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The Safety and Immunogenicity of an Interleukin-12-Enhanced Multiantigen DNA Vaccine Delivered by Electroporation for the Treatment of HIV-1 Infection

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Conflicts of Interest For the remaining authors no conflicts of interest were declared.

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Abstract

Background—Therapeutic vaccination is being studied in eradication and “functional cure” strategies for HIV-1. The Profectus Biosciences (Tarrytown, NY) multiantigen (MAG) HIV-1 DNA vaccine encodes HIV-1 Gag/Pol, Nef/Tat/Vif, and Envelope, and interleukin-12 (IL-12) and is delivered by electroporation (EP) combined with intramuscular injection (IM-EP).

Methods—Sixty-two HIV-1-infected patients on antiretroviral therapy (plasma HIV-1 RNA levels 200 copies/mL; CD4⁺ T-cell counts 500 cells/mm³) were randomly allocated 5:1 to receive vaccine or placebo. At weeks 0, 4 and 12, four consecutive cohorts received 3000 µg HIV MAG pDNA with 0, 50, 250, or 1000 µg of IL-12 pDNA by IM-EP. A 5th cohort received HIV MAG pDNA plus 1000 µg of IL-12 pDNA by standard IM injection.

Results—CD4⁺ T cells expressing IL-2 in response to Gag and Pol and interferon-γ responses to Gag, Pol, and Env increased from baseline to week 14 in the low-dose (50-µg) IL-12 arm vs. placebo ($P < 0.05$; intracellular cytokine staining). The total increase in the IL-2 expressing CD4⁺ T-cell responses to any antigen was also higher in the low-dose IL-12 arm vs. placebo ($P = 0.04$). Cytokine responses by CD8 T cells to HIV antigens were not increased in any vaccine arm relative to placebo.

Conclusions—HIV-1 MAG/low-dose IL-12 DNA vaccine delivered by IM-EP augmented CD4⁺ but not CD8⁺ T-cell responses to multiple HIV-1 antigens.

Keywords

HIV; therapeutic vaccination; DNA vaccine; interleukin-12; electroporation

INTRODUCTION

As a component of a multipronged strategy to eliminate cells latently infected with HIV, therapeutic vaccination has a potential role in achieving eradication of HIV from an infected person.¹ Recent *ex vivo* evidence suggests that an enhanced anti-HIV cellular immune response is critical to kill cells from which the expression of latent HIV is induced.² Therapeutic immunization might also promote a “functional cure” of HIV infection by inducing indefinite host control of HIV replication to undetectable levels off antiretroviral therapy (ART).³

One approach to HIV therapeutic vaccination under study is the priming of the immune response with a plasmid DNA vaccine that contains genes encoding HIV proteins followed by boosting HIV-specific immunity with a viral vector delivering HIV genes. Plasmid DNA priming has been shown to improve the frequency, strength, and breadth of anti-HIV CD4⁺ and CD8⁺ T-cell responses; to improve the persistence of these anti-HIV T-cell responses; and to avoid antivector T-cell immune responses that otherwise could dampen the HIV-specific responses.^{4,5} These effects are seen even when no T-cell responses are detected after the DNA prime.⁵ Alternatively, the augmentation of immune responses seen when DNA vaccines are administered by electroporation could be comparable to that seen when DNA is delivered by viral vectors⁶ and could thus serve to substitute for the boost from viral

vector–delivered genes. Electroporation delivers a small electrical pulse along with the intramuscular (IM) injection, resulting in increased penetration of the DNA into cells.^{7,8}

The Profectus BioSciences (Tarrytown, NY) multiantigen (MAG) DNA vaccine consists of two plasmid DNA expression vectors: a single promoter expression vector encoding an HIV-1 clade B Gag/Pol fusion, and a dual promoter expression vector encoding an HIV-1 clade B Nef/Tat/Vif fusion and a clade B primary isolate (6101) Envelope gp160. The Profectus MAG DNA vaccine is co-delivered with a 3rd expression plasmid encoding the p35 and p40 subunits of human interleukin-12 (IL-12). IL-12 is a cytokine that promotes the maturation and function of T cells.⁹ In pre-clinical studies in rhesus macaques the pDNA vaccine delivered by electroporation after intramuscular injection (IM-EP) led to stronger cellular immune responses compared to the IM injection alone, as measured by IFN-gamma ELISPOT assay¹⁰. The inclusion of the IL-12 plasmid as an adjuvant improved the magnitude, quality and breadth of the antigen-specific cellular immune responses in the same animal model^{11,12}.

