A randomized placebo-controlled efficacy study of a prime boost therapeutic vaccination strategy in HIV-1 infected individuals: VRI02 ANRS 149 LIGHT Phase II trial

Y. Levy, C. Lacabaratz, Edouard Lhomme, A. Wiedemann, Claire Bauduin, C. Fenwick, E. Foucat, M. Surenaud, L. Guillaumat, Valerie Boilet, et al.

To cite this version:

Y. Levy, C. Lacabaratz, Edouard Lhomme, A. Wiedemann, Claire Bauduin, et al.. A randomized placebo-controlled efficacy study of a prime boost therapeutic vaccination strategy in HIV-1 infected individuals: VRI02 ANRS 149 LIGHT Phase II trial. Journal of Virology, American Society for Microbiology, In press, 10.1128/jvi.02165-20. hal-03188939

HAL Id: hal-03188939
https://hal.archives-ouvertes.fr/hal-03188939
Submitted on 2 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
A randomized placebo-controlled efficacy study of a prime boost therapeutic vaccination strategy in HIV-1 infected individuals: VRI02 ANRS 149 LIGHT Phase II trial

Y. Lévy,a,b,c Y. Lévy and C. Lacabaratz are co-first authors and contributed equally to this work; they are listed in order of seniority. J. D. Lelièvre and R. Thiébaut are co-senior authors and contributed equally to this work.

Corresponding author: Yves Lévy, yves.levy@aphp.fr

Running title: DNA-GTU and Lipopeptide therapeutic HIV vaccine

Keywords: HIV, antiretroviral therapy interruption, therapeutic vaccine

Abbreviations: cART, combined antiretroviral therapy; ATI, analytical treatment interruption

Abstract: 250; Importance: 141

Text: 5,528 (excluding the references and figure legends)

JVI Accepted Manuscript Posted Online 10 February 2021

J Virol doi:10.1128/JVI.02165-20

Copyright © 2021 Lévy et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.
ABSTRACT

In this placebo-controlled phase II randomized clinical trial, 103 HIV-1 infected patients under cART (combined antiretroviral treatment) were randomized 2:1 to receive 3 doses of DNA GTU-MultiHIV B (coding for Rev, Nef, Tat, Gag and gp160) at Week (W)0, W4 and W12 followed by 2 doses of LIPO-5 vaccine containing long peptides from Gag, Pol and Nef at W20 and W24 or placebos. Analytical treatment interruption (ATI) was performed between W36 to W48. At W28, vaccinees experienced an increase in functional CD4+ T cell responses measured (P<0.001 for each cytokine compared to W0) predominantly against Gag and Pol/Env and an increase in HIV-specific CD8+ T cells producing IL-2 and TNF-α (P=0.001 and 0.013, respectively), predominantly against Pol/Env and Nef. However, analysis of T cell subsets by mass cytometry in a subpopulation showed an increase of W28/W0 ratio for memory CD8+ T cells co-expressing exhaustion and senescence markers such as PD-1/TIGIT (P=0.004) and CD27/CD57 (P=0.044) in vaccinees compared to placebo. During ATI, all patients experienced viral rebound with a maximum observed HIV RNA level at W42 (median: 4.63 log_{10} cp/ml; IQR 4.00-5.09) without any difference between arms. No patient resumed cART for CD4 cell count drop. Globally, the vaccine strategy was safe. However, a secondary HIV transmission during ATI was observed.

These data show that the prime-boost combination of DNA and LIPO-5 vaccines elicited broad and polyfunctional T cells. The contrast between the quality of immune responses and the lack of potent viral control underscores the need of combined immunomodulatory strategies.
IMPORTANCE

In this placebo-controlled phase II randomized clinical trial, we evaluated the safety and immunogenicity of a therapeutic prime-boost vaccine strategy using a recombinant DNA vaccine (GTU®-MultiHIV B clade) followed by a boost vaccination by a lipopeptide vaccine (HIV-LIPO-5) in HIV-infected patients while on combined antiretroviral therapy. We show that this prime-boost strategy is well tolerated, consistently with previous studies in HIV-1 infected individuals and healthy volunteers who received each vaccine component individually. Compared to placebo group, vaccines elicited strong and polyfunctional HIV-specific CD4+ and CD8+ T cell responses. However, these immune responses presenting some qualitative defects were not able to control viremia following antiretroviral treatment interruption as no difference in HIV viral rebound was observed in vaccine and placebo groups. Several lessons were learned from these results pointing out the urgent need to combine the vaccine strategies with other immune-based interventions.
INTRODUCTION

Despite the beneficial effects of cART on HIV morbidity and mortality, these drugs do not eradicate the latent HIV reservoir resulting in a constant rebound in viremia after stopping cART (1). Several strategies are under development to clear latently infected cells, which contain integrated HIV DNA, capable to survive indefinitely in patients despite long-term cART. The concept behind these strategies, which needs to be proven, is that activation of these cells using latency reversing agents for instance, may lead to HIV reactivation, expression of HIV proteins and elimination of these cells by the immune system (2). Until now, clinical outcomes using this strategy have been disappointing. One possible obstacle is that the killing of cells harboring HIV requires robust and efficient T-cell responses making therapeutic vaccination central in strategies aiming at reducing the latent HIV reservoir and at achieving a functional cure (3).

In the last 25 years, several vaccine strategies to restore and improve HIV-specific functional immune responses have been developed, with varying results in terms of immunogenicity or HIV control when experimental designs comprised a period of ART interruption. In some trials, a partial effect on viral rebound was observed (4). Although promising, firm conclusions on the efficacy of these strategies are difficult to draw when they are based on non-controlled studies (4).

In the present study, we sought to address some of these issues by designing a randomized, placebo-controlled therapeutic vaccination trial combining two different HIV vaccines, GTU®-MultiHIV B clade and long HIV lipopeptide
sequences (HIV LIPO-5 vaccine) in a prime-boost regimen. These two vaccines share homologous HIV sequences and strong CTL epitopes. The GTU®-MultiHIV B encodes for a MultiHIV antigen (Rev, Nef, Tat, Gag p17/p24 proteins and an epitope stretch of previously identified CTL epitope-rich regions encoded by pol and env of a subtype B HIV-1 isolate Han-2) and lipopeptides are composed of 5 synthetic peptides (Nef 66-97, Nef 116-145, Gag 17-35, Gag 253-284, Pol 325-355 also from a clade B strain) to which lipid chains are covalently bound. GTU®-MultiHIV B has been evaluated in HIV untreated patients where it led to HIV-specific sustained CD4+ and CD8+ T cell response as well as a significant decline of plasma HIV viral load (5). More recent results combining transcutaneous (TC) and intramuscular (IM) injection of GTU®-MultiHIV B showed a lack of improvement of immune responses in HIV treated patients and concluded for the need of a combinatory approach (6). LIPO-5 has been evaluated in healthy adults where it led to a sustained HIV-specific CD4+ and CD8+ T-cell responses (7, 8).

Correlates of protection/control/cure are supposed to be different from HIV prophylactic interventions and especially T cell responses are supposed to play a key role in the clearance of infected cells. Successful approaches in SIV such as the CMV-based vaccine have given new bases on the key role of CD8+ T cell response (9). There is a consensus in the field to propose ATI for evaluation of the virological efficiency of an immune intervention on the HIV reservoir since the ultimate objective of any intervention in HIV treated patients is to maintain a low viral replication after cART withdrawal (10). The efficacy endpoints of our study
included T-cell immunogenicity and viral kinetics following a 12-week period of antiretroviral treatment interruption (ATI).
RESULTS

Study participants

One hundred and thirty-three HIV-infected individuals were screened, and 103 were enrolled and randomized in 18 centers in France between September 2013 and May 2015 (Figure 1B). Five participants withdrew consent before receiving any intervention, 98 received at least one injection of placebo (n=35) or vaccine strategy (n=63) and were included in the mITT analysis of the study. Baseline characteristics of participants are reported in Table 1. The two study arms were balanced at baseline. Nine participants (2 and 7 in placebo and vaccine groups, respectively) withdrew from further follow up after week 0, the majority due to consent withdrawal.

