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Therapeutic Immunization In HIV Infected Ugandans Receiving Stable Antiretroviral Treatment: A Phase I Safety Study⁴

Cissy Kityo^{a,1}, Stephanie Bousheri^{b,1}, Juliette Akao^a, Francis Ssali^a, Rose Byaruhanga^a, Isaac Ssewanyana^a, Prossy Muloma^a, Sula Myalo^a, Rose Magala^a, Yichen Lu^{c,2}, Peter Mugenyi^a, and Huyen Cao^{b,2,*}

^aJoint Clinical Research Centre, Kampala, Uganda ^bCalifornia Department of Public Health, Richmond, California ^cHaikou VTI Biological Institute, Hainan, China

Abstract

Therapeutic immunizations in HIV infection may boost immunity during antiretroviral treatment. We report on the first therapeutic vaccine trial in Uganda, Africa. This open label Phase I trial was designed to assess the safety, tolerability and immunogenicity of a therapeutic HIV-1 vaccine candidate. Thirty HIV positive volunteers receiving a stable regimen of antiretroviral therapy with CD4 counts > 400 were recruited for the safety evaluation of LFn-p24C, a detoxified anthrax-derived polypeptide fused to the subtype C HIV gag protein p24. The vaccine was well tolerated and HIV RNA levels remained undetectable following three immunizations. CD4 counts in vaccine recipients were significantly higher compared to the control individuals after 12 months. HIV-specific responses were associated with higher gain in CD4 counts following LFn-p24C immunizations. Volunteers were subsequently asked to undergo a 30-day period of observed treatment interruption. 8/24 (30%) individuals showed no evidence of viral rebound during treatment interruption. All demonstrated prompt suppression of viral load following resumption of ART. Our data demonstrates the safety of LFn-p24C and suggests that adjunct therapeutic immunization may benefit select individuals in further boosting an immune response.

Background

An estimated 25 million people with HIV infection reside in sub-Saharan Africa. In resource poor settings, restricted treatment options and the cost of alternative treatment regimens are likely to magnify the limitations of antiretroviral treatment (ART) programs [1]. Uganda's current HIV infection rate of 6- 10% remains unacceptably high. The majority of ART regimens available in Uganda are restricted to low-cost, fixed-dose combination drugs[2] and options for second line treatment remain limited. Antiretroviral treatment can successfully reduce plasma HIV-1 RNA levels to < 50 copies/mL[3-5] although it is less effective in those with advanced immunosuppression and higher viral loads [6]. ART has

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*Address Correspondence and reprint requests to: Dr. Huyen Cao, California Department of Public Health, 850 Marina Bay Parkway, YRDL, Richmond, CA, 94804 Phone: (510) 307-8913, Huyen.Cao@cdph.ca.gov.

¹CK and SB contributed equally to the work

²YL and HC have shares in Vaccine Technologies, Inc.

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been linked to abnormalities of body fat metabolism and distribution, hyperlipidemia, insulin resistance, hyperglycemia, and lactic acidosis [7-9] requiring regimen change in 40% of individuals within 1 year of starting treatment[10,11].

Therapeutic interventions that enhance immunologic function with concurrent ART may improve the long-term outcome of HIV infection[12]. Immune recovery following appropriate ART is often incomplete and fails to elicit responses associated with protection from disease progression [13-16]. This failure has been associated with dysfunctional T cell responses [17-19]. Therefore, boosting the immune response via therapeutic intervention may significantly delay or inhibit progression to AIDS. Therapeutic vaccinations in rhesus macaques have demonstrated that immunity can be elicited, leading to lower viral load [20]. Prior studies have shown a reduction in plasma viremia in HIV infected individuals[21-23] and evidence of enhanced HIV specific T cell responses after immunization[21-24]. LFn-p24C consists of a detoxified anthrax-derived polypeptide, called lethal factor n-terminus (LFn), which has been fused to the HIV Subtype C gag protein p24. While subtypes A and D are most prominent in Uganda, use of a subtype C based immunogen elicits an immune response due to the fact that portions of the gag gene are conserved among virus subtypes. In vivo tests of this recombinant protein delivery approach have demonstrated cellular delivery of peptides that stimulate potent immune responses[21-23]. In this study, we report on the safety of LFn-p24C as a therapeutic immunogen in a two-phase, open label trial. Phase 1A evaluated the safety of the vaccine candidate followed by a short observed treatment interruption in Phase 1B.

