2 of 13) to the Env pools (1 and 2; fig. S3). Antibody responses to VSV were detected in all vaccine recipients after two vaccinations (fig. S4). No subject in the placebo group had detectable VSV antibodies.

**Effect of vaccination on CD4+ T cell reservoir**

To examine the impact of therapeutic vaccination on the size of the measurable persistent HIV reservoirs, a number of virologic parameters were evaluated. During the 16-week treatment interruption phase of the study, the horizontal axis gives the time in days since stopping ART. One subject in the placebo group broke protocol and prematurely restarted ART before day 112 (the end of the treatment interruption phase); data for this subject were censored at day 72. P value was calculated by exact log rank. Kaplan-Meier analysis of suppression of plasma viremia after cessation of ART was also shown. Duration of plasma viremia under 40 and 400 copies/ml after discontinuation of ART was compared between the groups. (D) Proportions of visits with plasma viremia of <400 copies/ml after discontinuation of ART were compared between the vaccine and placebo groups using the Wilcoxon rank sum test. ECD4+ T cell counts of study subjects before (baseline), at the end of the treatment interruption phase (ATI), and at the end of study (EOS). Vaccine and placebo arms were compared using a mixed model after log transformation of CD4+ T cell counts. Fixed effects included arm, time (treated as categorical), and arm-by-time interaction. The P value was based on the arm-by-time interaction.
were evaluated using CD4+ T cells obtained from the study subjects throughout the vaccination period up to the point of discontinuation of ART at study week 56. As shown in Fig. 3A, no significant changes in the frequency of CD4+ T cells carrying HIV proviral DNA, cell-associated HIV RNA, or replication-competent virus (P = 0.222, \( P = 0.583 \), and \( P = 0.314 \), respectively) were observed in either group over the specified time, indicating that the therapeutic vaccine regimen had no observable effect on the size of the HIV reservoir, as measured in peripheral blood CD4+ T cells. Having established that the therapeutic vaccination failed to have any measurable effect on persistent HIV reservoirs, we examined the relationship between the size of HIV reservoirs and the kinetics of plasma viral rebound in all study subjects (both vaccine and placebo arms) after discontinuation of ART. No correlation was found between the level of HIV proviral DNA or replication-competent virus in the CD4+ T cells and the timing of plasma viral rebound or duration of virologic control (<40 copies/ml) in the study participants as a whole after cessation of ART (\( P = 0.447, r = -0.147 \) and \( P = 0.103, r = -0.309 \), respectively). However, a weak correlation was noted between the level of cell-associated HIV RNA and the timing of plasma viral rebound (\( P = 0.046, r = -0.374 \); Fig. 3B), implying that lower viral RNA expression in infected CD4+ T cells could potentially contribute to longer periods of virologic control (<40 copies/ml). Finally, we examined the relationship between the size of HIV reservoirs and plasma viremia at the end of the treatment interruption period. As shown in Fig. 3C, the frequency of CD4+ T cells carrying HIV DNA, cell-associated HIV RNA, and replication-competent virus correlated positively with the level of plasma viremia at the end of the treatment interruption period (\( P = 0.011, P = 0.018 \), and \( P = 0.035 \), respectively). When the study arms were analyzed separately, significant correlations were observed in the placebo arm between the plasma viremia at the end of treatment interruption period and the frequency of CD4+ T cells carrying HIV DNA (\( P = 0.033 \)) and cell-associated HIV RNA (\( P = 0.013 \)) but not in replication-competent virus (\( P = 0.104 \)). Within the vaccine group, no correlations were found between any of the HIV reservoir measurements and plasma viremia at the end of the treatment interruption period.