Global overview of vaccine immunogenicity

Evaluation of vaccine-elicited T cell responses measured by flow cytometry-based intracellular cytokine staining (ICS) for antigen-specific IFN-γ and/or IL-2 and/or TNF-α was performed at baseline and week 28 on ninety-two participants who received the complete schedule of vaccination/placebo until week 28 in a per protocol analysis. Total CD4+ T cell responses to several HIV peptides pools showed no difference between groups at entry. We found a significant increase of CD4+ T cells producing cytokines to HIV peptide pools in the vaccine group at week 28 compared to baseline (P<0.001) while no difference was observed in the placebo group (Figure 2A). Detailed analysis for each cytokine and each HIV peptide pool showed a significant increase in IFN-γ, IL-2 and TNF-α production after Gag and Pol/Env stimulation, but not Nef, in the vaccine group (Figure 2C)
as well as increases of CD4+ T cells producing 1, 2 or 3 cytokines at W28 (P<0.001 for all comparisons to baseline) (Figure 2D), specially against Gag and Pol/Env pools (P<0.001 for each comparison) (Figure 2F). While CD8+ T cells producing cytokines against HIV peptides did not change in the placebo group, we found an increase of total cytokine in the vaccine group at week 28 compared to baseline (Figure 2B), and detailed analysis showed that these responses were directed against Pol/Env and Nef peptides, but not Gag (Figure 2C). The frequency of polyfunctional CD8+ T cells (producing at least 2 cytokines), but not of monofunctional CD3+CD8+ T cells (producing only 1 cytokine), increases significantly in the vaccine group compared to W0 (P=0.04 and 0.025 for production of 2 and 3 cytokines, respectively) (Figure 2E), especially after Pol/Env or Nef stimulation (Figure 2F).

To extend the analysis of immune cells, an ancillary analysis of T cell phenotypic profile was performed in 28 patients (12 placebo and 16 vaccinees) with mass cytometry allowing the detection of 40 cell surface markers. Figure 3 depicts the W28/W0 ratio of gated positive populations for each marker in vaccinees compared to placebo. Significant changes of CD8+ memory T cell subsets were observed in the vaccine group after vaccination with a higher frequency of memory CD8+ T cells coexpressing PD-1 and TIGIT (Figure 3A), and coexpressing CD27 and CD57 (Figure 3B). Changes in the population of CD8+ T cell exhibiting markers of activation were also observed as memory HLA DR+ CD38+ CD8+ T cells were increased in the vaccine group (Figure 3C) without any modification of the memory CD4+ T cells (Figure 3D). No change was observed in the different CD4+ T cell subsets, including Treg or CD32a+ expressed on
CD4+ T cell HIV reservoir (not shown).

Analytical Treatment Interruption

ATI was proposed in both arms to individuals with plasma HIV RNA < 50 copies/ml at week 36. Eighty-nine participants (n=32 (91%) and 57 (90%) in placebo and vaccine groups, respectively) started ATI according to study protocol and were followed until week 48, the final study end point. Sixty-five participants (n=23 (66%) and 42 (67%) in placebo and vaccine groups, respectively) resumed ART according to the study protocol at week 48. Eighteen participants (n=7 (20%) and 11 (17%) in placebo and vaccine groups, respectively) resumed ART before week 48 for participants or doctors’ decisions. Two participants from each group resumed ART after week 48, and two participants from the vaccine group did not resume ART at the end of the follow up after week 48. Figure 4A shows longitudinal evolution of HIV plasma viral loads (VL) in the two study arms during the ATI period. The maximum level of viral load was observed at week 42 in both groups. mITT analysis did not show any significant differences between groups in terms of maximum observed (peak) viral load: median peak VL (Q1;Q3) between weeks 36-48 were 5.26 (4.58;7) and 5.15 (4.73;7) in placebo and vaccine groups, respectively (P=0.9). The frequency of participants with VL below 10 000 copies/ml at week 48, defined as the virological success, was 50% and 44% in placebo and vaccine groups, respectively. In total, seventy-one patients met this predefined success criterion of the strategy without any significant difference between groups: 25 and 46 in placebo and vaccine groups, respectively (Table 2).
The kinetics of the peak of VL looks slightly different between groups having experienced ATI. At week 40, the maximum peak of VL was observed in 44% and 28% of participants in the placebo and vaccine groups, respectively (P=0.27). They were 8% and 20% at week 44, respectively (Table 2). At the end of the ATI phase (week 48), two participants from the vaccine group did not resume ART because of plasma VL below 50 copies/ml. These participants had an initial VL rebound at week 42 and 44, and then exhibited a spontaneous suppression of viremia which remained undetectable without ART at the end of the study. Among the 91 participants restarting cART, 75 (31 and 44 in placebo and vaccine group, respectively) participants suppressed viremia (<50 copies/ml) at W74.

Figure 4B shows patterns of CD4+ T cell changes in participants during the ATI period. The evolution was similar in both groups of participants with a nadir (median, IQR) at week 44 of 657 cells/mm³ (556-832) and 661 (584-930) in placebo and vaccine groups, respectively. CD4+ T cell counts remain similar in both groups at the end of the study and after resuming ART at week 48.

Relationship between polyfunctionality of HIV-specific T cell responses and viral parameters following ATI

A principal component analysis (PCA) was conducted to illustrate the interrelationships between vaccine-induced T cell responses measured by ICS before ATI (polyf CD4, polyf CD8) and viral parameters during ATI. Figure 5A is a projection of variables on the first two axes. The first principal component (x-axis) represented 59% of the variability while the second principal...
component represented 21%. All immunological variables were on the right side of the figure, illustrating their trend to be positively correlated. The y-axis allowed to differentiate the CD8⁺ T cell responses (top) and the CD4⁺ T cell responses (bottom). Results showed that maximal viral load, viral load slope and viral load AUC were projected at the opposite direction of the immune markers, indicating a trend towards negative correlations between the magnitude of viral load after ATI and T cell responses before ATI. In Fig. 5B, the representation of the patients on the PCA illustrates the poor immunological status of some patients (left side) and the CD4⁺ and CD8⁺ T cell-oriented response of the others (middle right). Vaccinated participants seemed to be slightly more numerous on the right part of the plan (as shown by the distribution blue curve on the top) corresponding to good responders, while those who were on the left part exhibited poorer immunological responses (being vaccinated or not) with a higher maximum viral load.

**Safety**

Ninety-eight individuals received at least one injection, 93 received all injections. The majority of participants (96%) experienced at least one Adverse Events (AE) being transient (median duration 15 days, IQR 3-62). As shown in Table 3, most of the AE were grade 1 or 2 and there were no marked differences between arms. Among 15 Serious Adverse Events (SAE) (Table 4), one was possibly related to the GTU®-Multi-HIV B vaccine (arthritis) and one to the research: secondary HIV transmission during the ATI period confirmed by phylogenetic analysis of the HIV in the placebo arm (11). Primary infection-like symptoms,
usually mild, were observed in 23% of the individuals after ATI. There was no resumption of ART due to CD4 cell count drop during ATI.

