

# Immunization of HIV-1-Infected Persons With Autologous Dendritic Cells Transfected With mRNA Encoding HIV-1 Gag and Nef: Results of a Randomized, Placebo-Controlled Clinical Trial

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**Background:** HIV-1 eradication may require reactivation of latent virus along with stimulation of HIV-1-specific immune responses to clear infected cells. Immunization with autologous dendritic cells (DCs) transfected with viral mRNA is a promising strategy for eliciting HIV-1-specific immune responses. We performed a randomized controlled clinical trial to evaluate the immunogenicity of this approach in HIV-1-infected persons on antiretroviral therapy.

**Methods:** Fifteen participants were randomized 2:1 to receive intradermal immunization with HIV-1 Gag- and Nef-transfected DCs (vaccine) or mock-transfected DCs (placebo) at weeks 0, 2, 6, and 10. All participants also received DCs pulsed with keyhole limpet hemocyanin (KLH) to assess whether responses to a neo-antigen could be induced.

**Results:** After immunization, there were no differences in interferon-gamma enzyme-linked immunospot responses to HIV-1 Gag or Nef in the vaccine or placebo group. CD4 proliferative responses to KLH increased 2.4-fold ( $P = 0.026$ ) and CD8 proliferative responses to KLH increased 2.5-fold ( $P = 0.053$ ) after vaccination. There were increases in CD4 proliferative responses to HIV-1 Gag (2.5-fold vs. baseline, 3.4-fold vs. placebo,  $P = 0.054$ ) and HIV-1 Nef (2.3-fold vs. baseline, 6.3-fold vs. placebo,  $P = 0.009$ ) among vaccine recipients, but these responses were short-lived.

**Conclusion:** Immunization with DCs transfected with mRNA encoding HIV-1 Gag and Nef did not induce significant interferon-gamma enzyme-linked immunospot responses. There were increases in proliferative responses to HIV-1 antigens and to a neo-antigen, KLH, but the effects were transient. Dendritic cell vaccination should be optimized to elicit stronger and long-lasting immune responses for this strategy to be effective as an HIV-1 therapeutic vaccine.

**Key Words:** HIV-1, mRNA-transfected dendritic cell, immunization, randomized controlled trial, therapeutic vaccine

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## INTRODUCTION

Eradication of HIV-1 may require a combination of reactivation of latently infected virus and stimulation of HIV-1-specific immune responses to clear infected cells. Because of the potential role of the immune system in killing cells that are expressing virus antigens, there is substantial interest in developing strategies to elicit T cell responses against HIV-1. One promising method for stimulating T cell immunity is dendritic cell (DC) vaccination, in which autologous dendritic cells are derived from blood monocytes, manipulated *ex vivo*, exposed to specific antigens, and then injected back into the person.

One of the main challenges for this strategy is how to optimally deliver antigen to DC *ex vivo*. Transfection with mRNA is an efficient method of delivering antigens to DCs<sup>1</sup> and has been shown to stimulate antigen-specific CD4 and CD8 T cell responses *in vitro* and *in vivo*.<sup>2,3</sup> We and others

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have shown that transfection of mRNA encoding HIV-1 gene products into DCs induces CD8 and CD4 T cell responses *in vitro*.<sup>4-6</sup> More recently, this approach has been demonstrated in clinical trials to elicit antigen-specific immune responses in HIV-1-infected participants (reviewed by Garcia et al<sup>7</sup>).