In AIDS Clinical Trials Group (ACTG) A5281, we studied the safety, tolerability, and immunogenicity of the Profectus MAG DNA vaccine combined with escalating doses of the IL-12 plasmid and delivered by IM-EP (TriGrid Delivery System, Ichor Medical Systems, San Diego, CA) in persons with HIV-1 infection on effective ART.

METHODS

Study Design

We conducted a phase I randomized, partially double-blind (with respect to study agent, but open-label with respect to administration/delivery), placebo-controlled, dose escalation study. Eligible study participants were HIV-1-infected men and women between the ages of 18 and 55 years on ART who met the following requirements: plasma HIV-1 RNA levels ≤ 200 copies/mL for at least 6 months prior to study entry and <50 copies/mL at screening; CD4⁺ T-cell counts ≥ 500 cells/mm³ within 30 days prior to study entry; and nadir CD4⁺ T-cell counts ≥ 200 cells/mm³.

Four consecutive cohorts of 12 study participants were allocated to receive by IM-EP progressively higher doses of the IL-12 plasmid (0, 50, 250, 1000 μ g) along with 3000 μ g of HIV MAG plasmid DNA (pDNA). The 5th cohort received the highest tolerated dose of the IL-12 plasmid (assessed in real-time during the trial) along with HIV MAG pDNA by standard IM injection without EP. In each cohort, 10 participants were randomly assigned to receive active vaccine and 2 were assigned to receive sodium chloride 0.9% as placebo. The ability of the IL-12 pDNA to express biologically active IL-12 protein *in vitro* was confirmed prior to the release of clinical trial material. The study vaccine was administered, divided into two injections, at 0, 4, and 12 weeks. Participants were followed on study for an additional 24 weeks after the last immunization (Table 1).

The protocol was approved by each study site's institutional review board. Written informed consent was obtained from all participants. The study was conducted according to human

experimentation guidelines of the US Department of Health and Human Services and was monitored by an Interim Monitoring Committee of the ACTG.

Evaluation of Participants and Follow-up

Study participants were clinically evaluated, and laboratory safety monitoring (hematology, serum chemistries, and liver enzymes) was performed at baseline and at weeks 1, 4, 6, 8, 12, 14, 16, 24, and 36. Safety of each vaccine dosing was assessed 2 to 3 days later by telephone contact. Participants used vaccine report cards after each vaccination to record daily temperatures, injection site reactions, and adverse systemic signs and symptoms. Plasma HIV-1 RNA levels were measured at a single ACTG laboratory (Johns Hopkins University) using the Amplicor HIV-1 Monitor test (version 1.5; Roche Diagnostics, Indianapolis, IN) at baseline and at weeks 4, 12, 16, and 36. CD4⁺ and CD8⁺ T-cell counts were assayed at baseline and weeks 8, 16, and 36. Plasma and peripheral blood mononuclear cells were drawn for frozen storage to perform subsequent immunologic assays within 14 days before study entry (preentry), at study entry, and at weeks 4, 8, 12, 14, 16, and 36. Serum samples were obtained for anti-IL-12 antibodies at entry and at weeks 16 and 36.

Participants completed a tolerability questionnaire at weeks 0, 4, and 12 after vaccination administration, rating the pain they experienced at various steps of vaccine administration if an EP device was used to administer the vaccine. All participants were asked whether the study vaccination procedure they experienced would be acceptable to them as a part of a treatment for HIV infection or would be acceptable if their participation contributed to increased scientific knowledge about vaccine administration to prevent or treat infection.

Information on the perceived pain associated with use of the EP device was collected by asking participants to write down a number and make a vertical mark on a line that represented the pain scale from 0 (no pain) to 10 (worst possible pain), respectively for each time point.

