HIV

A Dendritic Cell–Based Vaccine Elicits T Cell Responses Associated with Control of HIV-1 Replication

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Combination antiretroviral therapy (cART) greatly improves survival and quality of life of HIV-1–infected patients; however, cART must be continued indefinitely to prevent viral rebound and associated disease progression. Inducing HIV-1–specific immune responses with a therapeutic immunization has been proposed to control viral replication after discontinuation of cART as an alternative to "cART for life." We report safety, tolerability, and immunogenicity results associated with a control of viral replication for a therapeutic vaccine using autologous monocyte-derived dendritic cells (MD-DCs) pulsed with autologous heat-inactivated whole HIV. Patients on cART with CD4$^+$ >450 cells/mm^3 were randomized to receive three immunizations with MD-DCs or with nonpulsed MD-DCs. Vaccination was feasible, safe, and well tolerated and shifted the virus/host balance. At weeks 12 and 24 after cART interruption, a decrease of plasma viral load setpoint ≥1 log was observed in 12 of 22 (55%) versus 1 of 11 (9%) and in 7 of 20 (35%) versus 0 of 10 (0%) patients in the DC–HIV-1 and DC-control groups, respectively. This significant decrease in plasma viral load observed in immunized recipients was associated with a consistent increase in HIV-1–specific T cell responses. These data suggest that HIV-1–specific immune responses elicited by therapeutic DC vaccines could significantly change plasma viral load setpoint after cART interruption in chronic HIV-1–infected patients treated in early stages. This proof of concept supports further investigation of new candidates and/or new optimized strategies of vaccination with the final objective of obtaining a functional cure as an alternative to cART for life.

INTRODUCTION

Although combination antiretroviral therapy (cART) improves survival and quality of life of HIV-infected patients, cART does not represent definitive therapy for HIV infection, in part because treatment must be continued indefinitely to prevent viral recrudescence and associated disease progression. Viral eradication and functional cure have been proposed as two alternatives to "cART for life" (1). The concept of "functional cure"—defined as control of HIV replication without cART—is based on the observation that a limited number of HIV-infected patients (termed as long-term nonprogressors or elite controllers) can remain persistently infected for decades but are able to control HIV replication effectively enough to remain aviremic as measured by standard assays and without appreciable depletion of CD4$^+$ T cells. It has been proposed that the strong HIV-specific immune responses observed in these patients explain this natural functional cure (2), raising the possibility that therapeutic immunization of patients in whom HIV replication is suppressed by cART might result in a similar outcome, obviating the need for lifelong cART (3, 4).

Therapeutic immunization is intended to increase existing immune responses against HIV or induce de novo beneficial antiviral immune responses by vaccination with a suitable immunogen (3, 4). In the ideal scenario, these responses would be able to control viral replication in at least a proportion of patients, even after discontinuation of cART.

Even if it fell short of achieving such a functional cure if a therapeutic vaccine were shown to be capable of changing plasma viral load (pVL) setpoint after a diagnostic interruption of cART, it would provide a proof of concept that would justify further investigation of new candidate therapeutic immunization approaches.

Autologous myeloid dendritic cells (DCs), such as monocyte-derived DCs (MD-DCs) (5), pulsed ex vivo with a variety of inactivated pathogens and tumor antigens, have been shown to induce a potent protective immunity in experimental murine models of human infections and tumors (6–8). Some studies in animal models suggest that animals immunized with DCs loaded with HIV-1 viral lysate, envelope glycoproteins, or inactivated virus mount a potent immune response against HIV-1 (9–12). Although at least 12 published clinical trials of DC-based immunotherapy for HIV infection have been performed in humans (13–24) [reviewed in (25)], most of them were noncontrolled, nonrandomized studies. In the present blinded placebo-controlled clinical trial, we successfully immunized antiretroviral-treated chronic HIV-1–infected patients with a high dose of MD-DCs pulsed with heat-inactivated autologous virus to assess whether induced HIV-specific immune responses were able to change significantly pVL setpoint after cART interruption.

RESULTS

Characteristics of study patients and side effects of immunizations

Thirty-six patients were enrolled. The baseline characteristics of the patients in each arm, shown in Table 1, were well balanced. One patient in the DC-control group was excluded from the analysis because of consent withdrawal before receiving any immunization. Seven patients in the DC–HIV-1 and five in the DC-control started cART (DC–HIV-1:

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at week 4 due to consent withdrawal; at weeks 8, 12, 12, 36, and 36 due to a drop in CD4 T cell count below specified criteria; and at week 36 due to a drop in platelets; DC-control: at weeks 16, 24, 36, and 36 due to a drop in CD4 T cell count below specified criteria and at week 36 due to consent withdrawal) (Fig. 1). Overall, the immunizations were well tolerated, with asymptomatic enlargement of local lymph nodes after vaccination in four patients (in three DC–HIV-1 patients after 6 to 12 hours after three immunizations, and in one DC-control recipient after the first injection), one episode of mild local redness (one in DC–HIV-1 patients after the second immunization), and one episode of flu-like symptoms (one in DC–HIV-1 patients after the second immunization). The enlargement of lymph nodes regressed after 48 to 72 hours, without intervention, in each instance. The redness and flu-like symptoms subsided after 24 hours. Eleven DC–HIV-1 patients reported at least one adverse event during the follow-up, compared with five patients in the DC-control group. Except for the lymph node enlargement and the episode of local redness and flu-like symptoms, the adverse events were classified as unrelated to immunizations. The DC injections were not associated with any clinical or serologic evidence of autoimmunity, and no rheumatoid factor or antinuclear antibodies were detected after DC injections.

Changes in viral load and CD4+ T cell count after DC immunizations and cART interruption

DC–HIV-1 patients experienced significant changes of pVL setpoint after cART interruption compared with DC-controls. The drop in pVL setpoint after the second cART interruption is shown in Table 2 and Fig. 2 (A and B). The results of the analysis did not change at week 24, when a sensitivity-type analysis using the last observation carried forward for the missing pVLs was performed [mean (SE) change pVL ($\log_{10}$ RNA copies/ml), −0.80 versus −0.19 in DC–HIV-1 versus DC-control, respectively ($P = 0.01$)]. At weeks 12 and 24, a decrease of pVL setpoint ≥ 1 log was observed in 12 of 22 (55%) versus 1 of 11 (9%) individuals, and in 7 of 20 (35%) versus 0 of 10 (0%) individuals in DC–HIV-1 and DC-control, respectively ($P = 0.02$ and $P = 0.03$) (Fig. 3 and Table 2). Proportions of patients with a drop of pVL ≥ 0.5 log in both arms at different time points are shown in Fig. 3 and Table 2. Over a 48-week period, the AUC analysis showed a significant difference for a change of pVL setpoint between the groups [mean (SE), −0.73 (0.14) versus −0.24 (0.16), DC–HIV-1 and DC-control, respectively; $P = 0.04$]. The highest and lowest decreases in pVL setpoint (defined as the highest and lowest differences between pVL setpoint at any time point after cART interruption and baseline pVL setpoint before any cART) were also significantly different between arms [highest decrease: mean (SE), −1.2 (0.14) versus −0.56 (0.21), in DC–HIV-1 versus DC-control, $P = 0.01$; lowest decrease: mean (SE), −0.54 (0.11) versus −0.06 (0.19), in DC–HIV-1 versus DC-control, $P = 0.04$]. Finally, a pairwise analysis with a Wilcoxon test comparing pVL at baseline with pVL at weeks 12, 24, 36, and 48 showed that the differences were significant in the DC–HIV-1 group ($P < 0.0001$, $P < 0.0001$, $P = 0.0006$, and $P = 0.0028$, respectively) but not in the DC-control group ($P = 0.06$, $P = 0.44$, $P = 0.69$, and $P = 0.10$, respectively). Despite these reductions in pVL setpoint, VL rebounded to detectable levels in all patients. pVL after the first interruption of cART before any immunization was similar to baseline pVL before any cART (see Table 1).