There are several reasons why transfecting DCs with mRNAs encoding specific antigens is a particularly appealing strategy in HIV-1-infected patients.<sup>8</sup> First, by including lysosomal targeting sequences in the transfected mRNA, antigen can be directed into lysosomal compartments for degradation, which has been shown to stimulate antigen-specific CD4 cell responses.<sup>9-13</sup> Because most HIV-1-infected patients on antiretroviral therapy (ART) lack virus-specific CD4 cell proliferative responses,<sup>14-17</sup> the ability to stimulate these responses may be critical in providing help to dysfunctional CD8 cells in these patients.<sup>18</sup> Second, because transfected mRNA is delivered directly into the cytoplasm where it is translated, this obviates the need for codon optimization of the HIV-1 sequence,<sup>19</sup> because the main effect of codon optimization is to improve stability and export of mRNA from the nucleus.<sup>20,21</sup> Finally, immunization with mRNA-transfected DCs allows one to deliver whole viral gene products, which should allow recognition by persons of diverse human leukocyte antigen (HLA) types. This strategy overcomes the limitation of approaches that use specific peptides as the antigen, which restricts the immunogenicity of such vaccines to a particular subset of subjects who have HLA types that present those epitopes. Transfection of mRNA encoding whole HIV-1 gene products also obviates the need to synthesize, purify, and characterize vaccine-grade protein immunogens, which is technically and financially challenging.

Based on this rationale, we investigated whether transfection of DCs with mRNA encoding specific HIV-1 antigens is able to elicit T cell responses *in vivo*. Unlike a recent trial of this approach, which included HIV-1 Tat, Rev, and Nef as vaccine antigens,<sup>22</sup> we chose to transfect HIV-1 *Gag* and *Nef* because T cell responses against *Gag* have been associated with immune control of the virus<sup>23</sup> and because of the high density of HIV-1 epitopes in *Nef*. We also included lysosomal targeting sequences in the transfected mRNAs to improve the likelihood of stimulating antigen-specific CD4 cell responses. Here, we report the results of a randomized, placebo-controlled trial of the safety and immunogenicity of vaccination of HIV-1-infected persons on ART with autologous DCs transfected with mRNA encoding HIV-1 *Gag* and *Nef*.

## MATERIALS AND METHODS

This single-site study was conducted according to Declaration of Helsinki principles, and was approved by our Institutional Review Board. All participants provided written, informed consent for the study. The NCT number for this study is NCT00833781.

### Study Population

The main inclusion criteria for the study were (1) HIV-1-infected persons ages 18–60 years; (2) receiving combination antiretroviral therapy for a minimum of 12 months; (3)

documentation of HIV-1 RNA levels below the limits of quantification for at least 6 months; (4) CD4 cell counts  $>200/\text{mm}^3$  for at least 1 year. The main exclusion criteria included (1) HCV antibody positivity; (2) history of clinically significant cardiac, gastrointestinal, hepatic, renal, pancreatic, neurologic, or autoimmune disease; (3) positive rheumatoid factor or anti-dsDNA antibody; or (4) initiation of ART within 12 months of documented HIV-1 seroconversion.

### Vaccine Preparation

Participants underwent leukapheresis to obtain large numbers of peripheral blood mononuclear cells (PBMCs). Monocyte-derived dendritic cells were expanded *in vitro* by culture with granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4 (both from CellGenix, Freiberg, Germany and Portsmouth, NH) using published methods.<sup>24</sup> On day 5 of culture, immature monocyte-derived dendritic cells were resuspended in Belzer UW solution, mixed with synthetic mRNA encoding HIV-1 *Gag* and *Nef* or were mixed with saline, and subjected to electroporation to induce RNA uptake. The electroporation conditions were 900 V, 0.75 mS, square wave, using a Gene-Pulse II Electroporator (Bio-Rad, Hercules, CA). Two different synthetic mRNA products were used in this study: *LysGag* mRNA and *LysNef* mRNA (manufactured by Asuragen, Austin, TX). Each mRNA molecule was monocistronic and contained a single Kozak consensus ribosome binding site at the 5' end to promote ribosome binding and translation from the mRNA. Each mRNA also included a codon-optimized polypeptide coding sequence with an endoplasmic reticulum translocation signal peptide, the antigen polypeptide, and a human lysosome-associated membrane protein-1 (Genbank accession NM\_005561) targeting sequence, including the transmembrane domain. The purpose of the lysosome-associated membrane protein-1 targeting sequence was to direct transport of the antigenic polypeptide into a lysosomal/endosomal compartment where it would be digested to provide antigenic peptides to be loaded onto MHC class II molecules.<sup>9</sup> The sequences of the *Gag* and *Nef* polypeptides were designed to correspond at the amino acid level to the 2001 HIV-1 Clade B consensus sequence.<sup>19</sup> Transfected DCs were then matured with a cytokine cocktail consisting of IL-1- $\beta$ , IL-6, tumor necrosis factor- $\alpha$  (all from CellGenix), and prostaglandin E2 (Sigma, St. Louis, MO). After maturation, the DCs were frozen. To confirm the *in vitro* immunogenicity of DCs prepared according to this protocol, DCs were prepared and transfected with the clinical preparation of mRNA, and cocultured with autologous PBMCs to measure expansion of antigen-specific CD4 and CD8 T cells as previously described<sup>25</sup> (data not shown). Before injection, an aliquot of DCs was confirmed to be sterile [negative bacterial and fungal cultures; negative *Mycoplasma* PCR and culture (performed by Bionique Testing Laboratories, Saranac Lake, NY)] and endotoxin-free (negative limulus amebocyte lysate testing). An aliquot of DCs also underwent testing to confirm that the cells met prespecified release criteria for viability and maturation:  $>75\%$  viability, CD83<sup>+</sup>, CD86<sup>+</sup>, HLA-DR<sup>+</sup>, and CD14 negative. All DC preparations met these criteria.