Immunologic Evaluations

Intracellular cytokine staining—Peripheral blood mononuclear cells were assessed for *ex vivo* responses to 11 pools of HIV-1 15-mer peptides (Bio-Synthesis, Inc., Lewisville, TX) covering global potential T-cell epitopes across all HIV-1 proteins contained within the HIV MAG pDNA vaccine (Gag1-2, Pol1-3, Env1-3, Nef, Tat, and Vif).¹³ The final concentration for each peptide was 1 µg/mL during stimulations. Staphylococcal enterotoxin B (Sigma-Aldrich, St. Louis, MO) stimulation was the positive control, whereas peptide diluent (dimethyl sulfoxide, final concentration of 1%) was the negative control. The 6-hour stimulation included brefeldin A (10 µg/mL; Sigma-Aldrich) and anti-CD28/anti-CD49d antibodies (each at 1 µg/mL; BD Biosciences, Franklin Lakes, NJ).

A validated intracellular cytokine staining (ICS) protocol was used as described previously¹⁴ with minor modifications.¹⁵ Cells were first stained with Violet Live/Dead Fixable Dead Cell Stain (Life Technologies, Carlsbad, CA),¹⁶ then fixed, permeabilized, and stained intracellularly with fluorescent-labeled antibody reagents detecting CD14 (exclusion

gate), CD3, CD4, CD8, interferon- γ (IFN- γ), IL-2, tumor necrosis factor- α (TNF- α), and CD107a.

The ACTG has a standard operating procedure for specimen acquisition, storage, and transport from sites to the laboratory performing immunologic assays. Assays were performed in batch at the end of participant completion of the trial.

Anti-IL12 antibodies—The IL-12 neutralization assay was a sandwich enzyme-linked immunosorbent assay that measured IFN- γ secreted by a natural killer cell line, NK92-MI, in the presence of IL-12. Assays on prevaccination serum samples were conducted to determine baseline variance in response, and quantities up to 25 NU/mL were considered negative.

Statistical Methods

Participants who received at least one vaccination were included in the analyses. ICS assay results were background subtracted. The response to any single peptide pool of a specific HIV-1 antigen was defined as the change in percentage of CD4 or CD8 T cells generating cytokine from baseline (average of preentry and entry values) to week 14. The total response was the sum of all antigen-specific responses (defined as the greatest response against a single peptide pool of all the pools specific for that antigen). Pairwise comparisons between vaccine dose cohorts and combined placebo were conducted using the Wilcoxon rank sum and Fisher's exact tests. All statistical tests were 2-sided at the 5% nominal level of significance without adjustment for multiple testing.

RESULTS

Study Population

ACTG A5281 enrolled 62 study participants into 5 cohorts at 13 clinical sites between April 2011 and July 2012. In the first 4 study cohorts, the vaccine was administered by IM-EP. In the first cohort, 10 participants received the HIV MAG vaccine without IL-12 plasmid; in the 2nd cohort, 10 participants received the HIV MAG vaccine with 50 μ g IL-12 plasmid; in the 3rd cohort, 11 participants received the HIV MAG vaccine with 250 μ g IL-12 plasmid (one participant received only partial vaccination at week 0; one additional participant did not receive any vaccination before going off study; both were replaced); and in the 4th cohort, 10 participants received the HIV MAG vaccine with 1000 μ g IL-12 plasmid. Ten participants in the 5th cohort received the HIV MAG vaccine with 1000 μ g IL-12 plasmid by standard IM injection. A total of 10 participants received placebo (8 by IM-EP and 2 by standard IM injection). The participant who did not receive any vaccination was excluded from analyses.

Baseline characteristics of the evaluable study population ($N = 61$) are shown in Table 2. The median CD4 T-lymphocyte count was 729 cells/mm³. Fifty-eight of 61 participants (95%) had an entry HIV-1 RNA level available: 57 had HIV-1 RNA <50 copies/mL and one participant had an entry HIV-1 RNA of 58 copies/mL.

No deaths occurred during the study. Fifty-seven of the 61 participants who had received at least one dose of vaccine completed study-defined follow-up. Four participants went off study prematurely (2 in the 250- μ g IL-12 arm, one in the placebo arm, and one in the 1000- μ g IL-12/IM arm) due to withdrawal of consent (2), difficulty adhering to requirements of the protocol (1), or deportation from the country (1). Fifty-two of 61 participants (85%) completed 3 vaccine administrations with 2 injections each time. Nine participants missed at least one injection.