DISCUSSION

In this study, we show that a therapeutic immunization strategy combining a DNA prime followed by a boost with long HIV lipopeptides is well tolerated in chronically HIV-1 infected individuals treated with cART. These safety data are consistent with previous studies in HIV-1 infected individuals and healthy volunteers who received each vaccine component individually (8, 12, 13). This study comprised two phases, a vaccination period followed by an ATI phase of 12 weeks to evaluate both the immunogenicity and virologic efficacy of the vaccine strategy. At the end of the vaccination period, the immunogenicity of the vaccine strategy was clearly demonstrated. Vaccinees exhibited significant changes in the frequency and the functionality of HIV-specific T cell responses. However, these changes in the immune status of individuals did not translate into any differences in the kinetics and magnitude of viral rebound following ATI. Consistently, we found that the vaccine strategy did not impact significantly the levels of cellular HIV-DNA measured before ATI (14). Nevertheless, integrative analysis of virological and immunological parameters showed a trend toward an association between good vaccine responders and a lower viral load after ATI, while individuals with poorer immunological responses (being vaccinated or not) exhibited a higher maximum viral load.
These findings might have important implications in the design and evaluation of future studies testing immunological interventions aimed at sustainably control viral replication without cART.

The rationale to combine a DNA GTU prime and HIV long lipopeptides was based on previous results obtained with each individual vaccine component. Administration of DNA GTU in cART naïve individuals resulted in a modest, but significant, decrease of plasma HIV viral load (up to 0.5 log_{10} copies/ml) in a large therapeutic study performed in South African individuals (5). Previous therapeutic vaccine studies centered around HIV lipopeptides provided also encouraging results (7, 8). Combination of ALVAC/HIV lipopeptide and IL-2 preceding ATI in chronically HIV-1 infected patients resulted in a greater chance to maintain a viral load during a 24 weeks ATI period (HIV RNA below 10 000 copies/ml as predefined in the present study) compared to individuals from a control arm (12, 15). In a recent non randomized vaccine study, we showed that vaccination with ex vivo generated Dendritic Cells (DC) loaded with HIV-lipopeptides (the Dalia trial) elicited strong CD4^{+} and CD8^{+} T cell responses associated with a control of viral replication following ATI in chronically HIV-1 infected individuals (16). In these two previous studies, we found a correlation between vaccine elicited responses and the magnitude of viral replication or the frequency of individuals maintaining plasma HIV viral load below a predefined threshold following ATI (12, 15–18).
Here, the combination of these two vaccine components in a prime boost strategy was also supported by the sharing of several HIV T-cell epitopes in common, raising the hypothesis of a stronger induction of HIV-specific T cell responses. Indeed, immunogenicity analysis showed a significant expansion of functional T-cell responses (producing at least two cytokines) against HIV-1 Gag, Env and Pol antigens for CD4+ T cells while CD8+ T cells were directed against Env, Pol, Nef but not Gag antigens. Despite this broad repertoire, these responses did not impact significantly HIV replication throughout the 12-weeks of ATI. The failure to show an association between vaccine immunological efficacy and the kinetics of viral rebound raises several questions about the repertoire, functionality of these responses and the immunological context following vaccination. In depth analysis and epitope mapping of T-cell responses elicited by the DC-based vaccine delivering HIV lipopeptides revealed an inverse correlation between the functionality of CD4+ T cell responses (production of IL-2 and IL-13), the repertoire of these responses directed against HIV Gag, Nef and Pol dominant epitopes, and the magnitude of viral rebound (17). These results are supported by several previous studies showing that robust HIV-1-specific T cell responses are associated with a better control of infection in LTNP (19). In the present study we did not investigate the precise repertoire of CD4+ T cell responses against individuals HIV epitopes. Whether the lack of antiviral effect of HIV-specific CD4+ T cell responses to vaccine regimen containing HIV lipopeptides delivered through IM route, as compared to DC delivery, could be explained by a difference in the immune profile (cytokine pattern) or repertoire of vaccine elicited CD4+ T-cell responses warrants further analyses.
We also show that the vaccine regimen elicited expansion of memory CD8+ T cell responses. Surprisingly, responses against Gag epitopes contained in the DNA GTU and Lipopeptide sequences were not amplified. Several teams, including our group, have shown the importance of CD8+ T cell responses to Gag in the control of HIV (17). Our results, from a subgroup of individuals, show also changes in the population of CD8+ T cell exhibiting markers of activation (increase of memory HLA DR+ CD38- CD8+ T cells in the vaccine group) and more importantly markers of exhaustion (TIGIT and PD-1) and senescence (CD57), which might indicate the low capacity of these cells to control viral replication. These inhibitory immune receptors have been previously shown to regulate antiviral and antitumor CD8+ T cell effector function in mice model of LCMV and in human with advanced melanoma (20–22). It has been shown that TIGIT and PD-1 blockade additively increased proliferation, cytokine production, and degranulation of tumor antigen-specific CD8+ T cells. One limitation of this observation is that we did not look at the expression of these markers on HIV-specific CD8+ T cells. However, as already described in cancer patients, we cannot rule out that these specific CD8+ T cells would exhibit a low killing capacity of HIV infected cells (20). Regarding the design of future studies, these results underscore the need to include functional killing assays in the evaluation of the efficacy of vaccine trials (23).

One intriguing question, beyond the results of this trial, is why despite the capability of eliciting strong immune responses, several candidate vaccines
tested showed disappointing results and failed to control HIV replication in cART-free individuals. We, and others, have already raised the hypothesis that the balance between inflammatory responses and activation of effector T cells seems crucial in this setting (24). The deleterious association of persistent inflammation signature after vaccination with the immune response to vaccine has been reported for several vaccine platforms (25, 26), including HIV (24). Recently, integrative analysis of a large set of arrays (T-cell responses, cytokine production, blood transcriptomic changes) evaluating immune responses in individuals receiving DC/HIV lipopeptide vaccine showed that inflammatory pathways related to Toll-Like Receptor signaling were associated with a poorer immune response to vaccination and poorer viral control after ATI (24). The similar involvement and impact of these pathways in responses to other vaccines indicates a potential broad mechanism driving the immune response to vaccine. Likely these data underscore the need to carefully investigate, besides the profile of effector specific T cells, the kinetics of inflammatory responses in future vaccine studies. Furthermore, these results point out the need to develop further strategies combining vaccines with adjuvants and/or immunomodulators (3).

The lack of immune correlates, or robust markers, predicting virologic control implies that a period of antiretroviral treatment interruption remains necessary to assess the efficacy of immune interventions in HIV-infected patients. Our study comprised a 12-weeks ATI period and an arbitrary threshold of plasma viral load defining the success of the strategy (i.e: frequency of individuals maintaining plasma viral load below 10 000 copies/ml). One month following cART
interruption, the maximum peak of plasma viral load concerned a higher percentage of placebo as compared to vaccinees (44% and 28% of participants, respectively). At the end of the ATI phase (week 48), two participants from the vaccine groups maintained a suppressed viral load below 50 copies/ml and remained without cART at week 74. Interestingly these participants had an initial VL rebound at week 42 and 44 which makes unlikely that these two subjects were elite HIV controllers. However, we were unable to demonstrate the efficacy of vaccine regimen in an intent-to-treat analysis and according to the predefined criteria of success. This underscores the added value of the comparison to a well-controlled placebo group (4) to limit the risk of misinterpreting results. The decision to propose ATI to individuals receiving placebo should be carefully balanced by the risk to miss the demonstration of efficacy or to erroneously conclude on existing efficacy of an immune intervention. Thus, the large heterogeneity of previous immunotherapeutic trials in terms of ATI duration, presence of a control group, threshold criteria for resuming cART, timeline of virologic evaluation might hinder the capacity to identify promising strategies. For example, the use of a conservative criterion for resuming cART, such as plasma viremia above 1 000-2 000 copies/ml, risks missing important positive effects of immune interventions on viral control (27). Likely, the recent consensus report on recommendations to optimize ATI strategies and to mitigate the risks for participants will help to better design future studies.