## Study Procedures

Participants were randomized 2:1 to receive 4 intradermal injections of 5–15 million mRNA-transfected DC (active vaccine;  $n = 10$ ) or mock-transfected DCs (placebo;  $n = 5$ ) at weeks 0, 2, 6, and 10. (The number of DCs injected within the specified range depended on the yield during their preparation.) The participants and the investigators were blinded to study assignment. All participants also received a second intradermal injection on the contralateral side of 1.5–6 million autologous DCs pulsed with 10  $\mu\text{g}/\text{mL}$  of keyhole limpet hemocyanin (KLH), a neo-antigen, at weeks 0 and 2. All injections were given 6–8 cm from the axilla in the inner aspect of the upper arm. Participants were monitored for at least 30 minutes after each injection. Between 24 and 48 hours after the injections, a study nurse contacted each participant to determine whether they had experienced any adverse events. All participants also completed vaccination diaries in which they documented any new signs or symptoms after the vaccinations. CD4 cell counts were measured at the following visits: weeks 0, 6, 10, 14, 18, 24, and 48. HIV-1 RNA was measured at the following visits: weeks 0, 2, 6, 10, 14, 18, 24, and 48. An autoimmunity panel, consisting of rheumatoid factor and anti-ds DNA antibody, was performed at weeks 6, 10, 14, and 48. Chemistries, tests of renal function, ALT, AST, alkaline phosphatase, complete blood count with differential were performed at weeks 0, 6, 10, 14, 18, 24, and 48.

## Primary Endpoints

The primary endpoints of the trial were safety and tolerability and T cell responses to HIV-1 Gag and Nef were measured 4 weeks after the final vaccination. The primary safety endpoint for the study was grade 3 or 4 adverse events that were possibly, probably, or definitely related to study vaccine or procedures. Immunogenicity was measured by interferon-gamma enzyme-linked immunospot (ELISPOT) assay, carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution, and intracellular cytokine staining (ICS) (see below). The primary immunogenicity endpoint was the change in ELISPOT responses to HIV-1 Gag and Nef from pretreatment (geometric mean of preentry and week 0 measurements) to 4 weeks after the last vaccination (week 14), comparing those participants who received active vaccine to those who received placebo.

## Immunologic Assays

For assays using peptide pools, peptides were based on the 2001 Consensus B HIV-1 sequence as previously described.<sup>19</sup> For the ELISPOT assays, PBMCs were thawed and interferon-gamma production against overlapping peptide pools (representing Gag and Nef) was determined as previously described.<sup>19</sup> For the intracellular cytokine staining assays, PBMCs were thawed and stimulated overnight in the presence of anti-CD107 antibody, with medium alone or with HIV-1 Gag or Nef peptide pools; no interleukin-2 was added. Cells were fixed and stained, and the following parameters were measured by flow cytometry: IFN-gamma, IL-2, TNF-alpha, and CD107