Safety and Tolerability

Three subjects had grade 3 or 4 adverse events considered as related to study treatment. These events were local injection site pain, with or without bruising and/or swelling. One of these participants received placebo, one received HIV MAG pDNA alone, and one received HIV MAG pDNA with 250- μ g IL-12, each by IM-EP. Two of these episodes resolved within 2 days; one resolved after 5 days. No clinical evidence of autoimmune disease occurred in any study participant.

The EP administration process was well tolerated, as measured by questionnaire and pain scale ratings. All 49 IM-EP participants completed the tolerability assessment question at least once. When asked, "In your opinion, would this study's vaccination procedure be acceptable as part of a treatment for HIV, if it proved to be effective?", 3 participants answered "no." When asked, "In your opinion, would this study's vaccination procedure be acceptable if it could contribute to increased scientific knowledge about how best to administer vaccines to prevent or treat infections?", no one answered "no." Of the 12 participants who received IM injections without EP, no one answered "no" to the first question, and one participant answered "no" to the second question.

Participants tended to rate the pain higher for IM-EP compared with IM injection (overall median [Q1, Q3]: 5[2,7] at the time of the first 2 IM-EP vaccinations and 4[3,6] at the time of the 3rd IM-EP vaccination, compared with 2[1,3] at the time of all 3 IM injections). Pain ratings decreased over time (1[0,3] after 10 minutes and 1[0,2] after 30 minutes). The median pain rating was not different between the vaccine and placebo arms. Minimal differences were observed among cohorts.

Plasma HIV RNA remained <50 copies/mL throughout the vaccination period in all participants except for one individual with 911 copies/mL at week 12 (placebo), one with 1460 copies/mL at week 4 and 205 copies/mL at week 12 (50- μ g IL-12, IM-EP), and 6 others with transient minor blips to values <100 copies/mL. All increased values returned to <50 copies/mL while subjects were on study without change in ART.

Immunologic Responses

Fifty-seven participants contributed data to the immunologic analyses (3 went off study prior to week 14, and one participant, in the 1000- μ g IL-12 EP/IM arm, did not have enough stored cells for the assay). When evaluating the results by study arm as a whole, we observed small increases from baseline to week 14 (2 weeks after the 3rd vaccination) in CD4⁺ T-cell responses to several HIV-1 peptide pools, as measured by expression of individual cytokines (ICS assay), in the low-dose (50- μ g) IL-12 arm vs. placebo (Figure 1).

We found statistically significant increases in IL-2-expressing CD4⁺ T cells to Gag2 ([baseline median=]0.022%; [median increase=]0.012%), Pol1(0.034%; 0.022%), and Pol2 (0.034%; 0.005%) and in IFN- γ -expressing CD4⁺ T cells to Gag2 (0.022%; 0.012%), Pol1 (0.003%; 0.024%), Pol3 (0.004%; 0.005%), and Env2 (0.002%; 0.008%) (all $P < 0.05$). There were trends toward increases in CD4⁺ T cells expressing TNF- α to Env1, Env3, and Pol1; and expressing IFN- γ to Pol2, Env1, and Nef ($P = 0.05$ and <0.20). There were no increases in the number of CD4⁺ T cells expressing an individual cytokine to any individual HIV-1 antigen in any of the other active treatment arms vs. placebo. Figure 2 illustrates changes in the CD4⁺ T-cell responses to selected antigens.

We found no significant increases from baseline to week 14 in CD8⁺ T-cell responses when stimulated by any HIV-1 peptide pool in the ICS assay in any treatment arm vs. placebo. Representative CD8⁺ T-cell responses are illustrated in Figure 3.

The total change from baseline to week 14 in CD4⁺ or CD8⁺ T-cell responses to any HIV-1 antigen was assessed (Supplementary Figures 1 and 2). The only statistically significant difference between a vaccine arm and placebo was in the change in IL-2-producing CD4⁺ T cells in the low-dose (50- μ g) IL-12 arm (median 0.082% vs. 0.019%; $P = 0.04$). There was also a trend in the change in IFN- γ -producing CD4⁺ T cells in the low-dose IL-12 arm compared with placebo (0.110% vs. 0.021%; $P = 0.09$).