CD4 T cell counts declined to pre-cART values without significant differences between groups (Fig. 4).

Changes in HIV-1–specific responses after DC immunizations

Baseline median values of the total sum of HIV-specific responses against HIV peptide pools were similar in both arms [2625 versus 2283 spot-forming cells (SFCs)/10^6 peripheral blood mononuclear cells

Table 1. Baseline pre-cART characteristics of vaccine recipients (DC–HIV-1) and placebo recipients (DC-control). DC–HIV-1: group of patients who received 10^7 MD-DCs pulsed with heat-inactivated autologous virus (10^9 copies per dose). DC-control: group of patients who received placebo nonpulsed 10^7 MD-DCs. MSM, men who have sex with men.

	DC–HIV-1	DC-control
n	24	12
Gender (male)	19/24	9/12
Age, median (IQR)	41 (39–46)	39 (32–45)
Risk HIV MSM	15/24	7/12
Baseline pre-cART pVL [$\log_{10}$ mean (SE) copies/ml]*	4.87 (0.11)	4.57 (0.18)
pVL STOP 1 [$\log_{10}$ mean (SE) copies/ml]†	4.92 (0.12)	4.59 (0.16)
Baseline pre-cART CD4 [median (IQR) cells/mm³]*	503 (353–644)	416 (345–470)
CD4 at day 0 (second interruption cART)‡	702 (568–900)	656 (505–728)

*Before any antiretroviral therapy: pVL, $P = 0.14$; CD4 T cell count, $P = 0.07$.   †pVL after first interruption of antiretroviral therapy.   ‡Day of interruption of cART after immunizations (STOP 2): day 0 CD4 T cell count: $P = 0.36$.

Table 2. Differences in changes in pVL setpoint, the proportion of patients with a drop of pVL setpoint $\geq 1 \log_{10}$ or $\geq 0.5 \log_{10}$, and HIV-specific responses against HIV peptide pools between arms.

	Changes in viral load setpoint [$\log_{10}$ mean (SE) RNA copies/ml]			Proportion of patients with a drop of pVL setpoint $\geq 1 \log_{10}$			Proportion of patients with a drop of pVL setpoint $\geq 0.5 \log_{10}$			Changes in HIV-specific responses [median (IQR)]		
	DC–HIV-1	DC-control	P	DC–HIV-1	DC-control	P	DC–HIV-1	DC-control	P	DC–HIV-1	DC-control	P
Week 12	−0.91 (0.11)	−0.39 (0.18)	0.01	12/22 (55%)	1/11 (9%)	0.02	18/22 (82%)	6/11 (55%)	0.09	1267 (−215 to 3130)	497 (−503 to 2084)	0.36
Week 24	−0.81 (0.16)	−0.13 (0.16)	0.01	7/20 (35%)	0/10 (0%)	0.03	13/20 (65%)	3/10 (30%)	0.07	3567 (1397– 6097)	513 (158–2729)	0.02
Week 36	−0.70 (0.17)	−0.10 (0.24)	0.05	7/20 (35%)	1/9 (11%)	0.4	9/20 (45%)	2/9 (22%)	0.24	723 (−503 to 2084)	1886 (−590 to 3153)	0.28
Week 48	−0.64 (0.18)	−0.49 (0.22)	0.66	3/17 (18%)	1/6 (17%)	0.95	9/17 (53%)	3/6 (50%)	0.9	1847 (1077–4533)	2206 (470 to 4031)	0.88

(PBMCs), $P = 0.462$]. The median [interquartile range (IQR)] change in the frequency of total HIV-specific T cell responses after immunizations is shown in Table 2 and Fig. 5A. This difference was more evident when analyzing responses at week 24 against Gag p17 and Nef peptide pools ($P = 0.0288$ and $P = 0.03615$, respectively). Nevertheless, we also observed at week 24 an increased response against Gag p24, Gag small proteins, and Env gp41 peptide pools in the immunized arm, although the difference was not statistically significant when compared to the placebo arm (852 versus 328 SFCs/10^6 PBMCs for Gag p24, $P = 0.705$; 426 versus 60 SFCs/10^6 PBMCs for Gag small proteins, $P = 0.909$; and 415 versus 73 SFCs/10^6 PBMCs for Env gp4, $P = 0.167$, respectively). A complete immunophenotype was performed on T cells from all individuals. We observed, at different time points, a significant increase in activation markers such as CD38 and human leukocyte antigen (HLA)–DR especially on CD4 T cells from the DC–HIV-1 group (Fig. 5B). On the other hand, CD8 T cells, which usually correlate with progression of HIV infection, did not change significantly between groups. The median increase of peptide pools recognized was similar between the two groups of patients.

HIV-specific T cell responses observed at week 24 tended to be inversely associated with the drop in pVL in DC–HIV-1 group ($r =$
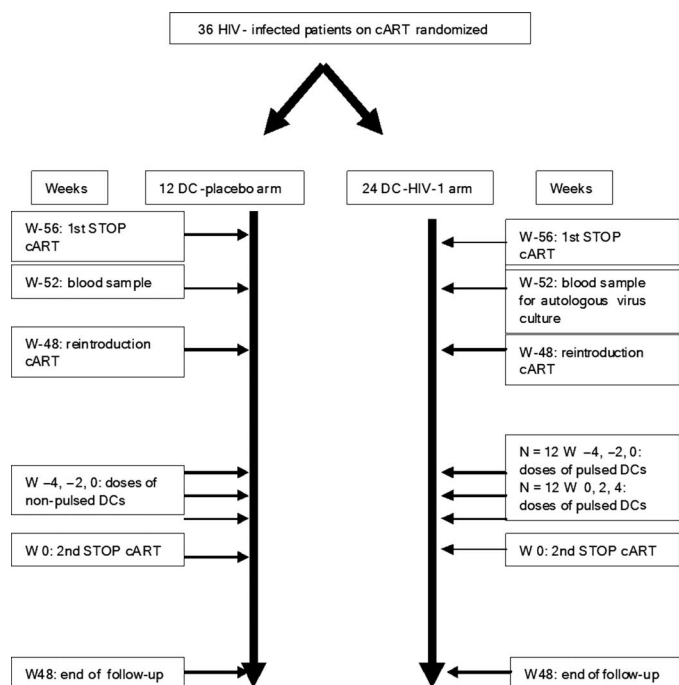
-0.38, $P = 0.08$); however, they were directly and significantly correlated with changes in pVL in the DC-control group ($r = 0.66$, $P = 0.03$) (Fig. 6, A and B).