surface expression. For the CFSE dilution assays, PBMCs were thawed and stained with CFSE. Stained cells were allowed to proliferate in the presence of media alone, or in the presence of KLH, or Gag peptide pool, or Nef peptide pool. After 7 days of proliferation, cells were stained for CD3, CD4, CD8, CD25, and viability. The percent proliferation for CD4 and CD8 T cells was calculated as the percent of all CD4<sup>+</sup> or CD8<sup>+</sup> T cells that were CFSE-low and CD25<sup>+</sup>. The instrument used for flow cytometry was a LSR Fortessa (Becton Dickinson, Inc., Franklin Lakes, NJ); the software used for analysis was Flowjo (Treestar, Inc., Ashland, OR)

## Statistical Methods

Demographic and baseline characteristics of the participants randomized to mRNA-transfected or mock-transfected DCs were compared by Wilcoxon rank-sum test and Fisher's exact test. The sample size was selected to assess safety. With  $n = 10$  participants exposed to mRNA-transfected DCs and safety declared only if no more than a single vaccine or procedure-related grade 3 or 4 adverse event was observed, the study had 91% power for declaring mRNA-transfected DCs safe if the true rate of such adverse events were 5% or less and less than a 5% probability of declaring mRNA-transfected DCs safe if the true rate were 40% or greater. Based on the observed standard error for the primary immunogenicity endpoint, the study had 80% power to detect a 1.5-fold treatment-dependent ELISPOT response. ELISPOT assay results were log-transformed after subtracting the number of spots in media-only wells from the number of spots in wells with peptide pools. For analysis of CFSE results, the percent proliferation for CD4 and CD8 T cells was log-transformed. For analysis of ICS results, the percent of CD4 or CD8 cells that produced the cytokine of interest was log-transformed. Serial measurements of ELISPOT, CFSE, and ICS were analyzed in shared-baseline, random-slope linear mixed effect models with fixed effects for visit and the interaction of treatment and postrandomization visit and random participant-specific intercepts and slopes with unstructured covariance. The shared baseline assumption, enforced by omitting a treatment main-effect term, reflects the homogeneous state of the population sampled before randomization and has the advantage of adjusting for any chance differences at baseline in a manner similar to analysis of covariance.<sup>26</sup> Estimates of change from baseline and treatment-dependent differences were obtained from linear contrasts of least-square means and then back-transformed. All analyses were performed in SAS (version 9.3; SAS Institute, Cary, NC). All tests were two-tailed, with significance declared for  $P < 0.05$  without correction for multiple comparisons.

## RESULTS

Fifteen participants were enrolled in the study (Table 1); 12 were male. The median age at consent was 47 years (range 33–55 years). At study entry, the median CD4 cell count was 556/ $\text{mm}^3$  (range 380–853/ $\text{mm}^3$ ). All participants had a HIV-1 RNA  $< 50$  copies per milliliter at study screening and entry.

**TABLE 1.** Baseline Characteristics

Characteristic	Overall (n = 15)	Active Vaccine (n = 10)	Placebo (n = 5)
Age (years), median (range)	47 (33–55)	44.0 (33–55)	49.0 (42–52)
Male sex (%)	12 (80%)	7 (70%)	5 (100%)
Ethnicity			
Hispanic	3 (20%)	2 (20%)	1 (20%)
Nonhispanic	12 (80%)	8 (80%)	4 (20%)
Race			
Caucasian	13 (87%)	9 (90%)	4 (80%)
Black	2 (13%)	1 (10%)	1 (20%)
Baseline CD4 count (cells/mm <sup>3</sup> ), median (range)	556 (380–853)	569 (380–853)	525 (388–679)
Nadir CD4 count (cells/mm <sup>3</sup> ), median (range)	279 (30–497)	278 (74–454)	311 (30–497)
Antiretroviral regimen*			
PI-based	5 (33%)	2 (20.0%)	3 (60.0%)
NNRTI-based	9 (60%)	7 (70.0%)	2 (40.0%)
INSTI-based	1 (7%)	1 (10.0%)	0 (0.0%)

\*For simplicity, participants receiving PIs plus an NNRTI, INSTI, or CCR5 receptor antagonist were classified as being on a PI-based regimen (this applied to 2 participants). INSTI, integrase strand transfer inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Baseline characteristics were well-balanced between the active vaccine and placebo recipients (all *P*-values >0.25 for comparisons between the 2 groups).