Detectable anti-IL-12 antibodies did not develop in any study participant.

DISCUSSION

This IL-12-enhanced HIV MAG pDNA vaccine was safe and well tolerated in HIV-infected persons, whether delivered by standard IM injection or by IM-EP. Electroporation induced substantial pain initially, but within 10 minutes, the pain subsided, and most study participants found the procedure to be acceptable.

To define a “responder” in an HIV vaccine trial is arbitrary, especially in the HIV-infected population, in whom background immune responses to HIV-1 antigens are present and fluctuate widely. Because of the high background variability, rather than depend on a definition of “responder” we chose as a primary endpoint to compare between study arms the change in the percentage of CD4⁺ or CD8⁺ T cells expressing an individual cytokine or marker in response to an individual HIV-1 antigen.

The vaccine induced small but significant increases in CD4⁺ T-cell responses against multiple HIV-1 antigens, individually and *in toto*. The clinical meaningfulness of the changes compared to placebo is open to interpretation, since they were small and were seen only in the low-dose (50- μ g) IL-12 plasmid adjuvant arm. However, there was a consistent pattern, and one might not expect a typical dose–response curve with immune-based therapies as is seen with standard drugs. The immune system has complex circuitry, and the effects of cytokines and other signaling molecules may vary depending on their concentrations, the cells being targeted, the tissue environment, and the extent to which counterregulatory pathways are induced at different time points. Thus, higher doses of IL12 might have induced counter-regulatory pathways that blunted the induction of adaptive

immune responses against HIV at the time point measured. It may be that that stronger immune responses were present at earlier time points. It could also be that the HIV-specific cells induced by higher doses of IL-12 were sequestered in lymphoid tissue and not in peripheral blood. The immune responses were not lower in any of the HIV antigen + IL-12 arms compared to those in the HIV antigen alone arm, so there is no evidence that IL-12 impaired the anti-HIV immune response. There was no induction of IL-12 antibodies to explain the lower responses at higher doses of IL-12. Finally, it should be noted that it is possible that IL-12 alone could have enhanced HIV-specific responses in the infected participants

In the CD8⁺ T-cell population, the vaccine did not induce significant increases in the percentage expressing an individual cytokine or marker in response to an individual HIV-1 antigen or to HIV-1 antigens combined. Of particular interest for designing HIV eradication strategies, there were no improvements in the CD107a-expressing CD8⁺ T cells. CD107a is a component of the membrane of cytotoxic granules that fuse with the cell membrane at the immunologic synapse with the target cell and is a marker of cell degranulation upon activation,^{17,18} usually but not always resulting in cell lytic activity.¹⁹ In the “kick-and-kill” strategy currently the focus of intensive research,¹ an enhanced CD8⁺ T-cell lytic activity targeted against latently infected cells induced to express HIV-1 antigens is crucial.² The inability of the DNA vaccine to enhance CD8⁺ T-cell degranulation in response to HIV-1 antigens argues against the potential role of the vaccine by itself in this strategy. However, it is conceivable that this DNA vaccine could serve to prime for a viral vector–delivered HIV DNA vaccine given later as a boost, and that this combination could prove more effective at inducing CD8⁺ T-cell lytic activity.^{4,5}

CD4⁺ T-cell help provides essential support for CD8⁺ T-cell function, maturation, and memory formation.²⁰⁻²³ The increases in CD4⁺ T-cell responsiveness against several HIV-1 antigens could prove important in augmenting anti-HIV CD8⁺ T-cell activity over time²⁴ and helpful in inducing CD8⁺ T-cell responses to a subsequent viral vector vaccine boost. This vaccine followed by a boost with an rVSV vectored HIV-1 Gag vaccine is being evaluated in HIV-uninfected healthy adults (HVTN087, NCT 01578889).