Subjects, Materials and Methods

Study Design

Eligible adult male and female HIV-1 infected volunteers were recruited from the HIV clinic at the Joint Clinical Research Centre (JCRC) in Kampala, Uganda. Recruitment was restricted to individuals with documentation of HIV status and evidence of ART-mediated viral suppression for at least six months prior to enrollment. Study eligibility criteria included: age 18 to 60 years, CD4+ T cell counts >400 cells/ μ L, normal complete blood count, chemistry, liver function tests and urinalysis. All female volunteers had a negative pregnancy test at baseline, agreed not to breast-feed and to use adequate birth control during the course of the study. All volunteers provided written informed consent. Reactogenicity and adverse events were assessed at 1 hour and 3 days after each immunization (day 0, 28 and 84). Subsequent follow-up visits were 6, 9 and 12 months after enrollment in Phase 1A. After twelve months, volunteers from Phase 1A were invited to enroll in a follow-up Phase 1B trial where a 4-week observed treatment interruption was initiated following a single LFn-p24C booster immunization (Table 1). HIV plasma RNA, CD4 count and clinical evaluation were performed every fourteen days. The COBAS AmpliPrep/ COBAS TaqMan kit was used for amplification and detection of HIV-1 viremia in our study volunteers. For this clinical trial we use 400 copies/mL as the lowest cut-off for detection. After 4 weeks (28 days) of treatment interruption, participants were asked to resume their prior antiretroviral treatment regimens and were carefully monitored every two weeks for 2 months and then again at three and six months. For antiretroviral treatments that included medications with a short plasma half-life drug concentration, all antiretrovirals were stopped simultaneously and restarted within 4 weeks. For antiretroviral treatments that included a single long acting reagent (such as Nevirapine or Efavirenz), staggered interruption was initiated with the long acting agent being discontinued 7-10 days prior to discontinuation of other ART.

Counseling sessions were conducted at each study visit to assess ART adherence, and HIV risk behavior. Safety laboratory testing was done throughout both phases of the study and blood samples designated for immunogenicity testing were collected. PBMC's were isolated

and cryopreserved at visit 11A in accordance with the study protocol. Because the phase I study primary objective was to determine the safety of therapeutic immunization in HIV infected Ugandans, the open label protocol did not include a placebo or control arm. As baseline samples were not included in the data analysis, we included as historical controls, thirty-one unvaccinated individuals (sample time: month 0 and month 12). These historical control volunteers were recruited from the JCRC and enrolled in an observational longitudinal study where counseling and blood draws were obtained every three months. The control cohort had a comparable clinical profile to that of the study volunteers. All had CD4+ T cell counts greater than 400 cells/ μ l with undetectable viremia and were on a stable regimen of ART for at least 6 months.

The protocol for this small open label phase 1 safety study without a placebo arm was approved by the US and JCRC IRB's, the Uganda National Council for Sciences and Technologies and the Uganda National Drug Authority.