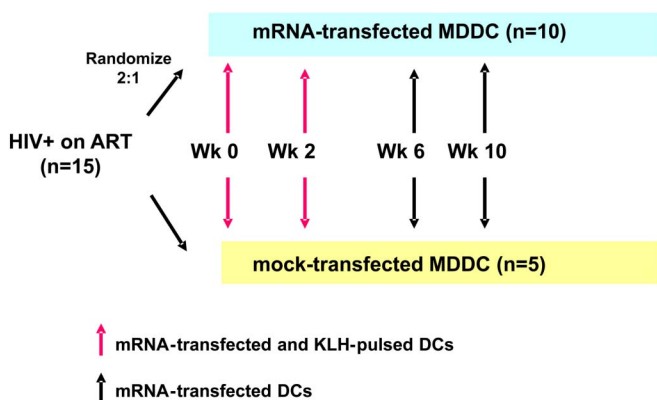
The participants were randomized 2:1 to receive 4 intradermal injections of 5–15 million mRNA-transfected autologous dendritic cells (n = 10) or mock-transfected autologous DCs (n = 5) at weeks 0 (entry), 2, 6, and 10 (Fig. 1). All of the participants completed all of the vaccinations. The vaccinations were well-tolerated with no grade 3 or 4 adverse events related to the immunizations. No participants had confirmed HIV-1 RNA levels >50 copies per milliliter. CD4 cell counts were stable throughout the study in both treatment groups (data not shown). An external safety monitoring committee that reviewed all of the data did not identify any safety concerns.

### ELISPOT Responses

The primary assessment of immunogenicity was the ELISPOT response to HIV-1 Gag and Nef overlapping

peptides. In the overall study population, the median pre-treatment Gag-specific ELISPOT response was 780 spot-forming cells per million PBMCs (range 33–4530) and the median Nef-specific ELISPOT response was 325 spot-forming cells per million PBMCs (range 37–2474); there was no difference in baseline Gag or Nef ELISPOT responses between participants who were randomized to active vaccination and those randomized to receive placebo.

After immunization with mRNA-transfected or mock-transfected DCs at study weeks 0, 2, 6, and 10, we assessed HIV-1 Gag and Nef responses at study weeks 2, 6, 14, 18, and 24 (Fig. 2). ELISPOT responses to HIV-1 Gag declined transiently at week 6 among placebo recipients and responses to HIV-1 Nef were modestly higher among active vaccine recipients as early as week 2, but treatment-dependent differences between the active vaccine group and the placebo group measured 4 weeks after the final vaccination (week 14) or at subsequent time points (weeks 18 and 24) were not significant (from week 0 to week 14, Gag fold ratio = 1.00, 95% CI: 0.76 to 1.32, *P* = 0.99; Nef fold ratio = 1.13, 95% CI: 0.85 to 1.49, *P* = 0.39).



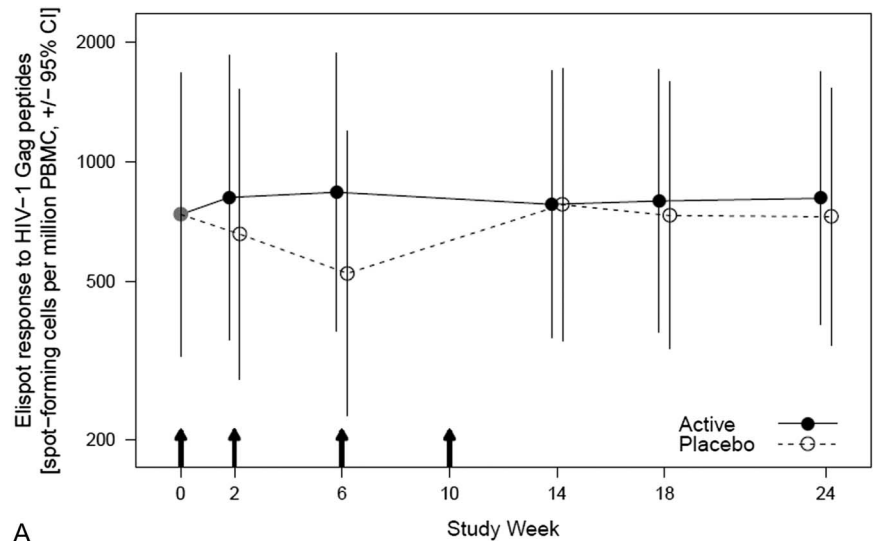
**FIGURE 1.** Study Schema. MDDC: Monocyte-derived dendritic cells.