**DISCUSSION**

Here, the administration of a DNA prime, live attenuated virus boost therapeutic HIV vaccine regimen in subjects who began ART during acute or early HIV infection was well tolerated, with no safety concerns identified. Our safety data are consistent with previous studies in HIV-infected individuals and normal volunteers who received the individual vaccine components contained in this regimen in phase I safety studies (17, 18). The vaccine regimen augmented existing HIV-1 Gag and Env-specific CD4+ T cell responses in a subset of vaccine recipients with little augmentation of CD8+ T cell responses. In persons who began ART during acute or early infection, the vaccination regimen had no significant effect on the kinetics or magnitude of viral rebound after interruption of ART. Compared to the placebo arm, subjects in the vaccine arm had a significantly greater decrease in CD4+ T cell counts during the ART interruption phase and a slightly higher rate of meeting criteria to prematurely restart ART, findings that could suggest that the vaccine regimen had a deleterious effect on immune control of viral replication in a subset of patients. A potential mechanism responsible for this effect is the possibility that the vaccine, in contrast to the placebo, activated CD4+ T cells, rendering them more susceptible to infection when the virus rebounded. This phenomenon has previously been reported in the context of clinical trials with preventive HIV vaccines (19). However, these results could also represent chance findings, given the number of comparisons made.

We were unable to demonstrate any impact of vaccination on the size of the HIV reservoir in the CD4+ T cell compartment, as measured by three different laboratory assays. There was a statistically significant correlation (independent of study arm) between the baseline size of the persistent viral reservoir and the plasma viral load at the end of the ART interruption phase. The clinical significance, if any, of this observation remains unclear. Nonetheless, within this study, we identified a subset of early treated infected individuals who were capable of achieving durable suppression of HIV replication in the absence of ART, as represented by patients in both the vaccine and placebo group who achieved prolonged viral suppression after discontinuation of ART. It is plausible that early ART suppression of HIV replication led to lower frequencies of persistently infected CD4+ T cells and effective antiretroviral immunity, which collectively contributed toward suppressing plasma viremia in the absence of ART.

Despite the failure to show any differences in control of viral rebound in the vaccine versus placebo group, our findings regarding the magnitude and kinetics of plasma viral rebound after ART interruption have important potential implications for the design of future trials testing interventions aimed at inducing ART-free virologic remissions. It has been well documented that the vast majority of HIV-infected individuals who discontinue ART experience rebound of viremia, usually within 4 to 6 weeks (20, 21). However, a small subset of individuals who initiate ART during early HIV infection appear to be able to maintain viral suppression for prolonged periods after discontinuation of therapy (22–25). The frequency of individuals initiating ART during early infection who subsequently maintain suppression of plasma viremia after stopping ART has been historically difficult to determine because there are few prospective trials of ART interruption with frequent virologic monitoring and predefined virologic endpoints for resuming treatment.

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**Table 2. Effect of therapeutic vaccination on immunologic responses to HIV.** The frequency of study subjects whose CD3+CD4+ and CD3+CD8+ T cells responded to overlapping HIV peptides during the vaccination phase before treatment interruption. Changes in the frequency of cells expressing intracellular cytokines upon peptide stimulation over baseline were determined as described in Materials and Methods. PTEg, global potential T cell epitopes.

<table>
<thead>
<tr>
<th>Peptide pool</th>
<th>CD3+CD4+ T cells</th>
<th>CD3+CD8+ T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (percentage) of responders to HIV antigens</td>
<td>Vaccine group</td>
<td>Placebo group</td>
</tr>
<tr>
<td>Env 1 PTEg</td>
<td>4/12 (33%)</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>Env 2 PTEg</td>
<td>4/13 (31%)</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>Gag 1 PTEg</td>
<td>7/13 (54%)</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>Gag 2 PTEg</td>
<td>5/13 (38%)</td>
<td>2/13 (15%)</td>
</tr>
<tr>
<td>Nef PTEg</td>
<td>0/13 (0%)</td>
<td>1/13 (8%)</td>
</tr>
<tr>
<td>Pol 1 PTEg</td>
<td>2/13 (15%)</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>Pol 2 PTEg</td>
<td>3/13 (23%)</td>
<td>1/13 (8%)</td>
</tr>
</tbody>
</table>
After ART interruption, 3 of 15 (20%) subjects in the placebo arm of our study had sustained suppression of viremia to <40 copies/ml. An additional subject in the placebo arm had virologic suppression ranging from <40 to 280 copies/ml before restarting ART at study week 72. The rate of post-ART control in our placebo arm is consistent with that recently reported in a retrospective analysis of transient virologic control in a subgroup of participants in the Short Pulse AntiRetroviral Therapy at Acute Seroconversion (SPARTAC) trial (26). SPARTAC was a randomized controlled trial of 0, 12, or 48 weeks of ART initiated at diagnosis of early or acute HIV-1 infection (27). In their retrospective analysis of the SPARTAC trial, Martin et al. reported that 18.6% of subjects who were randomized to the ART group and remained on treatment for >12 weeks exhibited sustained (>16 weeks) suppression of viremia to <400 copies/ml after treatment interruption (26). The virologic outcomes observed in the placebo arm of our trial confirm that a significant proportion of subjects who initiate ART during early HIV infection can exhibit prolonged suppression of viremia after treatment interruption without any other therapeutic intervention. It is unlikely that the subjects who controlled viremia after ART interruption were unrecognized elite HIV controllers (who suppress their plasma viremia below the limits of assay detection in the absence of ART). The estimated frequency of elite controllers in the HIV-infected population is <0.5% (28). In this 30-patient study, the probability that we would have by chance enrolled two or more elite controllers is <0.002. These findings emphasize the necessity of having an appropriate-sized control arm in clinical studies that use ART interruption to assess the efficacy of intervention studies aimed at achieving ART-free remissions in early treated patients. The importance of having a control arm for such studies is further illustrated by recently reported results of an ongoing single-arm, phase 1 trial of romidepsin combined with therapeutic vaccination in early treated subjects. That study reported that 5 of 13 (38%) subjects exhibited sustained suppression of plasma viremia to <2000 copies/ml for a median of 14 weeks after treatment interruption (29), a finding that suggested efficacy of the vaccine regimen when compared with historical controls. However, applying these same virologic criteria to our results, 6 of 15 (40%) subjects in the placebo arm of our trial exhibited suppression to <2000 copies/ml for at least 16 weeks after treatment interruption.