In order to minimize the risks for participants undergoing ATI, in our trial we used strict safety criteria for resuming cART before the end of the 12-week period of...
ATI, such as a confirmed >30% decline in CD4+ T cell count, an absolute CD4+ T cell count <350 cells/mm³, or the development of acute retroviral syndrome. Globally the strategy was well tolerated and no individuals reached these safety criteria for resuming cART. However, despite strong measures of counseling, ATI was associated with a secondary transmission to a sex partner of one participant from our study (11). This observation led our group to propose PrEP in our future HIV cure trial in France (28) but also at the European level (EHVA T02 trial NCT04120415; (29)). Although PrEP may mitigate the risk of secondary transmission, this strategy should be associated with strong counseling and additional measures of prevention because of the lack of clear data on the efficacy of PrEP against viral rebound to high levels of viremia following ATI. It would be also essential to closely monitor plasma viral load in participants during the ATI period and to adapt PrEP drugs to the resistant profile to the participant’s virus.

In conclusion, the prime boost regimen tested in this study was designed to maximize the immune response and to evaluate its virologic efficacy in a well-controlled design trial including a long-term ATI period. This study adds to the list of previous therapeutic vaccine trials showing that despite eliciting strong immune responses, no association to a long-term control of viremia was demonstrated. However, several lessons were learned from these results pointing out the urgent need to combine these vaccine strategies with other immune-based interventions.
MATERIALS AND METHODS

Study design and participants
The VRI02 ANRS 149 LIGHT trial is a phase II randomized, placebo-controlled, double-blinded, multicenter trial evaluating the safety and immunogenicity of a prime-boost vaccine strategy using a recombinant DNA prime vaccine (GTU®-MultiHIV B clade) followed by a boost vaccination by a lipopeptide vaccine (HIV-LIPO-5) in HIV-infected patients while on cART. Eligible patients were asymptomatic HIV-1-infected adults with CD4+ T-cell counts >600 cells/μL, plasma HIV RNA <50 copies/mL at screening and within the previous 6 months while on cART and were recruited in 18 hospitals in France. All study participants provided written informed consent before participation. The protocol was approved by the ethics committee of Ile de France 5 (Paris-Saint-Antoine) and authorized by the French regulatory authority (ANSM). The study is registered at www.ClinicalTrials.gov (NCT01492985) and EudraCT: 2009-018198-30.

Randomization and masking
Participants were randomized in a 1:2 ratio to receive either placebo or active vaccine. Randomization was done centrally one week before the first vaccination via the electronic case report software (Ennov clinical® software), on the basis of a randomization list generated by the unblinded statistician (CMG-EC, Inserm U1219, Bordeaux). Site staff and participants were both masked to the treatment assignment.
Procedures

DNA GTU MultiHIV and HIV LIPO-5 have been described elsewhere (5–7).

Briefly, GTU®-MultiHIV B clade, developed by FIT Biotech, encodes for a MultiHIV antigen (synthetic fusion protein built up by full-length polypeptides of Rev, Nef, Tat, p17 and p24 with more than 20 Th and CTL epitopes of protease, reverse transcriptase (RT) and env gp160 regions of the HAN2 HIV-1 B clade.

HIV-LIPO-5 vaccine consisted in 5 long HIV peptides from (Nef 66-97; Nef 116-145; Gag 17-35; Gag 253-284 and Pol 325-355) to which lipid tail are covalently bound. These lipopeptides which cover HIV epitopes binding to > 90% of HLA molecules, permit presentation of CD4+ and CD8+ T cell epitopes as well as generation of humoral immunity (17).

The DNA GTU MultiHIV at a dose of 1 mg or placebo priming vaccinations were administered IM using a biojector at study weeks 0, 4 and 12. HIV-LIPO-5 boosts, at a dose of 2.5 mg (0.5 mg of each lipopeptide) or placebo were given at weeks 20 and 24 (Figure 1A). For immunological analysis, PBMC samples were collected at entry (W0), four weeks following the last DNA GTU prime (W16) and the last LIPO-5 boost (W28), W48 (final end point) and W74. A cART interruption between W36 and W48 was proposed to individuals who had HIV-1 RNA <50 copies/mL and CD4+ T-cell counts >600 cells/µL. Clinical, immunological (CD4+ and CD8+ T cell counts) and virological (HIV viral load) follow-up was performed every 15 days for 2 months during ATI, then monthly. cART has to be resumed at W48 but could be resumed at any time according to the following criteria: (i) if the patients or their doctors wished so; (ii) if CD4+ T-cell count was <350 cells/µL at two consecutive measurements 2-weeks apart; and (iii) in the case of occurrence
of an opportunistic infection or a serious non-AIDS defining event. Patients were followed until W74 for final safety evaluation after resuming cART.

**Intracellular cytokine staining (ICS) assay**

Cell functionality was assessed by ICS, with Boolean gating to examine vaccine-induced HIV-1-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses after stimulation with 3 different HIV 15-mer peptide pools (1 pool Gag, 1 pool Pol/Env and 1 pool Nef peptides, JPT Peptide Technologies GmbH, Berlin, Germany). SEB-stimulated and unstimulated cells were used as positive and negative control, respectively. The flow cytometry panel included a viability marker, CD3, CD4 and CD8 to determine T-cell lineage, and IFN-γ, TNF-α, and IL-2 antibodies. Data were acquired on a LSRFortessa 4-laser (488, 640, 561 and 405 nm) cytometer (BD Biosciences) and analyzed using FlowJo software version 9.9.4 (Tree Star inc.).

**Mass cytometry staining and analyses**

In a subpopulation of patients from both groups selected among those presenting an ICS response, a mass Cytometry (CyTOF) analysis was performed at weeks 0 and 28. PBMC were thawed, rested and then stained using metal-conjugated antibodies according to the CyTOF manufacturer’s instructions (Fluidigm, San Francisco, CA). Cell viability staining was performed using the Cell-ID<sup>TM</sup>-103 Rh Intercallator at a final concentration of 1 µM that was incubated with PBMC for 15 minutes. PBMC from an individual donor were treated in parallel and multiplexed for staining and mass cytometry analysis to limit sample variation due to sample preparation and analysis. Multiplexed week 0 and week 28 PBMC were stained
for 20 minutes with either anti-CD45 89 Y or anti-CD45 194 Pt isotopes, respectively, and then washed with CSM buffer (PBS, 0.5% BSA, 0.02% sodium azide, all from Sigma) before combining the two samples. Pooled samples containing 2-4x10^6 cells were stained for 30 minutes using a cocktail of antibodies for cell surface markers in a total volume of 50 µl (Table 5). Cells were subsequently washed with CSM and PBS, fixed with 2.4% formaldehyde (Thermo Fisher) in PBS for 5 minutes and then resuspended in DNA-intercalation solution (PBS, 1 µM Ir-Intercalator, 1% formaldehyde, 0.3% saponin) before storage at 4°C until analysis. For CyTOF analysis, cells were washed 3 times with MilliQ water and resuspended at 0.5x10^6 cells/ml in 0.1% EQ™ Four Element Calibration Beads solution (Fluidigm). Samples were normalized for the EQ™ bead intensities using the matlab normalizer software to limit inter analysis staining intensities. Data were processed and analyzed with cytobank. Since W0 and W28 samples for a given donor were multiplexed and stained in parallel, the relative changes in marker intensities were determined using the W28/W0 ratio for the indicated gated positive populations.