Vaccine Candidate

The immunogen is composed of a combination of an anthrax-derived polypeptide lethal factor (LFn, from which the toxin domain has been removed) fused to a subtype C HIV-1 gag p24 protein. LFn-fusion proteins have been studied extensively as intracellular delivery agents because of their unique capability to translocate antigen across the cell membrane without affecting cell viability, using the classical MHC Class I and II pathways [25-27]. LFn-p24C was produced by Water Reed Army Institute of Research (WRAIR) in compliance with the US standard of Good Manufacturing Practices (GMP) and provided by Vaccine Technologies, Inc (VTI). The product underwent GLP (Good Laboratory Practices) grade animal toxicity studies and was previously used in an US FDA approved Phase 1 safety study involving HIV-1 negative healthy volunteers. This study took place in Maryland and was conducted by WRAIR (Deborah Birx and Shirley Lecher, personal communications). The protein expression vector for LFn and its fusion derivatives is the pET28b plasmid developed by Novagen (Madison, WI) [28]. The main features of this vector system include an inducible T7 promoter, an internal His.Tag for protein purification, and multiple cloning sites. The recombinant LFn is expressed in *E. coli* as an intracellular soluble protein with 6 tandem repeat Histidine (His) at its N-terminal end. The molecular weight of LFn is about 31 kiloDalton (kD). The LFn-p24C was diluted and administered intramuscularly in a volume of 1ml in a total dose of 300 μ g with Alhydrogel adjuvant. A total of 3 injections were administered intramuscularly in the deltoid region at 0, 1, and 3 months for Phase 1A, followed by single booster immunization at enrollment in Phase 1B.

Part of the rationale for use of subtype C *gag* in Uganda is that portions of the *gag* gene are conserved among virus subtypes. Multiple HIV subtypes (primarily A and D) are endemic in Uganda and a single-clade vaccine will not provide full subtype-match to the. Determining the general applicability of an effective HIV vaccine will require testing in populations with both matched and unmatched circulating virus.

CFSE Proliferation Assay

Cell proliferation was determined by carboxyfluoresceindiacetatesuccinimidyl ester (CFSE) dilution using the CellTrace™ CFSE Cell Proliferation Kit (Invitrogen, Carlsbad, CA), per the manufacturer's instructions. Cells were stimulated with peptides for five days at 37°C and 5% CO₂, then harvested and stained for surface markers with the following antibodies: CD3 APC, CD4 PE, CD8 PerCp-Cy5.5 (BD Biosciences, San Jose, CA). Samples were analyzed on an LSRII flow cytometer (BD Biosciences, San Jose, CA). Dead cells were excluded from analysis using a violet excited viability dye (LIVE/DEAD Fixable Dead Cell Stain; Invitrogen). All flow analysis was performed using FlowJo software (TreeStar,

Ashland, OR). Proliferation was measured by the extent of CFSE dilution. Staphylococcal Enterotoxin B (SEB Sigma-Aldrich, St. Louis, MO) stimulation was used as a positive control. All evaluable samples demonstrated significant proliferation following SEB stimulation. Only data with a minimum of 10,000 acquired events of CD3+CD4+ or CD3+CD8+ were analyzed and only results with less than 1% background response, and greater than 5% SEB response were considered valid. Results greater than twice the background values and more than 0.1% after subtraction of background were considered positive.

Immune Profile

Activation staining was performed by incubating PBMC with the following antibodies: CD3 AmCyan, CD4 APC-Cy7, CD8 PerCPCy5.5, HLADR FITC, CD38 PE, and PD-1 APC (BD Biosciences San Jose, CA). Dead cells were excluded from analysis using a violet excited viability dye (LIVE/DEAD Fixable Dead Cell Stain; Invitrogen). Immune activation was defined as the percent of CD38+ HLA DR+ T cells and PD-1 levels were defined as the percent expression of PD-1 APC on CD3+ CD8+ (or CD4+) T cells. Gating was standardized and set using fluorescence minus one controls for HLADR, CD38 and PD-1. Data was analyzed using FLOWJO software (TreeStar, Ashland, OR). A minimum of 30,000 CD4+ cells per sample were acquired and analyzed on an LSRII flow cytometer (BD Biosciences, San Jose, CA) [29].

Antigens

Peptides corresponding to the consensus subtype C Gag (122 peptides) were synthesized as 15 amino acids (a.a.) overlapping by 11 a.a. (NIH/NIAID repository). A single pool of overlapping peptides, corresponding to the amino acid sequence of the HCMV pp65 protein (JPT Peptide Technologies) was used to detect human CMV -specific responses. The final concentration of individual peptides was 1 µg/ml per peptide.