Although only transient positive responses to HIV p24 were observed, the median change in lymphoproliferative response (LPR) to HIV p24 at week 24 from baseline was 0.96 versus -0.50 ($P = 0.02$) in the DC–HIV-1 versus DC-control groups. Additionally, we found a significant positive correlation between changes in LPR to HIV p24 and changes in the magnitude of HIV-specific T cell responses measured by enzyme-linked immunospot (ELISPOT) in the DC-HIV group ($r = 0.86$, $P = 0.0045$), whereas in the case of the DC-control group, there was a negative tendency between these changes ($r = -0.6$, $P = 0.096$).

DISCUSSION

Here, therapeutic vaccination of antiretrovirally treated nonadvanced chronic HIV-1–infected patients with high doses of autologous MD-DCs
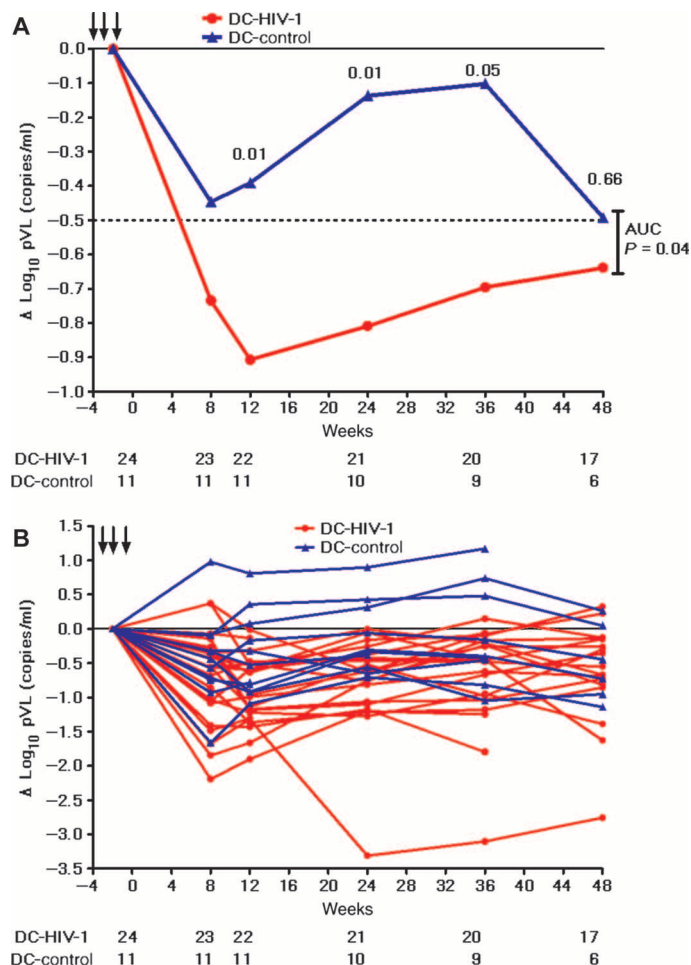

Fig. 1. Scheme of clinical trial design. Thirty-six antiretroviral-treated chronic HIV-1–infected patients were randomized to receive three immunizations with at least 10^7 MD-DCs pulsed with heat-inactivated autologous virus (10^9 copies per dose). Patients were followed up to 48 weeks after the first immunization. Week 0 was considered the day of second interruption of cART (2nd STOP). The DC–HIV-1 group received immunizations at weeks −4, −2, and 0 in 12 patients and at weeks 0, 2, and 4 in 12 patients. These two different schedules were selected to assess whether cART could have any influence in the response to immunizations. Because a significant difference in pVL changes or HIV-specific T cell responses between these two schedules was not observed, immunized patients have been analyzed as a single group. DC-control group patients received injection at weeks −4, −2, and 0.

Fig. 2. Change in pVL from baseline (before any antiretroviral therapy) after immunizations and second interruption of antiretroviral therapy. (**A**) Median values. (**B**) Individual values. Numbers at the bottom represent patients at risk. P values of Mann-Whitney U test are shown at weeks 12, 24, 36, and 48. P value of area under the curve (AUC) is also shown.

pulsed with high doses of autologous heat-inactivated HIV-1 was feasible, safe, and well tolerated. A significant decrease in VL was observed in immunized recipients combined with a consistent increase in HIV-1–specific T cell responses. Although our data fell short of achieving a functional cure, this therapeutic vaccine was capable of changing pVL setpoint after a diagnostic interruption of cART.

To date, results have been reported for 191 patients (60 off cART and 131 on cART) recruited into 12 clinical trials with DC-based immunotherapeutic vaccines (25). The safety profile has been excellent, with only minor local side effects reported in some studies. No severe side events or induction of autoimmunity has been reported. This excellent tolerance profile for DC administration to HIV-1–infected patients corresponds with experiences with DC-based cancer therapy. Our clinical trial confirms that a therapeutic MD-DC vaccine in chronically HIV-1–infected patients receiving antiretroviral therapy is feasible, safe, and well tolerated. However, this is a small trial in a highly select group of patients,

and the overall safety over the long term and in a substantial number of unselected participants remains to be established.