**Study endpoints**

The primary endpoint was the maximum observed plasma HIV-1 RNA load (in log_{10} copies/mL) during the ATI period between W36 and W48. Participants not having interrupted cART at W36, or having resumed their treatment before W48, were imputed with the maximum plasma HIV-1 RNA load observed among all the participants during the ATI. The delay to the maximum plasma viral load was also described in participants having experienced the ATI between W36 and W48.
Clinical and virological secondary endpoints were as follows: the frequency of clinical and biological adverse events occurring during the trial; CD4+ T cell counts at W40, W44, W48, W74; HIV-1 RNA loads at W40, W44, W48, W74; the virological success, assessed as the percentage of participants with plasma HIV-1 RNA load below 10,000 copies/mL at W48 considering virological failure for participants not having interrupted cART at W36; proportion of participants who reinitiated ARTs after W36; proportion of participants with CD4+ T cell counts < 350/mm³.

Secondary immunological end points were ICS based on the boolean and the marginal percentages of cells producing IFN-γ, IL-2 and TNF-α per T cell population (CD3+CD4+ and CD3+CD8+) after HIV stimulation (Gag, Pol/Env, Nef and total HIV) with background subtraction (negative values obtained after removing background were imputed to zero). The percentages of cells producing at least one cytokine among IFN-γ, IL-2 and TNF-α and polyfunctional cells (cells producing at least two or three cytokines) were also described.

**Statistical analysis**

The sample size calculation was based on the assumption that a reduction by at least 0.7 log₁₀ copies/mL in plasma HIV-1 RNA level at the end of the ATI in the vaccinated group compared to the placebo group (standard deviation of the viral load at the end of the interruption estimated at 1.0 log₁₀ copies/mL in the Window/ANRS 106 trial). With a two-sided type I error of 5% and a power of at least 90% (Wilcoxon-rank test), the targeted number of participants was 35 in the placebo group and 70 in the vaccine group.
All efficacy and safety analyses were carried out as modified intention to treat (mITT), in which participants who received no vaccine dose were excluded from the analysis. The immunological analyses were based on the per protocol population defined as exclusion from the analyses of participants with any discontinuation regarding either the vaccine therapy or the ATI. Quantitative and qualitative variables were respectively described by median and inter-quartile range and by frequency and proportion.

The primary endpoint expressed as the maximal plasma viral load during the ATI was compared between the placebo and the vaccine groups with the two-sided Wilcoxon-rank test. The immune ICS responses were compared between W0 and W28 in each arm using the Wilcoxon signed-rank test. A principal component analysis across ICS responses at W28 (log-transformed marginal % of positive cells for IFN-γ, IL-2, TNF-α, per T cell population, CD4⁺ and CD8⁺ respectively) was performed with a projection of highest viral load during ATI, viral load slope, time to rebound and viral load AUC as supplementary variable.

Statistical analyses were performed using SAS (version 9.3 or higher, SAS Institute, Cary, NC, USA) and R (version 3.6.0, The R Foundation for Statistical Computing, Vienna, Austria). Tests with a two-sided $P$ value of $<.05$ were considered statistically significant.

ACKNOWLEDGMENTS

Special thanks to all the patients included in this trial and to members of all clinical sites:
Pr. O. Bouchaud (Avicenne Hospital, Bobigny), Dr. L. Cotte (Croix Rousse Hospital, Lyon), Dr. L. Cuzin (Purpan Hospital, Toulouse), Pr. M. Dupon (Pellegrin Hospital, Bordeaux), Dr. V. Garrait (CHIC Hospital, Créteil), Dr. P. Genet (Victor Dupouy Hospital, Argenteuil), Pr. P-M. Girard (Saint Antoine Hospital, Paris), Pr. C. Goujard (Kremlin Bicêtre Hospital, Le Kremlin Bicêtre), Dr. L. Hocqueloux (La Source Hospital, Orléans), Dr. V. Joly (Bichat Hospital, Paris), Pr. O. Launay (Cochin Hospital, Paris), Pr. J-D. Lelièvre (Henri Mondor Hospital, Créteil), Dr. J-M. Livrozet (Edouard Herriot Hospital, Lyon), Pr. F. Lucht (North Hospital, St Etienne), Pr. J-M. Molina (Saint Louis Hospital, Paris), Pr. P. Morlat (Saint André Hospital, Bordeaux), Pr. J-P. Viard (Hôtel Dieu Hospital, Paris), Dr. D. Zucman (Foch Hospital, Suresnes).

We thank members of the scientific and independent committees: S. Abgrall, C. Delaugerre, M-L. Gougeon, J. Izopet, J-M. Molina, S. Paul, J-P. Viard, S. Grabar, A-G. Marcellin, I. Poizot-Martin and H. Pollard.

We thank G. Chêne and R. Thiebaut (methodologists), V. Boilet, V. Rieux and P. Reboud (project managers), I. Amri and A. Diallo (safety officers), C. Boucherie, A. Assuied, A. Perrier and C. Bauduin (statisticians), M. Soussi, S. Aït-Ouferoukf, F. Paraina, M. Badets and M. Desvallées (clinical research assistants), E. Grellet and M. Termote (data managers), C. Lacabarat, A. Wiedemann, M. Surenaud, E. Foucat, C. Krief, L. Guillaumat and G. Fenwick (Immunologists), H. Hocini, C. Lefebvre and P. Tisserand, B. Hejblum, S. Delahaye, and E. Lhomme (biostatisticians).
Inserm-ANRS is the sponsor of the trial. It was funded by ANRS, VRI 582 (Investissements d’Avenir program managed by the ANR under reference ANR-10-LABX-77-01) and conducted with the support of FIT Biotech Oy (Finland) for supplying the GTU-MultiHIV B vaccine candidate.

YL, CL, LR, JDL and RT contributed to study conception and design. VB, VR and CB contributed to trial coordination, monitoring, and data management. AW, CF, EF, MS and LG performed experiments and analyzed the data. YL, CL, EL, AW, CB, CF, LR, GP, JDL and RT participated to the statistical analysis and the interpretation of data. YL, CL, EL, CB, VB, LR, JDL and RT contributed to drafting of manuscript. AW, CF, VR and GP contributed to critical revision. All authors provided input into the report and approved the final version.

None of the authors declares a conflict of interest.
REFERENCES

1. Siliciano RF. 2014. Targeting reservoirs to clear and cure. Nat Med 20:480-481.

2. Margolis DM, Garcia JV, Hazuda DJ, Haynes BF. 2016. Latency reversal and viral clearance to cure HIV-1. Science 353:aaf6517.

3. Pantaleo G, Levy Y. 2016. Therapeutic vaccines and immunological intervention in HIV infection: A paradigm change. Curr Opin HIV AIDS 11:576-584.

4. Stephenson KE. 2018. Therapeutic vaccination for HIV. Curr Opin HIV AIDS 13:408–415.

5. Vardas E, Stanescu I, Leinonen M, Ellefsen K, Pantaleo G, Valtavaara M, Ustav M, Reijonen K. 2012. Indicators of therapeutic effect in FIT-06, a Phase II trial of a DNA vaccine, GTU®-Multi-HIVB, in untreated HIV-1 infected subjects. Vaccine 30:4046–4054.

6. Haidari G, Day S, Wood M, Ridgers H, Cope A V., Fleck S, Yan C, Reijonen K, Hannaman D, Spentzou A, Hayes P, Vogt A, Combadiere B, Cook A, McCormack S, Shattock RJ. 2019. The Safety and Immunogenicity of GTU®MultiHIV DNA Vaccine Delivered by Transcutaneous and Intramuscular Injection With or Without Electroporation in HIV-1 Positive Subjects on Suppressive ART. Front Immunol 10:2911.