Statistical Analysis

Statistical analyses were performed using Prism Version 4.0 (GraphPad Software Inc. San Diego, CA). Paired t-tests were used to compare data between time points and Mann-Whitney tests were used to compare the differences between control and study groups. *P* values <0.05 were considered statistically significant.

Results

Demographics

Screening and enrollment into Phase 1A occurred from April 2008 to September 2008. Of the 153 volunteers screened at the JCRC, 30 HIV positive volunteers were identified and enrolled (25 women and 5 men). The mean age of the volunteers was 41 years (range 29-55). All participants were stably suppressed on ART for 6 months or greater, had undetectable viral load (<400 copies/mL) and a mean CD4+T cell count of 520 (range 400-1100). Nadir CD4 information was not available. Most volunteers have received ART for at least 12 months prior to enrollment and no subtyping information was available. There were 31 HIV positive, unvaccinated volunteers in the historical control group (13 male and 18 female). The mean age of the unvaccinated control group was 45 (range 22-55). The mean CD4+ T cell count was 540 (range 400-1370 cells/µL). All maintained an undetectable viral load on a stable regimen of ART. No significant differences in age and CD4+ T cell counts were found between the two groups (*p*>0.05, data not shown).

A total of 29 out of 30 volunteers completed the Phase 1A study. One individual relocated outside of the country and was not able to complete her last visit at 12 months. Twenty-

seven of the thirty volunteers from Phase 1A agreed to participate in Phase IB. Of these, twenty-four fully evaluable volunteers received a booster immunization and underwent closely monitored treatment interruption twenty-one days after receiving the LFn-p24C booster injection.

Vaccine Safety

Local and systemic reactogenicity for both Phase 1A and 1B is shown in Figure 1. The most commonly reported local symptoms were pain and tenderness at the site of injection. The systemic symptoms related to LFn-p24C were malaise, myalgia and arthralgia. These local and systemic events were mostly mild and usually resolved prior to the subsequent visit (within 3–14 days). The majority of the self reported symptoms were mild 24/840 (2.9%) or moderate 1/840 (0.001%). There were no severe adverse events attributable to the immunogen and none of the volunteers discontinued the study due to adverse events. Other events not considered related to LFn-p24C included urinary tract infection, influenza infection, low back pain, pharyngitis, and acute malaria.

We carefully monitored CD4 cell counts and viral loads after LFn-p24C administration throughout the course of the phase 1A study. All thirty volunteers continued to have undetectable viral loads at all evaluated time points throughout the duration of the phase 1A study. Administration of LFn-p24C was associated with a significant increase in CD4 cell counts at and after 12 months compared to the historical, unvaccinated control cohort ($p=0.02$, $p=0.0006$ and $p=0.4$, respectively, Figure 2). This increase was not observed in the vaccinated cohort during the 12 months prior to immunization and enrollment into the study ($p=0.2$, Figure 2).

T Cell Profile of Vaccine Responders

HIV preferentially infects activated CD4⁺ T helper cells and this has previously raised concerns over whether an AIDS vaccine can generate more targets for the virus [30–32], particularly in HIV infected individuals. We examined both CD8 and CD4 T-cell immune activation after three immunizations (visit 11A) and compared the levels to our unvaccinated control samples. We found no significant differences in CD4 and CD8 immune activation between vaccine recipients and historical control samples (Figure 3A, $p>0.5$).

Functional impairment of T cells during chronic HIV infection is associated with higher expression of programmed death 1 (PD-1), and up regulation of PD-1 is also a predictor of disease progression [33–35]. Surprisingly, therapeutic immunization was associated with lower PD-1 expression in both CD4⁺ and CD8⁺ T cells at visit 11A compared to unvaccinated control samples (Figure 3B, $p=0.016$ and 0.041 , respectively). No significant changes in the level of activation and PD-1 expression were observed in the unvaccinated control group within twelve months ($p>0.5$, data not shown).