The 12 clinical trials published so far suggest that DC immunotherapy in HIV-1 infection can elicit HIV-1–specific immunological responses. However, only four of these studies reported virological responses to immunization (14, 15, 21, 22), three have not assessed virological responses (17, 20, 23), and five failed to show any response (13, 16, 18, 19, 24). The most promising results have been reported in two noncontrolled, nonrandomized studies (14, 22). The first preliminary noncontrolled, nonrandomized clinical trial described by Lu et al. (14) found that, after administration of three immunizations with DCs loaded with AT-2–inactivated autologous virus, VL decreased a mean of 0.7 $\log_{10}$ copies/ml in antiretroviral treatment of naïve HIV-1–infected patients. Moreover, they found a drop of 1 $\log_{10}$ in the viral load of 8 of 18 (44%) patients. Similar results were found by Routy et al. (22) in antiretroviral-treated patients. Conversely, in the other two randomized clinical trials performed previously by our group [one in patients off cART (21) and the other in patients on cART (15)], only a modest virological response associated with weak increase in HIV-1–specific CD8+ T cell responses was observed (15, 21, 26, 27).
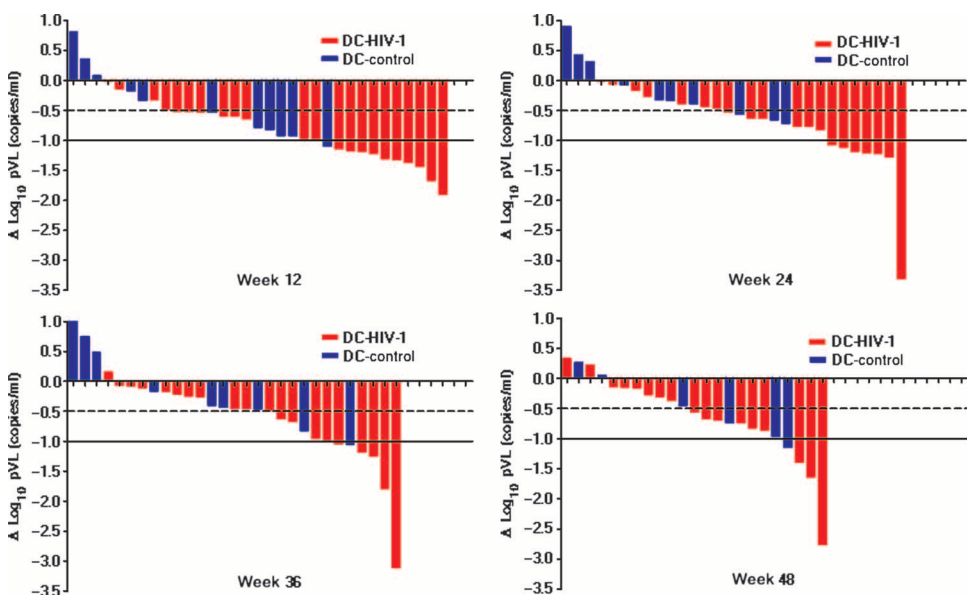
In the present blinded placebo-controlled study in antiretroviral-treated patients, we found a significant and consistent virological response that was maintained for at least 24 weeks in the vaccinated group and seemed to wane thereafter. Immunization shifted the virus/host balance; the change in pVL setpoint in the immunized patients compared to the control group was significant (mean peak drop of pVL of −1.2 $\log_{10}$ copies/ml and mean drop of pVL at weeks 12 and 24 of −0.91 and −0.81 $\log_{10}$ copies/ml). The protocol endpoint for control of pVL (≥1 $\log_{10}$) was achieved in 12 of 22 (55%) vaccine recipients at week 12 after cART interruption and was observed in only 1 of the control recipients during the 48 weeks of follow-up. Moreover, over this 48-week period, the AUC analysis showed a significant difference for a change of pVL setpoint between the groups with a mean drop of −0.73 in vaccinated and −0.24 in controls. To our knowledge, these are the best virological responses to any therapeutic vaccine used to date. However, comparison between the results of this clinical trial and those of published studies is difficult because, although the baseline characteristics of the patients were similar between different clinical trials, the design of these studies (including route of administration, type of immunogen, number of doses, and schedule) varied (25). In any case, it is worth considering whether autologous virus is a

Fig. 3. Drop of pVL setpoint at weeks 12, 24, 36, and 48. Each bar represents an individual. Red bars, DC–HIV-1 group; blue bars, DC-control group. The continuous line represents a drop of pVL setpoint ≥1 $\log_{10}$, and the dashed line represents a drop of pVL setpoint ≥0.5 $\log_{10}$.
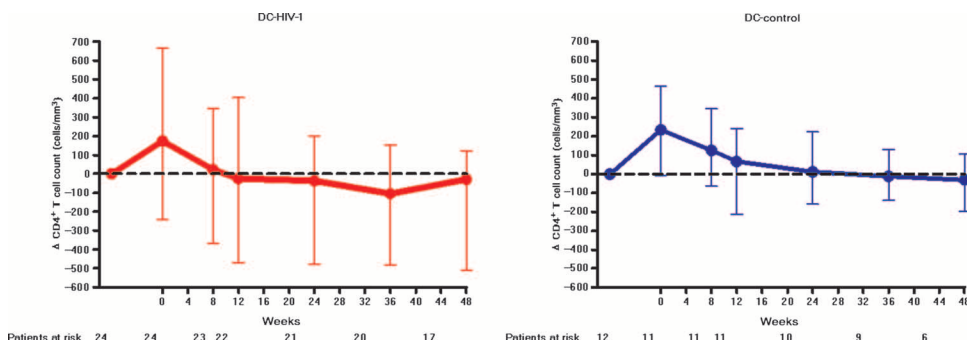

Fig. 4. Change in CD4 T cell count from baseline (before any antiretroviral therapy) after immunizations and second interruption of antiretroviral therapy. Median values are shown. Numbers at the bottom represent patients at risk. Week 0 was considered the day of second interruption of cART (2nd STOP). Dashed line represents baseline value before any antiretroviral therapy.

good immunogen. Clinical trials performed with heterologous antigens (13, 16–19, 23, 24) did not find any virological response. Conversely, the four studies reporting virological responses to immunization (14, 15, 21, 22) used autologous virus. Recently, Routy et al. (20, 28) reported the preliminary results of an immunotherapy noncontrolled, nonrandomized trial consisting of MD-DCs and RNA encoding autologous HIV-1 antigens (Gag, Nef, Rev, and Vpr) administered monthly in 33 subjects in four intradermal doses in combination with cART, followed by two more doses during a 12-week cART interruption. They found that this strategy induced a control of pVL similar to that reported by Lu et al. (14). These and our current data suggest that a combination of autologous antigens administered to patients on cART could have a higher virological effect. In addition, the difference in

Fig. 5. Changes in the frequency of HIV-1–specific T cell responses and T cell activation markers from baseline during the study period. (**A**) Median changes in total HIV-specific T cell responses (defined as the sum of positive individual responses per patient). (**B**) Percentages of CD38$^+$ and HLA-DR$^+$ surface activation markers on CD4$^+$ T cells in the DC–HIV-1 and DC-control groups during the study period. P values of Mann-Whitney U test are shown over significant time points.

efficacy of this current trial compared with our previous clinical trials (15, 21) could be explained by the much lower quantities of MD-DCs and virus used in the first trial [10^6 versus 10^7 (MD-DCs) and 10^6 versus 10^9 (virus) in the first and current trial, respectively] (15) and that the second trial was performed in antiretroviral untreated patients (21).