7. Salmon-Ceron D, Durier C, Desaint C, Cuzin L, Surenaud M, Hamouda NB, Lelievre JD, Bonnet B, Pialoux G, Poizot-Martin I, Aboulker JP, Levy Y, Launay O. 2010. Immunogenicity and safety of an HIV-1 lipopeptide
8. Richert L, Hue S, Hocini H, Raimbault M, Lacabaratz C, Surenaud M, Wiedemann A, Tisserand P, Durier C, Salmon D, Lelievre JD, Chene G, Thiebaut R, Levy Y. 2013. Cytokine and gene transcription profiles of immune responses elicited by HIV lipopeptide vaccine in HIV-negative volunteers. AIDS 27:1421–1431.

9. Hansen SG, Marshall EE, Malouli D, Ventura AB, Hughes CM, Ainslie E, Ford JC, Morrow D, Gilbride RM, Bae JY, Legasse AW, Oswald K, Shoemaker R, Berkemeier B, Bosche WJ, Hull M, Womack J, Shao J, Edlefsen PT, Reed JS, Burwitz BJ, Sacha JB, Axthelm MK, Früh K, Lifson JD, Picker LJ. 2019. A live-attenuated RhCMV/SIV vaccine shows long-term efficacy against heterologous SIV challenge. Sci Transl Med 11:eaaw2607.

10. Julg B, Dee L, Ananworanich J, Barouch DH, Bar K, Caskey M, Colby DJ, Dawson L, Dong KL, Dubé K, Eron J, Frater J, Gandhi RT, Geleziunas R, Goulder P, Hanna GJ, Jefferys R, Johnston R, Kuritzkes D, Li JZ, Likhitwonnawut U, van Lunzen J, Martinez-Picado J, Miller V, Montaner LJ, Nixon DF, Palm D, Pantaleo G, Peay H, Persaud D, Salzwedel J, Salzwedel K, Schacker T, Sheikh V, Søgaard OS, Spudich S, Stephenson K, Sugarman J, Taylor J, Tebas P, Tiemessen CT, Tressler R, Weiss CD, Zheng L, Robb ML, Michael NL, Mellors JW, Deeks SG, Walker BD. 2019. Recommendations for analytical antiretroviral treatment interruptions in HIV research trials—report of a consensus meeting. Lancet HIV 6:e259-e268.
11. Lelièvre J-D, Hocqueloux L. 2019. Unintended HIV-1 Transmission to a Sex Partner in a Study of a Therapeutic Vaccine Candidate. J Infect Dis 220:S5–S6.

12. Lévy Y, Gahéry-Ségard H, Durier C, Lascaux AS, Goujard C, Meiffrédy V, Rouzioux C, El Habib R, Beumont-Mauviel M, Guillet JG, Delfraissy JF, Aboulker JP. 2005. Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. AIDS 19:279–286.

13. Goujard C, Marcellin F, Hendel-Chavez H, Burgard M, Meiffrédy V, Venet A, Rouzioux C, Taoufik Y, El Habib R, Beumont-Mauviel M, Aboulker J-P, Lévy Y, Delfraissy J-F. 2007. Interruption of Antiretroviral Therapy Initiated during Primary HIV-1 Infection: Impact of a Therapeutic Vaccination Strategy Combined with Interleukin (IL)-2 Compared with IL-2 Alone in the ANRS 095 Randomized Study. AIDS Res Hum Retroviruses 23:1105–1113.

14. Palich R, Ghosn J, Chaillon A, Boilet V, Nere M-L, Chaix M-L, Delobel P, Molina J-M, Lucht F, Bouchaud O, Rieux V, Thiebaut R, Levy Y, Delaugerre C, Lelievre J-D. 2019. Viral rebound in semen after antiretroviral treatment interruption in an HIV therapeutic vaccine double-blind trial. AIDS 33:279–284.

15. Lévy Y, Durier C, Lascaux AS, Meiffrédy V, Gahéry-Ségard H, Goujard C, Rouzioux C, Resch M, Guillet JG, Kazatchkine M, Delfraissy JF, Aboulker JP. 2006. Sustained control of viremia following therapeutic immunization in chronically HIV-1-infected individuals. AIDS 20:405–413.
16. Lévy Y, Thiébaut R, Montes M, Lacabaratz C, Sloan L, King B, Pérusat S, Harrod C, Cobb A, Roberts LK, Surenaud M, Boucherie C, Zurawski S, Delaugerre C, Richert L, Cîne G, Banchereau J, Palucka K. 2014. Dendritic cell-based therapeutic vaccine elicits polyfunctional HIV-specific T-cell immunity associated with control of viral load. Eur J Immunol 44:2802–2810.

17. Surenaud M, Lacabaratz C, Zurawski G, Lévy Y, Lelièvre JD. 2017. Development of an epitope-based HIV-1 vaccine strategy from HIV-1 lipopeptide to dendritic-based vaccines. Expert Rev Vaccines 16:955-972.

18. Surenaud M, Montes M, Lindestam Arlehamn CS, Sette A, Banchereau J, Palucka K, Lelièvre J-D, Lacabaratz C, Lévy Y. 2019. Anti-HIV potency of T-cell responses elicited by dendritic cell therapeutic vaccination. PLoS Pathog 15:e1008011.

19. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA. 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107:4781–4789.

20. Johnston RJ, Comps-Agrar L, Hackney J, Yu X, Huseni M, Yang Y, Park S, Javinal V, Chiu H, Irving B, Eaton DL, Grogan JL. 2014. The Immunoreceptor TIGIT Regulates Antitumor and Antiviral CD8+ T Cell Effector Function. Cancer Cell 26:923–937.

21. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, Kirkwood JM, Chen THT, Maurer M, Korman AJ, Zarour HM. 2015. TIGIT and PD-1 impair tumor antigen-specific CD8+ T cells in melanoma patients. J Clin
22. Chew GM, Fujita T, Webb GM, Burwitz BJ, Wu HL, Reed JS, Hammond KB, Clayton KL, Ishii N, Abdel-Mohsen M, Liegler T, Mitchell BI, Hecht FM, Ostrowski M, Shikuma CM, Hansen SG, Maurer M, Korman AJ, Deeks SG, Sacha JB, Ndlovu LC. 2016. TIGIT Marks Exhausted T Cells, Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. PLoS Pathog 12:e1005349.

23. Sáez-Cirión A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, Barré-Sinoussi F, Delfraissy JF, Sinet M, Pancino G, Venet A. 2007. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A 104:6776–6781.

24. Thiébaut R, Hejblum BP, Hocini H, Bonnabau H, Skinner J, Montes M, Lacabaratz C, Richert L, Palucka K, Banchereau J, Lévy Y. 2019. Gene expression signatures associated with immune and virological responses to therapeutic vaccination with dendritic cells in HIV-infected individuals. Front Immunol 10:874.

25. Obermoser G, Presnell S, Domico K, Xu H, Wang Y, Anguiano E, Thompson-Snipes LA, Ranganathan R, Zeitner B, Bjork A, Anderson D, Speake C, Ruchaud E, Skinner J, Alsina L, Sharma M, Dutartre H, Cepika A, Israelsson E, Nguyen P, Nguyen QA, Harrod AC, Zurawski SM, Pascual V, Ueno H, Nepom GT, Quinn C, Blankenship D, Palucka K, Banchereau J, Chaussabel D. 2013. Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and
pneumococcal vaccines. Immunity 38:831–844.

26. Kazmin D, Nakaya HI, Lee EK, Johnson MJ, Van Der Most R, Van Den Berg RA, Ballou WR, Jongert E, Wille-Reece U, Ockenhouse C, Aderem A, Zak DE, Sadoff J, Hendriks J, Wrammert J, Ahmed R, Pulendran B. 2017. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci U S A 114:2425–2430.

27. Sneller MC, Clarridge KE, Seamon C, Shi V, Zorawski MD, Justement JS, Blazkova J, Huiting ED, Proschan MA, Mora JR, Shetzline M, Moir S, Lane HC, Chun TW, Fauci AS. 2019. An open-label phase 1 clinical trial of the anti-α4β7 monoclonal antibody vedolizumab in HIV-infected individuals. Sci Transl Med 11:eaax3447.