A second noteworthy observation in our study is the pattern of plasma viral rebound and spontaneous resuppression seen in select study subjects after treatment interruption. All of the subjects in our study who exhibited sustained suppression of viremia in the ART interruption phase experienced an initial rebound of viremia, followed by spontaneous resuppression. In several of these subjects, the magnitude of the initial plasma viral rebound would have triggered ending the
treatment interruption if criteria for restarting ART had been more conservative (for example, plasma viral load of >1000 to 2000 copies/ml). This pattern and magnitude of plasma viral rebound, followed by re-suppression, should be taken into account when designing interventional studies that use ART interruption to assess efficacy of immune-based therapies, especially in early treated subjects. Using overly conservative virologic criteria for restarting ART in future trials risks missing important positive effects of therapeutic interventions on rebound viremia.

Our study has limitations. Given the relatively small sample size, the occurrence of low-frequency AEs related to the vaccine regimen may have been missed. Likewise, small clinically insignificant effects of vaccination on the magnitude and kinetics of viral rebound after ART interruption may have gone undetected.

Despite using a vaccination strategy designed to maximize immunogenicity in a population of individuals who began ART during acute or early HIV infection, we were unable to demonstrate a beneficial effect of vaccination on maintaining suppression of HIV viremia after interruption of ART or on the size of the HIV reservoir of persistently infected CD4+ T cells. Our results add to a growing list of studies in which therapeutic vaccination alone failed to produce clinically significant suppression of HIV replication in the absence of ART. It remains to be established whether therapeutic vaccination alone can produce sustained suppression of HIV replication in the absence of ART. Future studies of therapeutic vaccination will likely need to incorporate other immune-based interventions into the vaccine regimens if sustained suppression of HIV replication in the absence of ART is to be achieved.

**MATERIALS AND METHODS**

**Study design**

This was a randomized, double-blind, placebo-controlled phase 1/2 clinical trial to assess the safety and virologic effect of an HIV-MAG DNA vaccine prime, rVSVN4CT1gag booster vaccine regimen in HIV-infected individuals who began ART during acute or early infection.

**Therapeutic vaccination phase (study weeks 0 to 48)**

Eligible subjects were randomized 1:1 by the National Institutes of Health (NIH) Clinical Center Pharmacy to receive either HIV-MAG pDNA, IL-12 pDNA priming vaccine, and the rVSVN4CT1gag booster or matching placebo injections. The investigators and subjects were blinded to treatment assignments for the duration of the study. For subjects randomized to the vaccine arm, the HIV-MAG pDNA vaccine prime was administered at a dose of 3000 μg (1500 μg of the HIV-1 gag/pol plasmid and 1500 μg of the HIV-1 nef/tat/vif and env/plasmid). Each construct of HIV-MAG pDNA vaccine (1500 μg each) was mixed and combined with 1000 μg of the IL-12 pDNA adjuvant. The resulting mixture was divided into two intramuscular injections and administered as 0.75-ml intramuscular injection in the left deltoid and 0.75-ml intramuscular injection in the right deltoid with in vivo electroportation using the Ichor TriGrid delivery system (Ichor Medical Systems) at weeks 24 and 48.