The vaccine tested here induced moderate HIV-specific T cell responses, as measured by ELISPOT. At week 24 after cART interruption, the pVL in immunized patients tended to be inversely correlated with HIV-1–specific T cell responses. In contrast, as other authors have reported previously, in the controls, HIV-1–specific T cells responses were directly correlated with an increase in pVL (29). We also observed a clear increase of activated CD4$^+$ T lymphocytes in vaccinated patients. The latter suggests that pulsed DCs may be able to appropriately stimulate T cells to obtain an efficient immune response. The clear positive correlation we found between the increase in HIV-1–specific T cell proliferative response and interferon-γ (IFN-γ) production in immunized patients suggests a coordinated response to the therapeutic immunization and reinforces this idea. We have previously reported that a transient activation of HIV-specific CD4$^+$, but not CD8$^+$ T cells with HIV-1 recombinant canarypox vaccine (vCP1452), might have a detrimental effect on HIV outcomes (30). Conversely, the present data suggest that limited activation of HIV-specific CD4$^+$ associated with stimulation of CD8$^+$ T cells might have a beneficial effect in the control of viral replication.

Our study has a number of drawbacks. First, despite important reductions in pVL setpoint, VL rebounded to detectable levels in all patients. The goal of any therapeutic vaccine would be to control viral replication to an undetectable level in at least a proportion of patients in the absence of cART (functional cure), and this objective has not been reached with this vaccine. A replenishment of the reservoir during the first interruption of cART (performed to isolate autologous virus for pulsing MD-DCs) could be a major problem with the study design. After this interruption, patients were on cART for only 48 additional weeks. It could be expected to cause significant persistent viral replication despite a decline in viral RNA to <50 copies/ml and may have compromised the results. Regretfully, we have not been able to perform any other measures of the reservoir at the time of second treatment interruption after vaccination. Second, immunization did not prevent the drop of CD4$^+$ T cells, and a third of randomized patients had to reinitiate cART during the follow-up. The observed decreases in CD4$^+$ T cells are likely due to persistent viral replication, and it is possible that, if a therapeutic vaccine would be able to control viral replication to undetectable level, this deleterious effect in CD4 T cell count would not be observed. Finally, viral load response to vaccine waned with time. A new strategy including booster doses after cART interruption could potentially avoid this decline of the vaccine effect. This is suggested by the preliminary data reported by Routy et al. (22). The results of an ongoing clinical trial using four immunizations while on cART plus two additional booster doses after cART interruption could help to answer this question (25).

In summary, these results suggest that HIV-1–specific immune responses elicited by therapeutic DC vaccines could significantly change pVL setpoint after cART interruption in most chronic HIV-1–infected patients treated in early stages. This is a proof of concept justifying further investigation of new candidates and/or new optimized strategies of vaccination with the final objective of obtaining a functional cure as an alternative to cART for life.
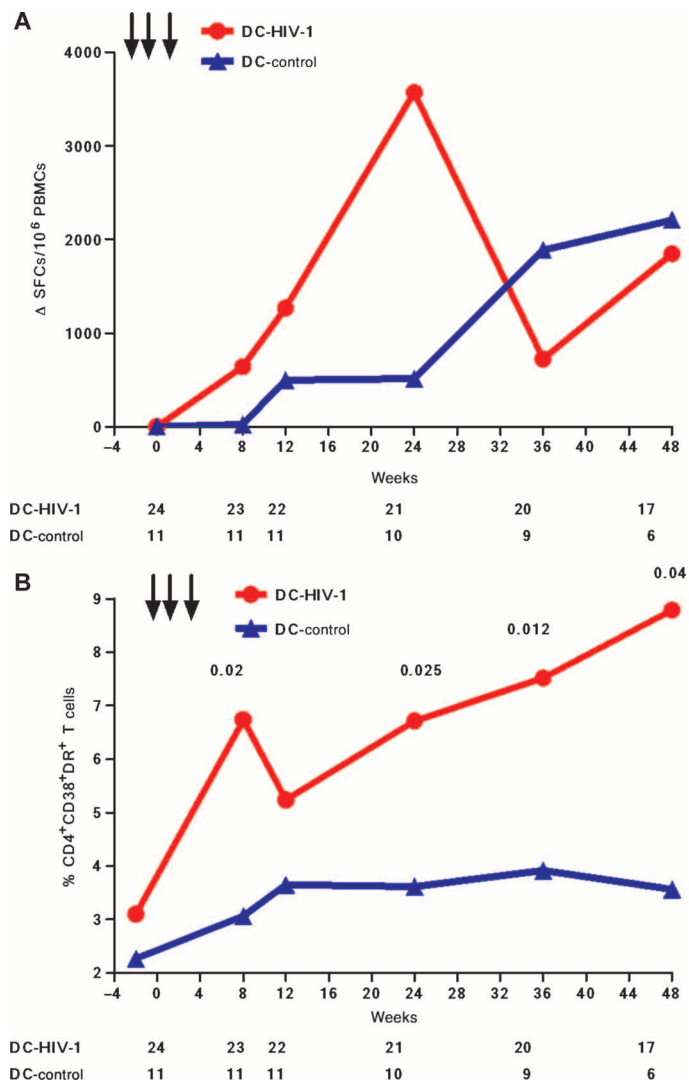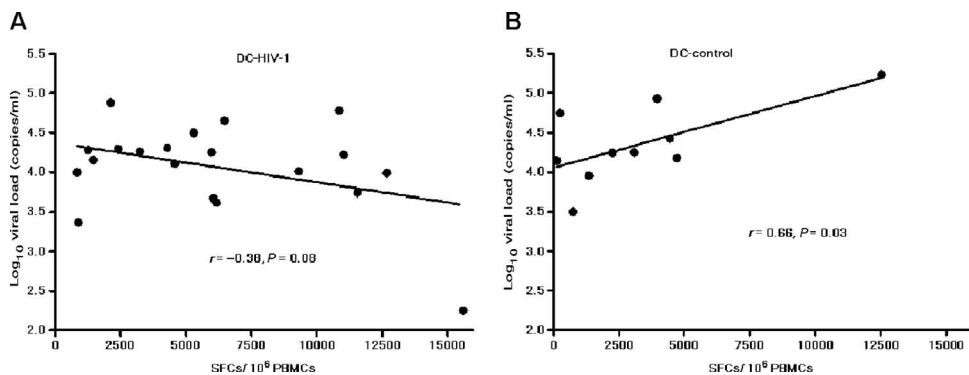
Fig. 6. (**A** and **B**) Correlation between the HIV-1–specific T cell responses and pVL in DC–HIV-1 patients (A) and DC-control recipients (B) at week 24 after antiretroviral therapy interruption. Each dot represents an individual.

MATERIALS AND METHODS

Patients and samples

Chronically HIV-1–infected patients on cART with baseline $CD4^+$ T lymphocytes above 450 cells/mm^3, nadir $CD4^+$ T cell count above 350 cells/mm^3, and undetectable pVL (<37 copies/ml) were enrolled. Patients had been on cART for at least the last 2 years before enrollment. The study design is shown in Fig. 1. All the immunologic and virologic determinations were performed in a blinded fashion. The study was explained to all patients in detail, and all patients gave written informed consent. The study was approved by the institutional ethical review board and by the Spanish Regulatory Authorities.