In addition, it is conceivable that the augmented anti-HIV CD4⁺ T-cell activity itself could have clinical significance. Importantly, HIV-specific IL-2-producing CD4 responses are largely lost during chronic infection,²⁵ so the ability to see these responses after vaccination is probably important. Therapeutic immunization clinical trials have found increased HIV-specific CD4⁺ T-cell responses to be associated with the control of viremia during analytical treatment interruptions (ATI) of antiretroviral therapy.^{26,27} In fact, induced HIV-specific CD4⁺ T-cell responses of a similar magnitude as seen in this trial correlated with viral load set point at the end of the ATI in the ACTG A5187 trial of an Ad5-HIV-Gag vaccine.²⁷ In that trial, the difference between the median percentage of CD4⁺ T cells expressing IFN- γ to Gag in the vaccine treatment arm compared to placebo was 0.01%. The increases compared to placebo that we saw in our study were 0.01% each for IFN- γ and IL-2 responses to Gag, and 0.02% each for IFN- γ and IL-2 responses to Pol. Thus, augmenting HIV-specific CD4⁺ T-cell activity may be an important goal of strategies to achieve a “functional” rather than an

eradication cure of HIV infection, in which the host immune system is reeducated to contain, and not necessarily eliminate, a persistent infection.³

A similar HIV-1 DNA vaccine delivered by electroporation elicited statistically significant increases in HIV-specific cellular immune responses above those seen at baseline in a majority of HIV-negative participants.⁶ Consistent with our study, CD4⁺ T-cell response rates (80.8%) were higher than CD8⁺ T-cell response rates (50.7%).⁶ The magnitude of the CD4⁺ T-cell responses in the IM-EP study arms of the HIV-negative study (with and without IL-12), as assessed by the median percentage of cells expressing IFN- γ and/or IL-2 to HIV-1 peptide pools, was similar to the median change in IL-2 responses to peptide pools in the low-dose IL-12 IM-EP arm of our study in HIV-infected individuals. As noted above, we found it more difficult to determine rates of vaccine responders in the HIV-infected population because of the background fluctuation in the ICS assay in the placebo controls.

Nonetheless, CD8⁺ T-cell responses were induced in a majority of study participants in the IM-EP study arms of the HIV-negative study,⁶ whereas we did not detect vaccine-induced CD8⁺ T-cell responses above background noise in our study. It may be that the damaged immune system in HIV-infected individuals blunts responsiveness to vaccines,²⁸ and T-cell exhaustion from chronic exposure to HIV antigens may also render the immune system less capable of responding to HIV vaccines.²⁹

The use of pooled overlapping peptides covering T-cell epitopes across all HIV-1 proteins contained within the HIV MAG pDNA vaccine should have allowed us to detect both CD4 and CD8 T cell responses. Nonetheless, it is conceivable that performing the ICC assays focused on “beneficial peptides” found to be associated with *ex vivo* viral inhibition, or performing the viral inhibition assay itself, could have produced different results.^{30,31}

Our study was unable to evaluate whether the use of IM-EP improved the immunogenicity of this DNA vaccine over standard IM administration, since the highest dose delivered by IM-EP used as the comparator with standard IM did not elicit increased immune responses.

In sum, this IL-12-enhanced HIV MAG DNA vaccine delivered by electroporation was safe and well tolerated. When we evaluated group responses in the treatment arms, the lowest dose of IL-12 (50 μ g) appeared to be the best at inducing small increases in CD4⁺ T-cell expression of IL-2 or IFN- γ directed against multiple HIV-1 antigens. Whether the magnitude of the increases translates into clinically meaningful effects at achieving a “functional cure” is yet to be determined. However, by itself, this HIV DNA vaccine administered by electroporation is unlikely to be useful in viral eradication strategies, given that it did not enhance anti-HIV CD8⁺ T-cell responses. Perhaps, as noted above, in a combination vaccine approach that boosts with a viral vector-delivered HIV-1 DNA vaccine, better responses could be elicited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 2.