**ATI phase (study weeks 56 to 72)**

At study week 56, 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 is roughly equal. During the treatment interruption phase, subjects were closely monitored every 2 weeks with measurement of CD4+ T cell counts and plasma HIV-1 RNA loads. Criteria to restart ART before the end of the 16-week treatment interruption phase were as follows: a sustained (≥4 weeks) plasma HIV-1 RNA load of >50,000 copies/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.

**Study subjects**

The study was conducted at the NIH Clinical Center in Bethesda, MD (ClinicalTrials.gov number NCT01859325). HIV-infected subjects were eligible if they were in general good health and had begun ART within 90 days of being diagnosed with acute or early HIV infection. Acute infection was defined as a plasma HIV RNA load of >2000 copies/ml with a negative HIV-1 enzyme immunoassay (EIA; criterion 1), a positive result from an HIV-1 EIA with a negative or indeterminate HIV-1 Western blot that subsequently evolves to a confirmed positive result (criterion 2), or negative result from an HIV-1 EIA within the past 4 months and HIV-1 RNA loads of >400,000 copies/ml in the setting of a potential exposure to HIV-1 (criterion 3). Early infection was defined as a negative result from an HIV-1 EIA within 6 months before a positive result from an HIV-1 EIA and confirmatory HIV-1 Western blot (criterion 4), a negative result from a rapid HIV-1 test within 1 month before a positive result from an HIV-1 EIA and Western blot (criterion 5), or the presence of low level of HIV antibodies as determined by having a positive EIA and Western blot with a non-reactive detuned EIA according to a multia assay testing algorithm for recent infection (criterion 6) (30). 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 1 year before enrollment. Patients with hepatitis B or hepatitis C virus co-infection were excluded. The clinical protocol was approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. All subjects provided written informed consent. Primary data are located in table S2.

**Vaccines**

The study vaccines and plasmid IL-12 (pIL-12) were supplied by Profectus BioSciences. The HIV-MAG DNA vaccine (HIV-MAG pDNA) consists of two pDNA expression vectors: a single-promoter expression vector encoding an HIV clade B gag/pol fusion and a dual-promoter expression vector encoding an HIV clade B nef/tat/vif fusion and a clade B primary isolate (HXB2) Gag protein (17).
Study endpoints
The primary safety endpoint was the rate of occurrence of grade 3 or higher AEs (including SAEs), which were at least possibly related to the study injections. AEs were graded according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0, December 2004 (clarification August 2009; accessible online at https://tsr.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables). The virologic endpoint was the difference in plasma viral loads at the end of the treatment interruption phase (week 72) between the vaccine and placebo groups. For subjects who met criteria to restart ART before study week 72, the viral load obtained immediately before restarting ART was used for the virologic endpoint analysis. The safety and virologic endpoints were defined before study initiation.

The exploratory endpoints were the frequency and rate of decay of the infectious HIV reservoir between the vaccine and placebo groups, and the change from baseline to postvaccination in the frequency of IFN-γ and/or IL-2–generating CD3+CD8+ and CD3+CD4+ T cells from peripheral blood mononuclear cells (PBMCs) in response to Gag, Pol, Env, and Nef, as measured by ICS.

Study oversight
The study team conducted a weekly review of all AEs. The study team remained blinded to treatment assignment for the duration of the study. After reviewing reported events, the study team assessed the relationship of the AE to the study vaccine/placebo injections. An independent data safety monitoring board also reviewed safety data broken down by placebo and vaccine group at 6-month intervals until study completion.