The co-primary endpoints were safety, change in pVL setpoint at week 24, and proportion of patients with a drop in pVL of at least 1 log$_{10}$ at week 24. Secondary endpoints were pVL setpoint change at weeks 12, 36, and 48; proportion of patients with a drop in pVL of at least 1 log$_{10}$ at weeks 12, 36, and 48; proportion of patients with a drop in pVL of at least 0.5 log$_{10}$ at weeks 12, 24, 36, and 48; proportion of patients who started therapy (cART) based on $CD4^+$ T cell count below 300 cells/mm^3 in two determinations separated by at least 1 month; and changes in $CD4^+$ T cells and in HIV-1–specific immune responses.

Preparation of inactivated autologous viral stocks for pulsing autologous MD-DCs

For each subject, one lot of autologous inactivated HIV-1 stock was prepared fulfilling clinical-grade good manufacturing practice (cGMP) requirements and complying with the predetermined specifications in the approved investigational medicine product dossier. Briefly, for each infected patient, a primary virus isolate was obtained and propagated by means of coculture of CD4-enriched PBMCs from both the HIV-1–infected subject and the HIV-seronegative unrelated, healthy volunteer donors. The virus-containing culture supernatants were collected and heat-inactivated twice at 56°C for 30 min and concentrated by ultracentrifugation to a final volume of 1 ml, which was divided into five 0.2-ml aliquots and stored frozen at −80°C until use for pulsing immature MD-DCs. To ensure that no interpatient cross-contaminations had occurred during the immunogen manufacturing, we compared the HIV-1 protease gene of the virus present in the patient plasma (extracted at the time of autologous HIV-1 isolation for the virus stock production) and that of the virus isolated to prepare the immunogen before heat treatment. We did

not find significant differences between these paired samples for any patient. The final products contained a median (IQR) VL of 11.0 (10.9 to 11.0) log$_{10}$ HIV-1 RNA copies/ml and a high infectivity reduction after heat treatment (median of 5.5 log$_{10}$) and were free from adventitious agents. The relevant quality control procedures have been explained in detail elsewhere (31).

In vitro generation of MD-DCs, their pulsing with inactivated HIV-1 under cGMP conditions, and immunizations

PBMCs were isolated, within 1 hour of the blood extraction, from a 150-ml sample of EDTA-treated venous blood by means of standard Ficoll gradient centrifugation. After being washed four times in cGMP phosphate-buffered saline (PBS) (Lonza), PBMCs were resuspended in MD-DC culture medium including X-VIVO 15 culture medium (Lonza) supplemented with 1% heat-inactivated autologous serum and pharmaceutical gentamicin (B. Braun Medical Supplies), fungizone (Bristol-Myers Squibb), and 1 μM zidovudine (Retrovir from GlaxoSmithKline); settled in sterile apyrogenic culture flasks; and incubated for 2 hours in a cell culture incubator (37°C, humidified atmosphere with 5% CO_2 in air). Thereafter, nonadherent cells were discarded, and adherent cells (enriched monocytes) were immediately washed gently (three times) with cGMP PBS to eliminate residual contaminating lymphocytes, and then cultured for 5 days in MD-DC culture medium with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (1000 IU/ml) (CellGenix) and cGMP recombinant human interleukin-4 (IL-4) (1000 IU/ml) (CellGenix). Cytokines at the indicated doses were added at 2- to 3-day intervals. On day 5 of culture, immature MD-DCs were collected, washed (three times) with PBS, pulsed for 2 to 3 hours with freshly thawed frozen aliquot of heat-inactivated autologous HIV-1 (0.2 ml containing >10^9 HIV-1 copies/ml) in a final concentration of 2×10^6 MD-DCs/ml in MD-DC culture medium with GM-CSF and IL-4, and cultured in an ultra-low attachment flasks to prevent cell adhesion. After viral pulsing, MD-DCs were fully matured during a total of 48 hours with a cocktail of cytokines containing IL-1β (300 IU/ml) (CellGenix), IL-6 (1000 IU/ml) (CellGenix), tumor necrosis factor–α (1000 IU/ml) (CellGenix) and prostaglandin E_2 (1 μg/ml) (Dinoprostona, Pfizer) and MD-DC medium with GM-CSF and IL-4 was added to a concentration of 0.5×10^6 MD-DCs/ml.

Immediately after pulsing with inactivated autologous HIV, cells were washed once and resuspended in 0.5 ml of pharmaceutical saline solution supplemented with 1% of pharmaceutical human albumin and immediately injected subcutaneously or intradermally in the upper-inner part of both arms (0.25 ml each injection). A small aliquot, removed immediately before injection, was used for immunophenotyping, Gram staining, and *Mycoplasma* detection. Before pulsing, microbiological culture was performed with uniform negative results. The amount of cells obtained and the maturation, as assessed by flow cytometry, was consistent throughout all the immunization periods.

ELISPOT assay

ELISPOT assays were performed to measure the numbers of IFN-γ–producing PBMCs directed against HIV-1 sequences. Briefly, assays

were performed with cryopreserved PBMCs using 22 pools of peptides, consisting of 15-nucleotide oligomers overlapping by 11, grouped in pools of 10 to 12 peptides each, covering the whole HIV-1 Gag, Nef, and Env gp41 sequences [provided by ORVACS (Objectif Recherche VACcin Sida)]. Negative control responses were obtained with unstimulated cells. Positive controls included cells stimulated with phytohemagglutinin A (Sigma) and a CEF pool containing 32 HLA class I–restricted peptides from cytomegalovirus, Epstein-Barr virus, and flu virus (National Institutes of Health AIDS Research and Reference Research Program). All time points for an individual patient were tested in a single assay. The ELISPOT assay was done in 96-well polyvinylidene difluoride microtiter plates coated overnight with a monoclonal antibody (mAb) specific for human IFN-γ (mAb B-B1, Diaclone, BioNova). PBMCs resuspended in RPMI plus 10% fetal calf serum were plated in the presence of different peptide pools (2 μg/ml, final concentration) and incubated overnight at 37°C and 5% CO_2. Plates were developed with biotinylated anti-human IFN-γ, streptavidin conjugated to alkaline phosphatase (Amersham Biosciences), and chromogenic substrate bromochloroindolyl phosphate–nitro blue tetrazolium (Sigma). SFCs were counted with an AID ELISPOT reader (Autoimmun Diagnostika GmbH). Results were expressed as the number of SFCs per million of PBMCs after subtracting the background. The positivity threshold for each peptide pool or antigen was defined at 50 SFCs/10^6 PBMCs. At least 85% of the responding cells with this methodology are CD8 T cells, but because all studies were conducted with unfractionated PBMCs, responses are described as HIV-specific T cells.