Vaccine-specific T cell Proliferation

HIV-1-specific T cell responses as measured by interferon secretion do not differ in individuals with progressive and long-term non-progressive HIV-1 infection, and are not directly associated with the level of viral replication [36–38]. In contrast, HIV-1-specific proliferative responses are lost in individuals with progressive disease [39]. We measured T cell proliferation in vaccine recipients after 3 immunizations (example of plot is shown in Figure 4A). Flow-based proliferation, as measured by CFSE dilution, was measured in vaccine recipients at twelve months (visit 11A) and compared to unvaccinated controls. Results were valid in 23 vaccine and 20 control samples. Vaccine-specific CD4⁺ proliferation to Gag C was significantly higher in individuals who received the vaccine compared to the unvaccinated control group; 5/23 [21.7%] and 0/20[0%] respectively (Figure

4B, $p < 0.05$). No CD4-mediated proliferation was detected in the control group at the two evaluated time points (0 and 12 months, data not shown). In contrast, no significant differences in CMV-specific responses were observed between 12/23 [52.2%] of vaccine recipients and 13/20 [65%] control samples, respectively. Similarly, higher CD8+ vaccine-specific responses in 5/23 [21.7%] of vaccine recipients compared to 2/20 [10%] control samples, respectively (Figure 4B). No significant differences in CMV-specific, CD8- and CD4-mediated responses were detected between the two groups (Figure 4C, $p > 0.5$). Individuals with detectable vaccine-specific responses (CD4- and/or CD8-mediated) achieved a greater increase in CD4+ T cell counts after three immunizations compared to vaccine recipients without detectable responses (Figure 5).

Structured Treatment Interruption

In order to assess whether therapeutic vaccination can elicit an anti-HIV response that controls viral replication, volunteers who completed Phase 1A were subsequently asked to undergo monitored treatment interruption following a single booster immunization. Twenty-one days after receiving a fourth dose of LFn-p24C, volunteers were instructed not to take their ART (ART was not dispensed), but to continue with any other medication they were currently being prescribed. Viral rebound was observed two weeks after treatment interruption was initiated. Full suppression of viremia followed ART resumption (Figure 6A). CD4 cell counts were closely monitored throughout the course of this study (Figure 6B). An anticipated decrease in CD4 cell count was observed following treatment interruption and full recovery of CD4 to baseline was not achieved by visit 11B (six months after booster immunization). Eight individuals (33%) showed no evidence of viral rebound during treatment interruption nor any significant decline in CD4 cell count ($p = 0.45$, data not shown). Lack of viral rebound was not associated with T cell proliferation detected at visit 11A (data not shown).

Discussion

The collapse of the immune system following HIV infection stems largely from the continual destruction of T cells. Antiretroviral treatment can restore CD4+ T cells but requires lifelong use and full adherence to medication regimens. ART is also associated with potential side effects and secondary options remain costly for the majority of infected persons worldwide [40]. In addition, established viral latency raises the possibility that complete eradication of HIV may be impossible with antiretroviral drugs alone. The underlying consideration in therapeutic vaccination is that boosting immunity is beneficial to the individual on ART and may modify the natural history of HIV disease favorably. Based on our hypothesis that an effective therapeutic approach needs to elicit potent antiviral immune responses, we investigated the performance of LFn-p24C vaccine in asymptomatic HIV positive Ugandans who maintained a stable regimen of antiretroviral treatment. Individuals who received LFn-p24C demonstrated a significant increase in CD4+ T cell counts over twelve months compared to the unvaccinated historical control group. This increase was not observed in the study volunteers 12 months prior to immunization, supporting the role of LFn-p24C in boosting the immune system. Subsequent larger randomized trials that include placebo recipients are needed to confirm the role of therapeutic vaccine intervention in augmenting and sustaining the CD4 T cell count in ART treated individuals.