Quantitation of HIV DNA
The frequency of CD4+ T cells carrying HIV DNA was determined by droplet digital polymerase chain reaction (PCR). Genomic DNA was isolated from highly purified CD4+ T cells and subjected to restriction digestion (Msc I; New England BioLabs), followed by droplet digital PCR (Bio-Rad Laboratories) according to the manufacturer’s specifications. The following PCR primers and probe were used for amplification of HIV DNA: 5′-GRAACCACCTGCTTTAGCCTCAA-3′ (5′ primer), 5′-TGTTGCCGCCACTGCTAGAAGA-3′ (3′ primer), and 5′-6FAM-AGTAGTGGTGCCGCTCTGTTT-1ABkFQ-3′ (fluorescent probe). The following PCR primers and probe were used for amplification of housekeeping gene RPP30: 5′-GATTTGGACCTGACGAAT-3′ (5′ primer), 5′-GCGGCTGTCTCCACAAGT-3′ (3′ primer), and 5′-HEX-TTCTGACCTGAAGGTCGTCG-1ABkFQ-3′ (fluorescent probe). HIV DNA copy numbers were normalized per 1 × 106 copies of TBP.

Quantitation of cell-associated HIV RNA
Reverse transcription PCR was carried out to determine the frequency of CD4+ T cells carrying cell-associated HIV RNA. Total RNA was isolated from CD4+ T cells (RNeasy Mini kit, Qiagen) and subjected to complementary DNA (cDNA) synthesis using qScript XLT cDNA Master Mix (Quanta BioSciences) as per the manufacturer’s specifications. cDNA was subjected to droplet digital PCR (Bio-Rad Laboratories) using HIV-specific primers [5′-CTCTTAGAGTGG-CGCCAGAGA-3′ (5′ primer) and 5′-TCTCTTAGAGTGG-CGCCAGAGA-3′ (3′ primer)] and probe (5′-HEX-TGTGACAGGAGCCAA-GAGTGAAGA/3-IABkFQ-3′). HIV RNA copy numbers were normalized per 1 × 106 copies of TBP.

Measurements of replication-competent HIV
To determine the frequency of CD4+ T cells carrying replication-competent/infectious virus, standard (1 × 106, 200,000, 40,000, 8000, 1600, and 320 cells per well in duplicate in 24-well plates) and high-input (5 × 106 cells in replicate in 12-well tissue culture plates) quantitative coculture assays were carried out. Highly purified CD4+ T cells were counted (1000 cells per count in quadruplicate) using an automated cell counter (Muse, EMD Millipore) and placed in corresponding tissue culture plates. The above cultures were then incubated with irradiated PBMCs (6 × 106 to 8 × 106 cells per well) obtained from HIV-negative donors and anti-CD3 antibody. The next day, 1 × 106 CD8-depleted and anti-CD3–stimulated PBMCs from HIV-negative donors were added to each well, followed by periodic removal of cell suspensions and replenishment with fresh medium containing IL-2. HIV p24 enzyme-linked immunosorbent assay was performed on the culture supernatants between days 14 and 21. The infectious units per million cells from quantitative coculture assays were determined as described (31).

Detection of anti-VSV antibody in study subjects who received therapeutic vaccines
Serially diluted plasma samples from the study subjects were incubated with plate-coated rVSV NS4CT1-MSC1 lysates. The plate was subjected to washing and incubation with alkaline phosphatase–conjugated goat anti-human immunoglobulin G (IgG) F(ab′)2. After washing and incubation with p-nitrophenylphosphate, alkaline phosphatase substrate solution and then with stop solution, plates were read using dual absorbance at 405- and 690-nm wavelength. Antigen-specific serum IgG endpoint titers were defined as the reciprocal of the last serum dilution, giving an optical density greater than 0.1.

Intracellular cytokine staining
PBMCs were assessed for ex vivo responses to seven pools of HIV-1 15-mer peptides (Gag 1 and 2 PTEg, Pol 1 and 2 PTEg, Env 1 and 2 PTEg, and Nef PTEg; Bio-Synthesis Inc.) covering global potential T cell epitopes (PTEg) across all HIV-1 proteins contained within the vaccines (32). The final concentration for each peptide was 1 μg/ml during stimulation. Staphylococcal enterotoxin B (Sigma-Aldrich) stimulation was the positive control, whereas peptide diluent (dimethyl sulfoxide; final concentration of 0.5%) was the negative control. The 6-hour stimulation included brefeldin A (10 μg/ml; Sigma-Aldrich), monensin, and anti-CD28/anti-CD49d antibodies (each at 1 μg/ml; BD Biosciences).