Lymphoproliferative responses

PBMCs were washed twice and resuspended at 2×10^6/ml in serum-free medium X-VIVO 10 (BioWhittaker). Cultures were plated in triplicate at 2×10^5 per well in 7-day assays, in 96-well round-bottomed microplates (TPP). Cells were cultured in the absence or presence of pokeweed mitogen (10 μg/ml) (Sigma) as a polyclonal stimulus or recombinant HIV-1 proteins gp160 and p24 (5 μg/ml) (Protein Sciences). Incorporation of tritium-labeled thymidine during the last 18 hours of 7-day culture was evaluated with a betaplate scintillation counter (LKB Wallac). Results were expressed as mean counts per minute (cpm). The stimulation index (SI) was calculated for each sample as follows: cpm for cells with stimulus/cpm for cells without stimulus. Positive antigen-specific responses were defined as more than 3000 cpm and SI greater than 3.

Statistical analysis

The HIV RNA values were $\log_{10}$-transformed before analysis. Patients' measurements were censored after ART initiation in those patients who had to start ART according to preestablished criteria. Baseline pVL and CD4 T cell count were defined as the mean of all determinations available 1 year before any cART. The continuous variables were compared between groups with the nonparametric Mann-Whitney test. The continuous variables were compared within groups with the nonparametric Wilcoxon test. Categorical variables were compared between groups with the Fisher's exact test. Changes in plasma HIV-1 RNA viremia and the frequency of total HIV-specific T cell responses over the 48 weeks of the trial were analyzed by an AUC measurement. Spearman rank order correlations were calculated to assess the correlation between continuous variables. Corrections were performed for multiple comparisons.

REFERENCES AND NOTES

1. J. Cohen, The emerging race to cure HIV infections. *Science* **332**, 784–789 (2011).
2. E. S. Rosenberg, J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, B. D. Walker, Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* **278**, 1447–1450 (1997).
3. N. L. Letvin, B. D. Walker, Immunopathogenesis and immunotherapy in AIDS virus infections. *Nat. Med.* **9**, 861–866 (2003).
4. B. Autran, B. Debré, B. D. Walker, C. Katlama, Therapeutic vaccines against HIV need international partnerships. *Nat. Rev. Immunol.* **3**, 503–508 (2003).
5. F. Sallusto, A. Lanzavecchia, Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med.* **179**, 1109–1118 (1994).
6. J. Banchereau, R. M. Steinman, Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).
7. J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, K. Palucka, Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18**, 767–811 (2000).
8. M. Larsson, J. Fonteneau, N. Bhardwaj, Cross-presentation of cell-associated antigens by dendritic cells. *Curr. Top. Microbiol. Immunol.* **276**, 261–275 (2003).
9. C. Lapenta, S. Santini, M. Logozzi, M. Spada, M. Andreotti, T. Pucchio, S. Parlato, F. Belardelli, Potent immune response against HIV-1 and protection from virus challenge in hu-PBL-SCID mice immunized with inactivated virus-pulsed dendritic cells generated in the presence of IFN-α. *J. Exp. Med.* **198**, 361–367 (2003).
10. A. Yoshida, R. Tanaka, T. Murakami, Y. Takahashi, Y. Koyanagi, M. Nakamura, M. Ito, N. Yamamoto, Y. Tanaka, Induction of protective immune responses against R5 human immunodeficiency virus type 1 (HIV-1) infection in hu-PBL-SCID mice by intrasplenic immunization with HIV-1-pulsed dendritic cells: Possible involvement of a novel factor of human CD4+ T-cell origin. *J. Virol.* **77**, 8719–8728 (2003).
11. F. Aline, D. Brand, J. Pierre, J. Fouquenet, B. Verrier, I. Dimier-Poisson, Generation of specific Th1 and CD8+ T-cell responses by immunization with mouse CD8+ dendritic cells loaded with HIV-1 viral lysate or envelope glycoproteins. *Microbes Infect.* **9**, 536–543 (2007).
12. W. Lu, X. Wu, Y. Lu, Y. Guo, J. M. Andrieu, Therapeutic dendritic-cell vaccine for simian AIDS. *Nat. Med.* **9**, 27–32 (2003).
13. S. K. Kundu, E. Engleman, C. Benike, M. H. Shapero, M. Dupuis, W. C. van Schooten, M. Eibl, T. C. Merigan, A pilot clinical trial of HIV antigen-pulsed allogeneic and autologous dendritic cell therapy in HIV-infected patients. *AIDS Res. Hum. Retroviruses* **14**, 551–560 (1998).
14. W. Lu, L. C. Arraes, W. T. Ferreira, J. M. Andrieu, Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat. Med.* **10**, 1359–1365 (2004).
15. F. García, M. Lejeune, N. Climent, C. Gil, J. Alcamí, V. Morente, L. Alós, A. Ruiz, J. Setoain, E. Fumero, P. Castro, A. López, A. Cruceta, C. Piera, E. Florence, A. Pereira, A. Libois, N. González, M. Guilá, M. Caballero, F. Lomeña, J. Joseph, J. M. Miró, T. Pumarola, M. Plana, J. M. Gatell, T. Gallart, Therapeutic immunization with dendritic cells loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. *J. Infect. Dis.* **191**, 1680–1685 (2005).
16. F. Ide, T. Nakamura, M. Tomizawa, A. Kawana-Tachikawa, T. Odawara, N. Hosoya, A. Iwamoto, Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: A phase 1 trial. *J. Med. Virol.* **78**, 711–718 (2006).
17. N. C. Connolly, T. L. Whiteside, C. Wilson, V. Kondragunta, C. R. Rinaldo, S. A. Riddler, Therapeutic immunization with human immunodeficiency virus type 1 (HIV-1) peptide-loaded dendritic cells is safe and induces immunogenicity in HIV-1-infected individuals. *Clin. Vaccine Immunol.* **15**, 284–292 (2008).
18. R. T. Gandhi, D. O'Neill, R. J. Bosch, E. S. Chan, R. P. Bucy, J. Shopis, L. Baglyos, E. Adams, L. Fox, L. Purdue, A. Marshak, T. Flynn, R. Masih, B. Schock, D. Mildvan, S. J. Schlesinger, M. A. Marovich, N. Bhardwaj, J. M. Jacobson; AIDS Clinical Trials Group A5130 team, A randomized therapeutic vaccine trial of canarypox-HIV-pulsed dendritic cells vs. canarypox-HIV alone in HIV-1-infected patients on antiretroviral therapy. *Vaccine* **27**, 6088–6094 (2009).
19. H. Kloverpris, I. Karlsson, J. Bonde, M. Thorn, L. Vinner, A. E. Pedersen, J. L. Hentze, B. S. Andresen, I. M. Svane, J. Gerstoft, G. Kronborg, A. Fomsgaard, Induction of novel CD8+ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes. *AIDS* **23**, 1329–1340 (2009).
20. J. P. Routy, M. R. Boulassel, B. Yassine-Diab, C. Nicolette, D. Healey, R. Jain, C. Landry, O. Yegorov, I. Tcherepanova, T. Monesmith, L. Finke, R. P. Sékaly, Immunologic activity and safety of autologous HIV RNA-electroporated dendritic cells in HIV-1 infected patients receiving antiretroviral therapy. *Clin. Immunol.* **134**, 140–147 (2010).
21. F. García, N. Climent, L. Assoumou, C. Gil, N. González, J. Alcamí, A. León, J. Romeu, J. Dalmau, J. Martínez-Picado, J. Lifson, B. Autran, D. Costagliola, B. Clotet, J. M. Gatell, M. Plana, T. Gallart; DCV2/MANON07-AIDS Vaccine Research Objective Study Group, A therapeutic dendritic cell-based vaccine for HIV-1 infection. *J. Infect. Dis.* **203**, 473–478 (2011).
22. J. P. Routy, J. B. Angel, S. Vezina, C. Tremblay, J. Loutfy, J. Gill, J. G. Baril, F. S. Smaill, R. Jain, C. Nicolette, Final analysis of a phase 2 study of an autologous DC immunotherapy (AGS-004) showed positive outcomes in primary endpoint of viral load control, and favorable safety and