There are hints that therapeutic immunization may play a role as the era of highly active antiretroviral therapy progresses. Immune-based therapies in this population have the potential to be a crucial addition to currently available ART, particularly in regions where secondary ART options are limited. How does vaccine-specific T cell immunity prevent disease progression in chronic HIV infection? The presentation of antigens by a therapeutic

immunogen may induce a distinct functional quality of the T cell that is associated with protective antiviral immunity, unlike the immune responses detected in HIV infection in the presence of viral suppression. In our study, volunteers who have evidence of vaccine-specific responses appear to gain significantly more CD4⁺ T cells. However, these same individuals subsequently failed to control viremia during the scheduled treatment interruption, perhaps suggesting that distinct mechanisms may contribute to immune recovery and viral control. Vaccinations can augment HIV-1-specific T cell responses in chronically infected persons sufficiently to achieve significant effects on viral load during ART interruption [41,42]. Our data demonstrates that therapeutic immunization induces HIV-specific T-helper and effector responses consistent with earlier studies [24,43,44]. HIV-1-specific proliferative responses were associated with a greater increase in CD4⁺ T cell gain over 12 months. This would be consistent with other reports, which have shown that the preservation of the T cell proliferation capacity is generally associated with an apparently effective immune response in patients with HIV-1 infection. The importance of maintaining the self-renewing capacity of the CD4⁺ T cell population with regard to disease progression has been previously established and may play a role in explaining our findings. We limited our study to asymptomatic, seropositive individuals on stable ART regimens, who had CD4⁺ T cell counts >400 cells/ μ L for optimal results from immunization [45-47]. Nadir CD4 information was not captured for the volunteers in this study and length of ART as well as infecting subtypes may provide more information on the mechanism of immune boosting from therapeutic immunizations. Whether a favorable immune profile can be achieved in patients with more advanced HIV disease remains to be determined [48].

The functional impairment of T cells during chronic HIV infection is associated with T cell exhaustion. Dysfunctional T cells subsequently fail to eliminate the virus. However, the mechanism leading to this functional impairment has yet to be understood. PD-1 belongs to the B7:CD28 family, and plays an active and reversible role in virus-specific T cell exhaustion [34,35,49,50]. Blockade of the PD-1 pathway restores HIV-specific T cell function in HIV infection [35,49]. In this cohort of chronically infected HIV-1 positive individuals, the combination of an HIV-1 therapeutic vaccine and suppressive ART was associated with a decrease in PD-1 expression, a marker of immune dysfunction. Further evaluation of the role of vaccination in modulating immune dysfunction may provide important insight into the mechanism of viral induced immune impairment.

Few controlled studies on the clinical effectiveness of therapeutic immunization in HIV infected individuals exists, particularly in Africa. Our trial examined the safety of a therapeutic vaccine in HIV-1-infected Ugandans who are virally suppressed. We show here that therapeutic immunization with LFn-p24C is safe. Immunization improves CD4 counts and boosts T cell responses in chronically HIV-1-infected volunteers. The present study raises the possibility that augmentation of HIV-specific immunity may be beneficial and an important goal in chronic HIV infection. While interpretations should be tempered by the relatively small study size and use of non-placebo controls, there are a number of significant observations that merit additional investigation. These include optimizing strategies for identifying susceptible individuals and offering them sustained protection through a correct immunization schedule. Future studies with more effective immunogens and in different populations are worthy of consideration. Similarly, the benefit of therapeutic intervention in preventing opportunistic infections should be explored. Our study introduces cautious optimism for the future of therapeutic immunizations as integral components of treatment strategies, particularly in regions where the AIDS epidemic has hit the hardest.

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Figure 1.

Local and systemic reactogenicity following 3 immunizations and a booster dose, for Phase 1A and 1B, respectively. A total of 840 events were recorded. 24/840 (2.9%) of the AE's were documented as mild and 1/840 (0.1%) were recorded as moderate in severity. There were no severe adverse events deemed related to the study vaccine.

Figure 2.

CD4 count distribution in phase 1A for historical control individuals and vaccine recipients (dashed and clear box plots, respectively). Horizontal lines represent medians (whisker values for minimum and maximum values). No statistically significant differences in CD4+ T cell count distributions in the control group (12 months, $p=0.41$, paired-t test) or in vaccine recipients (6 months prior to enrollment, $p=0.2$, paired-t test) were observed. Significant increases in CD4 cell counts were observed after three immunizations at 12 months and after 15 months ($p=0.02$ and 0.006 , respectively).