28. Lelièvre J-D. 2019. Preexposure Prophylaxis for Mitigating Risk of HIV Transmission During HIV Cure–Related Clinical Trials With a Treatment Interruption. J Infect Dis 220:S16–S18.

29. Moore CL, Stöhr W, Crook AM, Richert L, Lelièvre JD, Pantaleo G, García F, Vella S, Lévy Y, Thiébaut R, McCormack S. 2019. Multi-arm, multi-stage randomised controlled trials for evaluating therapeutic HIV cure interventions. Lancet HIV 6:e334-e340.
**Figure legends**

**Figure 1: Trial design.** (A) Schematics of study design. Blue arrows indicate time of DNA GTU-MultiHIV B or placebo administrations. Red arrows indicate time of HIV LIPO-5 or placebo administrations. ART, antiretroviral therapy; ATI, analytical treatment interruption; (B) Consolidated Standards of Reporting Trials (CONSORT) flow diagram for the trial. CONSORT diagram delineates the study enrollment of 103 participants who underwent randomization to the placebo or vaccine groups.

**Figure 2: Functional profile of CD4\(^+\) and CD8\(^+\) T cell responses.** Production of IL-2, IFN-\(\gamma\) and TNF-\(\alpha\) as measured by intracellular cytokine staining (ICS) using multiparametric flow cytometry after cell stimulation before (W0) and after vaccination (W28) in placebo (red) and therapeutic vaccine (blue) groups: (A) HIV-specific CD3\(^+\)CD4\(^+\) T cell frequency; (B) HIV-specific CD3\(^+\)CD8\(^+\) T cell frequency; (C) Heatmap of P values between W28 and W0 of CD3\(^+\)CD4\(^+\) and CD3\(^+\)CD8\(^+\) marginal responses against Gag, Pol/Env, Nef and sum of HIV peptides (Total HIV); (D) Frequency of HIV-specific CD3\(^+\)CD4\(^+\) T cells producing 1, 2 or 3 cytokines in the vaccine group at W0 (light grey) and W28 (dark grey); (E) Frequency of HIV-specific CD3\(^+\)CD8\(^+\) T cells producing 1, 2 or 3 cytokines in the vaccine group at W0 (light grey) and W28 (dark grey); (F) Heatmap of P values between W28 and W0 of CD3\(^+\)CD4\(^+\) and CD3\(^+\)CD8\(^+\) polyfunctionality responses against Gag, Pol/Env, Nef and total HIV peptides.
**Figure 3: CyTOF phenotyping.** Ratio of memory CD8$^+$ T cells at W28 compared to W0 for several subsets according to PD-1 and TIGIT (A), CD27 and CD57 (B), or HLA-DR and CD38 (C) in placebo (red) and therapeutic vaccine (blue) groups. Ratio of memory CD4$^+$ T cells at W28 compared to W0 for HLA-DR and CD38 (D) in placebo (red) and therapeutic vaccine (blue) groups. P-values were calculated using the Mann-Whitney test, where P=0.017 (*); P=0.0022 (**).

**Figure 4: Plasma HIV viral load and CD4$^+$ T cell count changes throughout the study.** (A) Levels of plasma HIV RNA in the placebo (red) and therapeutic vaccine (blue) groups before and after ATI (week 36-48); (B) CD4$^+$ T cell count changes during the vaccination phase and following ATI in the placebo (red) and therapeutic vaccine (blue) groups before and after ATI (week 36-48).

**Figure 5: Integrative analysis of immune response to vaccine.** Principal component analysis of ICS responses at W28. Log-transformed marginal CD4$^+$ and CD8$^+$ T cell responses at W28 were included as active variables; virological markers during ATI (highest viral load, viral load slope, viral load AUC, time to rebound) were included as supplementary variables. (A) Projection of variables; (B) Projection of individuals represented into placebo (red) and therapeutic vaccine (blue) groups.
Table 1: Baseline characteristics of study participants

|                                | Placebo (n = 35) | Vaccine (n = 63) | Total (n = 98) |
|--------------------------------|------------------|------------------|---------------|
| Age in years*                  | 44 (38 ; 49)     | 46 (36 ; 51)     | 45 (38 ; 51)  |
| Male, n (%)                    | 30 (86)          | 56 (89)          | 86 (88)       |
| Time since first positive serology (in years)* | 7 (5 ; 13)     | 8 (4 ; 14)       | 7 (4 ; 14)    |
| Nadir CD4$^+$ (/mm$^3$)*       | 390 (335 ; 502)  | 389 (332 ; 480)  | 390 (334 ; 480) |
| CD4$^+$ count at baseline (/mm$^3$)* | 844 (684 ; 1060) | 840 (744 ; 1018) | 842 (733 ; 1045) |
| RNA zenith (log$_{10}$ cp/ml)* | 5.1 (4.8 ; 5.6)  | 5.0 (4.4 ; 5.4)  | 5.0 (4.5 ; 5.4) |
| RNA at baseline (log$_{10}$ cp/ml)* | 1.6 (1.6 ; 1.6) | 1.6 (1.6 ; 1.6) | 1.6 (1.6 ; 1.6) |

* Median (Q1;Q3)
Table 2: Plasma HIV RNA values during ATI period

|                      | Placebo (n = 35) | Vaccine (n = 63) | Total (n = 98) | P-value |
|----------------------|------------------|------------------|----------------|---------|
| Maximum VL during ATI (log₁₀ cp/ml) |                  |                  |                |         |
| Mean (SD)            | 5.39 (1.40)      | 5.42 (1.17)      | 5.41 (1.25)    | 0.878   |
| Median (IQR)         | 5.26 (4.58 ; 7.00) | 5.15 (4.73 ; 7.00) | 5.16 (4.70 ; 7.00) |         |
| Range [min-max]      | [1.60 – 7.00]    | [1.60 - 7.00]    | [1.60 - 7.00]  |         |
| ATI experience between W36 and W48, n (%) | 25 (71)         | 46 (73)         | 71 (72)        |         |
| Time of maximum VL during ATI in participants having experienced ATI, n (%) |                  |                  |                |         |
| W38                  | 2 (8)            | 3 (7)            | 5 (7)          |         |
| W40                  | 11 (44)          | 13 (28)          | 24 (34)        |         |
| W42                  | 8 (32)           | 15 (33)          | 23 (32)        |         |
| W44                  | 2 (8)            | 9 (20)           | 11 (15)        |         |
| W48                  | 2 (8)            | 6 (13)           | 8 (11)         |         |
| Maximum VL during ATI (log₁₀ cp/ml) in participants having experienced ATI | | | 6.12 | |
| Participants with VL below 10 000 cp/ml at W48*, n (%) | | | 6.12 | |
| No                   | 17 (50)          | 34 (56)          | 51 (54)        |         |
| Yes                  | 17 (50)          | 27 (44)          | 44 (46)        |         |