immunogenicity profile, in subjects undergoing structured treatment interruption of ART (abstract 385). 18th Conference on Retroviruses and Opportunistic Infections, Boston, MA, February 27 to March 2, 2011.

23. E. Van Gulck, E. Vlieghe, M. Vekemans, V. F. Van Tendeloo, A. Van De Velde, E. Smits, S. Anguille, N. Cools, H. Goossens, L. Mertens, W. De Haes, J. Wong, E. Florence, G. Vanham, Z. N. Berneman, mRNA-based dendritic cell vaccination induces potent antiviral T-cell responses in HIV-1-infected patients. *AIDS* **26**, F1–F12 (2012).

24. S. D. Allard, K. B. De, A. L. de Goede, E. J. Verschuren, J. Koetsveld, M. L. Reedijk, C. Wylock, A. V. De Bel, J. Vandeloo, F. Pistoor, C. Heirman, W. E. Beyer, P. H. Eilers, J. Corthals, I. Padmos, K. Thielemans, A. D. Osterhaus, P. Lacor, M. E. van der Ende, J. L. Aerts, C. A. van Baalen, R. A. Gruters, A phase I/IIa immunotherapy trial of HIV-1-infected patients with Tat, Rev and Nef expressing dendritic cells followed by treatment interruption. *Clin. Immunol.* **142**, 252–268 (2012).

25. F. García, J. P. Routy, Challenges in dendritic cells-based therapeutic vaccination in HIV-1 infection: Workshop in dendritic cell-based vaccine clinical trials in HIV-1. *Vaccine* **29**, 6454–6463 (2011).

26. A. López, N. van der Lubbe, S. Sánchez-Palomino, M. Arnedo, M. Nomdedeu, P. Castro, M. Guilà, M. J. Maleno, F. García, T. Gallart, J. M. Gatell, M. Plana, Phenotypic and functional characteristics of HIV-specific CD8 T cells and gag sequence variability after autologous dendritic cells based therapeutic vaccine. *Vaccine* **27**, 6166–6178 (2009).

27. A. Ruiz, M. Nomdedeu, M. Ortega, M. Lejeune, J. Setoain, N. Climent, E. Fumero, M. Plana, A. León, L. Alós, C. Piera, F. Lomeña, J. M. Gatell, T. Gallart, F. García, Assessment of migration of HIV-1-loaded dendritic cells labeled with ^{111}In-oxine used as a therapeutic vaccine in HIV-1-infected patients. *Immunotherapy* **1**, 347–354 (2009).

28. J. P. Routy, M. R. Boulassel, B. Yassine-Diab, C. Nicolette, D. Healey, R. Jain, C. Landry, O. Yegorov, I. Tcherepanova, T. Monesmith, L. Finke, R. P. Sékaly, Immunologic activity and safety of autologous HIV RNA-electroporated dendritic cells in HIV-1 infected patients receiving antiretroviral therapy. *Clin. Immunol.* **134**, 140–147 (2010).

29. M. Plana, F. Garcia, A. Oxenius, G. M. Ortiz, A. Lopez, A. Cruceta, G. Mestre, E. Fumero, C. Fagard, M. A. Sambeat, F. Segura, J. M. Miró, M. Arnedo, L. Lopalco, T. Pumarola, B. Hirschel, R. E. Phillips, D. F. Nixon, T. Gallart, J. M. Gatell, Relevance of HIV-1-specific CD4+ helper T-cell responses during structured treatment interruptions in patients with CD4+ T-cell nadir above 400/mm3. *J. Acquir. Immune Defic. Syndr.* **36**, 791–799 (2004).

30. L. Papagno, G. Alter, L. Assoumou, R. L. Murphy, F. Garcia, B. Clotet, M. Larsen, M. Braibant, A. G. Marcelin, D. Costagliola, M. Altfeld, C. Katlama, B. Autran; ORVACS Study Group, Comprehensive analysis of virus-specific T-cells provides clues for the failure of therapeutic immunization with ALVAC-HIV vaccine. *AIDS* **25**, 27–36 (2011).

31. C. Gil, N. Climent, F. García, C. Hurtado, S. Nieto-Márquez, A. León, M. T. García, C. Rovira, L. Miralles, J. Dalmau, T. Pumarola, M. Almela, J. Martinez-Picado, J. D. Lifson, L. Zamora, J. M. Miró, C. Brander, B. Clotet, T. Gallart, J. M. Gatell, Ex vivo production of autologous whole inactivated HIV-1 for clinical use in therapeutic vaccines. *Vaccine* **29**, 5711–5724 (2011).

Abstracts

One-sentence summary: Therapeutic DC vaccines elicit HIV-1–specific immune responses that change viral setpoint in patients of cART.

Editor's Summary:
Putting the Vaccine Before the cART

Combination antiretroviral therapy has turned HIV infection from a death sentence to a manageable disease. However, current treatment requires "cART for life," a less than ideal situation for HIV-infected individuals because of drug cost and worries about resistance. New vaccine strategies are attempting to control viral replication after infection, thus allowing discontinuation of cART and a "functional cure." Garcia *et al.* report a dendritic cell (DC)–based vaccine that elicits an HIV-1–specific immune response and may change the setpoint of viral load.

The authors pulsed the patient's own DCs with heat-inactivated whole HIV and then used these DCs as a therapeutic vaccine. The vaccine was safe and well tolerated. They observed a decrease in viral setpoint after cART interruption in vaccinated patients with a concomitant increase in HIV-1–specific T cell responses. Although not yet a functional cure, these results support future studies optimizing a therapeutic vaccine to maintain HIV-1–infected patients.