Changes in percentage of CD4⁺ cells generating cytokines in response to selected antigens. One outlier value (+0.7%) in the placebo arm for CD4⁺ cells generating interferon-γ (IFNγ)-positive responses in response to Gag2 is not plotted. The increase in response from baseline to week 14 for the low-dose (50-μg) interleukin-12 (IL-12) arm was significantly higher compared with the placebo arm for (a) IL-2⁺ response to (i) Gag2 (median = 0.01% vs. -0.01% [124 vs. -69 per 10⁶ CD4⁺ cells], $P = 0.001$); (ii) Pol1 (0.02% vs. 0.01% [216 vs. 102 per 10⁶ CD4⁺ cells] $P = 0.022$); and for (b) IFN-γ-positive response to (i) Gag2 (0.01%

vs. -0.003% [120 vs. -29 per 10^6 $CD4^+$ cells], $P = 0.019$); and (iii) Pol1 (0.02% vs. -0.001% [244 vs. -5 per 10^6 $CD4^+$ cells], $P = 0.008$). IM/EP, electroporation after intramuscular injection.

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Figure 3.

Changes in percentage of CD8⁺ cells generating cytokines/markers in response to selected antigens. One outlier value (+2.9%) in the placebo arm for CD8⁺ cells generating interferon- γ (IFN γ)-positive responses to Gag1 is not plotted. IL, interleukin; IM/EP, electroporation after intramuscular injection.

TABLE 1

Study Design

Cohort	HIV MAG pDNA Dose (µg)	IL-12 pDNA Dose (µg)	Administration Method	Vaccine / Placebo (N)	Vaccination schedule (weeks)		
					0	4	12
1	3000	—	IM-EP	10/2	HIV MAG pDNA alone or placebo	HIV MAG pDNA alone or placebo	HIV MAG pDNA alone or placebo
2	3000	50	IM-EP	10/2	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo
3	3000	250	IM-EP	12/2	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo
4	3000	1000	IM-EP	10/2	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo
5	3000	1000	IM only	10/2	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo	HIV MAG pDNA plus IL-12 pDNA or placebo

Total subjects = 62.

Abbreviations: IL-12, interleukin-12; MAG, multiantigen.

TABLE 2

Baseline Characteristics by Treatment Arm

Characteristic	Total (N=61)	Treatment Arm					
		pDNA IM/EP (N=10)	pDNA + 50 µg IL-12 IM/EP (N=10)	pDNA + 250 µg IL-12 IM/EP (N=11)	pDNA + 1000 µg IL-12 IM/EP (N=10)	pDNA + 1000 µg IL-12 IM (N=10)	Combined Placebo (Cohorts 1–5) (N=10)
Mean age (SD), y	42 (8)	41 (7)	44 (5)	42 (10)	44 (8)	39 (4)	42 (10)
Sex							
Male	51 (84%)	9 (90%)	9 (90%)	9 (82%)	9 (90%)	5 (50%)	10 (100%)
Female	10 (16%)	1 (10%)	1 (10%)	2 (18%)	1 (10%)	5 (50%)	0 (0%)
Race/ethnicity							
White non- Hispanic	27 (44%)	5 (50%)	6 (60%)	6 (55%)	5 (50%)	2 (20%)	3 (30%)
Black non- Hispanic	19 (31%)	3 (30%)	3 (30%)	4 (36%)	1 (10%)	4 (40%)	4 (40%)
Hispanic (regardless of race)	14 (23%)	1 (10%)	1 (10%)	1 (9%)	4 (40%)	4 (40%)	3 (30%)
More than one race	1 (2%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Median CD4 ⁺ cell count (Q1–Q3), cells/mm ³	729 (591- 972)	746 (713- 934)	769 (585-853)	728 (593- 1,063)	620 (570-860)	814 (557- 961)	706 (597- 1,224)
Median CD8 ⁺ cell count (Q1–Q3), cells/mm ³	781 (570- 998)	688 (581- 1,036)	903 (823- 1,098)	794 (584- 940)	518 (367- 757)	647 (500- 749)	900 (487- 1,234)
Entry HIV-1 RNA							
<50 c/mL	57 (93%)	10 (100%)	8 (80%)	10 (91%)	10 (100%)	9 (90%)	10 (100%)
50 c/mL	1 (2%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Missing/unknown	3 (5%)	0 (0%)	1 (10%)	1 (9%)	0 (0%)	1 (10%)	0 (0%)

Abbreviations: IL, interleukin; IM/EP, electroporation combined with intramuscular injection; IFN-γ, interferon-γ.