* 4 participants did not resume ART at W36 and were considered in virological failure at W48
Table 3: Adverse events (AE) after W0

|                                  | Placebo (n = 35) | Vaccine (n = 63) | Total (n = 98) |
|----------------------------------|------------------|------------------|---------------|
| Participants presenting at least one AE, n (%) | 35 (100)         | 59 (94)          | 94 (96)       |
| Participants presenting at least one biological AE, n (%) | 5 (14)           | 9 (14)           | 14 (14)       |
| Participants presenting at least one clinical AE, n (%) | 35 (100)         | 59 (94)          | 94 (96)       |
| AE by maximal grade, n (%)       |                  |                  |               |
| Grade 1: Mild                    | 84 (38)          | 217 (54)         | 301 (48)      |
| Grade 2: Moderate                | 123 (56)         | 170 (42)         | 293 (47)      |
| Grade 3: Severe                  | 12 (5)           | 18 (4)           | 30 (5)        |
| Grade 4: Life threatening        | 1 (0)            | -                | 1 (0)         |
| SAE among all AEs, n (%)         |                  |                  |               |
| No                               | 213 (97)         | 397 (98)         | 610 (98)      |
| Yes                              | 7 (3)            | 8 (2)            | 15 (2)        |
| Median duration of AE (in days) (Q1;Q3) | 24 (5;90)        | 12 (3;49)        | 15 (3;62)     |
| Participants presenting at least one AE related to vaccine, n (%) | 12 (34)          | 34 (54)          | 46 (47)       |
| AE related to vaccine by maximal grade, n (%) | 36               | 105              | 141           |
| Grade                  | No   | 100% | 99%  | 99%  |
|------------------------|------|------|------|------|
|                         | 36   | 85   | 104  |
| Yes                    | -    | 1    | 1    |

- SAE among AE related to vaccine, n (%)
Table 4: Description of severe adverse events (SAE) after W0 by System Organ Class (SOC) and preferred term (PT)

| SOC                                | PT                                            | Placebo (n = 7) | Vaccine (n = 8) | Total (n = 15) |
|------------------------------------|-----------------------------------------------|----------------|----------------|----------------|
| Infections and infestations        | Peritonitis, n (%)                            | 1 (14.3)       | .              | 1 (6.7)        |
|                                    | Bacterial rectitis, n (%)                      | .              | 1 (12.5)       | 1 (6.7)        |
|                                    | Transmitting the HIV infection, n (%)          | 1 (14.3)       | .              | 1 (6.7)        |
| Injury, poisoning and procedural   | Accident on the public highway, n (%)          | .              | 1 (12.5)       | 1 (6.7)        |
| complications                      | Artery stenosis, n (%)                         | .              | 1 (12.5)       | 1 (6.7)        |
|                                    | Toxicity of various agents, n (%)              | .              | 1 (12.5)       | 1 (6.7)        |
| Musculoskeletal and connective     | Arthralgia, n (%)                              | 1 (14.3)       | .              | 1 (6.7)        |
| disorders                          | Rheumatoid arthritis, n (%)                    | .              | 1 (12.5)       | 1 (6.7)        |
| Heart disorders                    | Congestive cardiomyopathy, n (%)               | 1 (14.3)       | .              | 1 (6.7)        |
| Reproductive system and breast     | Benign prostatic hypertrophy, n (%)            | 1 (14.3)       | .              | 1 (6.7)        |
| disorders                          | Craniocerebral injuries + loss of consciousness, n (%) | .          | 1 (12.5)       | 1 (6.7)        |
| Nervous system disorders           | Iron deficiency anemia, n (%)                  | 1 (14.3)       | .              | 1 (6.7)        |
| Blood and lymphatic system disorders | Suicide, n (%)                                | 1 (14.3)       | .              | 1 (6.7)        |
| Disorder                                      | n (%)     |
|----------------------------------------------|-----------|
| Respiratory, thoracic and mediastinal disorders | 1 (12.5) 1 (6.7) |
| Metabolism and nutrition disorders           | 1 (12.5) 1 (6.7) |
Table 5: Overview of the mass cytometry panel

| Marker | Isotope | Clone | Source | Vol (µl) per 50 µl |
|--------|---------|-------|--------|-------------------|
| CD45   | 89 Y    | HI30  | Fluidigm | 0.40             |
| CD8    | 113 In  | RPA-T8| Biolegend | 0.50             |
| CD4    | 115 In  | RPA-T4| Biolegend | 0.40             |
| CCR6   | 141 Pr  | 1IA9  | Fluidigm | 0.50             |
| CD19   | 142 Nd  | HB19  | Fluidigm | 0.80             |
| ICOS   | 143 Nd  | C398.4A| Biolegend | 0.80             |
| CD69   | 144 Nd  | FN50  | Fluidigm | 0.50             |
| CD31   | 145 Nd  | WM59  | Fluidigm | 0.60             |
| IgD    | 146 Nd  | IA6-2 | BD Biosciences | 0.70             |
| CD28   | 147 Sm  | L293  | BD Biosciences | 0.30             |
| CD57   | 148 Nd  | G10F5 | Biolegend | 0.25             |
| CCR4   | 149 Sm  | 205410| Fluidigm | 0.75             |
| OX40   | 150 Nd  | ACT35 | Fluidigm | 1.20             |
| CD103  | 151 Eu  | Ber-ACT8| Fluidigm | 0.80             |
| CD21   | 152 Sm  | BL13  | Fluidigm | 0.50             |
| TIGIT  | 153 Eu  | MBSA43| Fluidigm | 0.60             |
| TLR2   | 154 Sm  | TL2.1 | Fluidigm | 1.00             |
| CD27   | 155 Gd  | L128  | Fluidigm | 0.50             |
| CD11c  | 156 Gd  | 3.9   | Biolegend | 0.60             |
| CCR7   | 159 Tb  | G043H7| Biolegend | 0.30             |
| CD14   | 160 Gd  | M5E2  | Fluidigm | 0.97             |
| CD1c   | 161 Dy  | L161  | Biolegend | 0.30             |
| CD32a-APC | 162 Dy   | APC003| Fluidigm | 3.5/10             |
| CXCR3  | 163 Dy  | G025H7| Fluidigm | 0.60             |
| CD43RO | 165 Hs  | UCHL1 | Fluidigm | 0.36             |
| CD38   | 167 Er  | HT2   | Fluidigm | 0.30             |
| CD40L  | 168 Er  | 24-31 | Fluidigm | 1.20             |
| CD45RA | 169 Tm  | H100  | Fluidigm | 0.80             |
| CD3    | 170 Er  | UCHT1 | Fluidigm | 0.40             |
| LAG3   | 172 Yb  | BM5   | Biotechnie | 1.60             |
| HLA-DR | 173 Yb  | L243  | Fluidigm | 0.30             |
| PD1    | 174 Yb  | EH12.2H7| Fluidigm | 0.50             |
| CXCR4  | 175 Lu  | 12G5  | Fluidigm | 0.35             |
| CD127  | 176 Yb  | A019D5| Fluidigm | 0.70             |
| CD45   | 194 Pt  | H30   | Biolegend | 0.50             |
| CD16   | 209 Bi  | 3G8   | Fluidigm | 0.50             |
Figure 1

- Vaccination/ART phase (36 weeks)
- ART phase (12 weeks)
- Clinical care/ART phase (26 weeks)

A

DNA GTU-Multifly B

LIP0.5 Vaccine or placebo

0 4 12 20 24 36 48 74

Downloaded from https://jvi.asm.org on April 2, 2021 by guest
Figure 2

A

% Cyt+/T CD4+

V00

V28

V0

V28

p<0.01

NS

B

% Cyt+/T CD8+

V00

V28

V0

V28

NS

p=0.03
Figure 3

A

PD-1
TIGIT

+ + +

+ + +

****

0.5 1.0 1.5

Ratio of CD8 Memory T Cells Subsets at Week 28 vs Week 0

B

CD27
CD57

+ + +

+ + +

* *

0.5 1.0 1.5

Ratio of CD8 Memory T Cells Subsets at Week 28 vs Week 0

Placebo (n=12)
Vaccine (n=16)
Figure 3

C

Ratio of CD8 Memory T Cells Subsets at Week 28 vs Week 0

D

Ratio of CD4 Memory T Cells Subsets at Week 28 vs Week 0

Placebo (n=12)
Vaccine (n=16)
Figure 4

A

B

Placebo
Vaccine
Figure 5

A

B

Cellular Responses

Placebo
Vaccine