### Other Measures of Immunogenicity

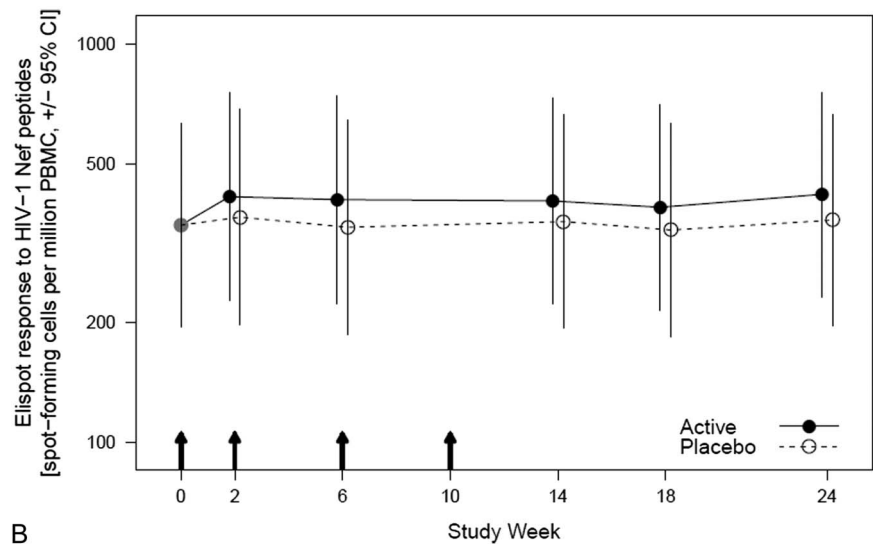
Secondary immunologic endpoints included T cell proliferation, assessed by CFSE dilution, and IL-2 and interferon-gamma production assessed by intracellular cytokine staining.

### Response to the Neoantigen, KLH, After Vaccination

In the overall study population (all of whom received KLH-pulsed DCs at study weeks 0 and 2), CD4 cell responses to KLH increased 2.4-fold and CD8 cell responses to KLH increased 2.5-fold, as measured by CFSE proliferation, from pretreatment (geometric mean of preentry and week



A



B

**FIGURE 2.** ELISpot responses against HIV-1 Gag and Nef overlapping peptides in HIV-infected recipients of mRNA-transfected (active) or mock-transfected (placebo) autologous DCs. Vaccination was performed at weeks 0, 2, 6, and 10 (indicated by the arrows). HIV-1 Gag-specific ELISpot responses declined transiently at week 6 among placebo recipients, but there were no significant differences at week 14 (the primary endpoint) in HIV-1 Gag- or Nef-specific ELISpot responses between the 2 groups.

0 measurements) to 4 weeks after the final vaccination (week 14) ( $P = 0.026$  for CD4 response and  $0.053$  for CD8 response). An example of a participant with a vaccine-induced KLH response is shown in Figure 3.