Figure 3A **Figure 3B** **Figure 3.**

(A) Immune Activation. PBMC were stained with HLADR FITC, CD38 PE, CD3 AmCyan, CD8 PerCPCy5.5, CD4 APC Cy7 and analyzed by flow cytometry. Samples were first gated on the CD3⁺/CD8⁺ and CD3⁺CD4⁺ lymphocyte populations, and the percent of CD38- and HLADR-positive cells were determined. No significant differences were observed in immune activation in CD4⁺/CD8⁺ T-cell subpopulations between vaccine and the historical control samples ($p > 0.5$, Mann-Whitney test). (B) Immune dysfunction was measured by PD-1 expression. PBMC were stained with CD3 AmCyan, CD8 PerCPCy5.5, CD4 APC Cy7 and PD-1 APC. Samples were first gated on the CD3⁺/CD4⁺ (and CD3⁺/CD8⁺) lymphocyte populations, and the percent of PD-1-positive cells were subsequently determined. CD4+PD1+ and CD8+PD1+ expression was significantly higher in control samples when compared to vaccine samples ($p = 0.016$ and 0.041 , Mann-Whitney test), respectively. Horizontal lines represent median values.

Figure 4A **Figure 4B**

Figure 4C **Figure 4.**

(A) Representative plot of Gag-specific CD4 proliferation. CFSE-labeled PBMC were stimulated with subtype C Gag peptides for 5 days then assessed for proliferation by flow cytometry. Results are expressed as the percent of proliferating CD4⁺ T-cells as measured by the extent of CFSE dilution. Positive proliferation is defined as >0.1% net and at least twice background. (B) Gag-specific and (C) CMV-specific CD4⁺ and CD8⁺ proliferation in vaccinees and historical control samples. A significant difference between the frequency of responses in CD4- and CD8- mediated proliferation was observed between control and vaccine recipients for Gag ($p < 0.05$, Mann-Whitney test) but not CMV ($p > 0.05$).

Figure 5. CD4+ T cell profile of Phase 1A vaccine recipients with (+) and without (-) evidence of vaccine-specific T cell proliferation. The mean CD4 gain in the (+) group was 255 compared to 122 (-) in the non-immunized group. Horizontal lines represent mean values.

Figure 6.

Immunological and virological characteristics of Phase 1B vaccine recipients. Twenty-four individuals discontinued ART for a period of 4 weeks after receiving a booster of LFn-p24C. (A) Viral load (HIV RNA copies/ml plasma) and (B) absolute numbers of CD4⁺ T cells per mm³ of blood were monitored throughout treatment interruption and cessation periods. Blue shading depicts periods off ART.

Table 1**Visit Designation**

Study	Visit	Schedule
Phase 1A		
Day 0	1A	First Immunization
Day 3-7	2A	Clinical evaluation
Day 14	3A	Laboratory & Clinical evaluation
Day 28	4A	Second Immunization
Day 31-35	5A	Clinical evaluation
Day 42	6A	Laboratory & Clinical evaluation
Day 84	7A	Third Immunization
Day 87-90	8A	Clinical evaluation
Day 98	9A	Laboratory & Clinical evaluation
Day 168	10A	Laboratory & Clinical evaluation
Day 365	11A	T cell profile evaluation
Phase 1B		
Day 0	1B	Booster Immunization
Day 3-7	2B	Clinical evaluation
Day 14	3B	Discontinuation of any NNRTI
Day 21	4B	Discontinuation of ART
Day 35	5B	Laboratory & Clinical evaluation
Day 49	6B	ART Resumption
Day 63	7B	Laboratory & Clinical evaluation
Day 77	8B	Laboratory & Clinical evaluation
Day 91	9B	Laboratory & Clinical evaluation
Day 105	10B	Laboratory & Clinical evaluation
Day 182	11B	Laboratory & Clinical evaluation