A validated ICS protocol was used as described previously (33), with minor modifications (34). Briefly, cells were first stained with Aqua LIVE/DEAD Fixable Dead Cell Stain (Life Technologies) (35), surface-stained with fluorescent-labeled antibody reagents detecting CD14 (exclusion gate), CD56, CXCXR5, PD-1, ICOS, CCR7, and CD45RA, and then fixed (BD FACS Lyse, BD Biosciences) and permeabilized (BD FACS Perm II, BD Biosciences). Cells were then stained intracellularly for expression of CD3, CD4, CD8, IFN-γ, IL-2, TNF-α, IL-4, IL-17, CD154, and granzyme B (fig. S2).

The differences in IFN-γ and/or IL-2 responses between the two baseline measurements were calculated for each T cell subset (CD4+ and CD8+), HIV antigen, and participant. Prediction intervals for each T cell subset and HIV antigen were estimated using the difference in
baseline measurements across all vaccine and placebo participants. In addition, for a response to be positive, the measurements at the post-vaccination time point had to be higher than the highest baseline measurement for that participant. Postvaccination responses were deemed positive if their change in antigen-specific cytokine response over mean baseline was higher than the high end of the prediction interval.

**Statistical analysis**

The safety endpoint of the study is the occurrence of grade 3 or higher AEs (including SAEs). A sample size of 14 vaccine recipients provides at least 90% chance of observing a grade 3 or higher AEs of a probability of 0.15 or greater.

To compute power for the virologic efficacy endpoint, we used data from Rosenberg et al. (9) and Grijsen et al. (36) to estimate the SD of rebound plasma viremia in log_{10} units in a population of early treated patients undergoing ART interruption. On the basis of the data in these two studies, we adopted an SD of 1. Although the Wilcoxon rank sum test was used to compare the two groups with respect to viremia, power for the Wilcoxon test is very close to that of the t test (the asymptotic relative efficiency is about 95%). Therefore, we approximated power for the Wilcoxon test by that of the t test. The power based on a two-sample t test with a two-tailed α of 0.05 and a total sample size of 29 is about 90% to detect a 1.25 log_{10} reduction in the rebound plasma viremia.

Plasma viral load at the end of ART interruption was compared between groups using a Wilcoxon rank sum test. Plasma viral loads and other continuous outcomes such as DNA, RNA, replication-competent HIV, and CD4+ T cell counts were also compared simultaneously at three time points: baseline, during ART interruption, and end of study using a mixed model after log transformation of the outcome. The model included arm, time (treated as categorical), and arm-by-time interaction as fixed effects and patient-by-time interaction as random effects. The pattern over time was declared different if the arm-by-time interaction was statistically significant. Associations between continuous variables (such as cell-associated HIV RNA and plasma viral load) were assessed using the Spearman correlation coefficient. All P values were two-tailed using α = 0.05.

**SUPPLEMENTARY MATERIALS**

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Fig. S1. Plasma viremia of individual study subjects after discontinuation of ART.

Fig. S2. Gating strategy for ICS by flow cytometry.

Fig. S3. Kinetics of HIV-specific T cell responses and immune parameters in placebo and vaccine recipients.

Fig. S4. Detection of anti-VSV antibody in study subjects who received therapeutic vaccines.

Table S1. Vaccine-related AEs.

Table S2. Primary data.

**REFERENCES AND NOTES**


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A randomized controlled safety/efficacy trial of therapeutic vaccination in HIV-infected individuals who initiated antiretroviral therapy early in infection


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Single-arm trials can leave you hanging

Depending on the study restraints and goals, not all clinical trials include a randomized placebo group. This is often done to minimize risk to patients but can also impair interpretation of the results. When Sneller et al. embarked on their therapeutic HIV vaccine trial, they chose to include a placebo group to get a better understanding of how their vaccine affected viral rebound upon therapy interruption. The vaccine itself generated minimal T cell activation and did not induce protective responses. Somewhat surprisingly, a proportion of individuals in the placebo arm demonstrated sustained viral suppression, although they were no longer being treated. These results suggest that any future HIV intervention trials would benefit from the inclusion of a placebo arm.