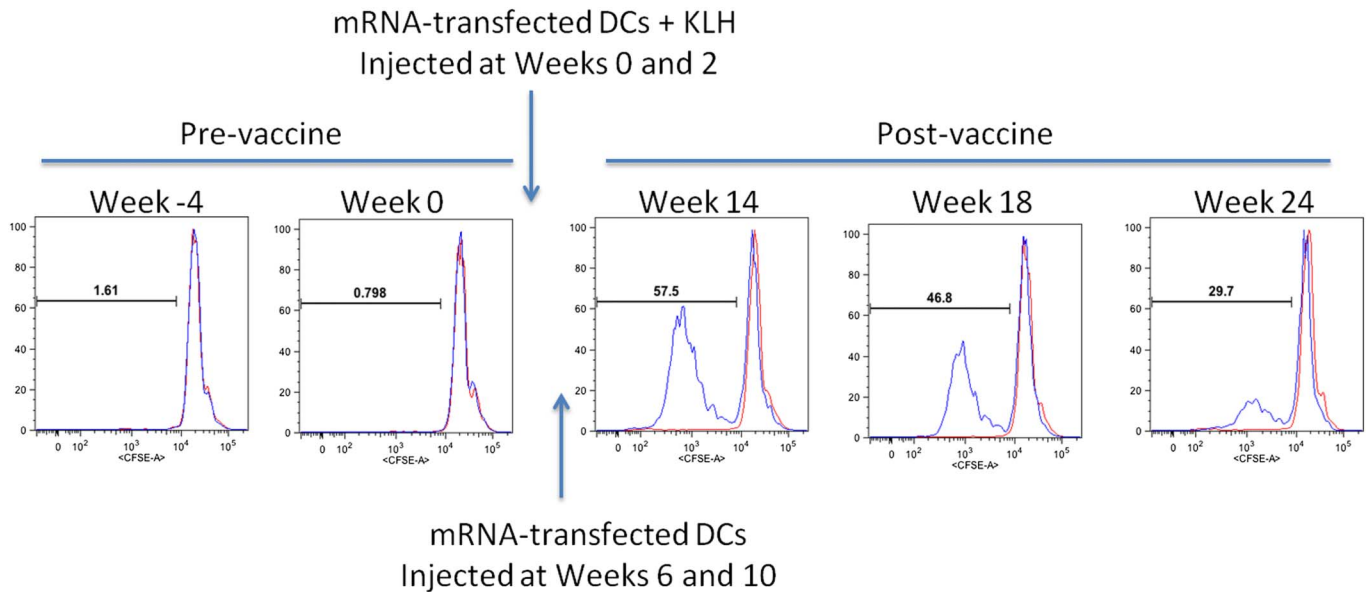
### Proliferative Response to HIV-1 Antigens After Vaccination

When comparing the change in CD4 T cell proliferative responses to HIV-1 Gag (measured by CFSE dilution) from week 0 to week 14, vaccine recipients had a 2.5-fold increase from baseline (95% CI: 1.17 to 5.21) and a 3.4-fold increase (95% CI: 0.98 to 12.1) compared with placebo recipients ( $P = 0.054$ ) (Fig. 4). Similarly, when comparing the change in CD4 T cell proliferative responses to Nef from week 0 to week 14, vaccine recipients had a 2.3-fold increase from baseline (95% CI: 1.04 to 5.04) and a 6.3-fold increase (95% CI: 1.6 to 24.6) compared with placebo recipients ( $P = 0.009$ ) (Fig. 4). In terms of CD8 T cell proliferative responses, there

was a trend towards a response to HIV-1 Nef: recipients of vaccine had a 4.8-fold increase (95% CI: 0.9 to 24.7,  $P = 0.062$ ) compared with those who received placebo (Fig. 4). However, these responses were transient, and at subsequent study weeks the changes were only 1.1–1.3-fold higher among vaccine vs. placebo recipients and were no longer significantly different. The variability in IL-2, interferon-gamma, CD107, and TNF-alpha expression assessed by intracellular cytokine staining was substantial, with no clear-cut vaccine-induced responses (data not shown).

### DISCUSSION

In this randomized controlled trial, mRNA-transfected DCs were safe and well-tolerated in HIV-infected patients on antiretroviral therapy. However, we did not detect significant vaccine-induced boosting of T cell responses, as measured by interferon-gamma ELISpot, against the transfected antigens. Participants developed de novo CD4 and CD8 proliferative



**FIGURE 3.** Induction of a response to a neoantigen, KLH, after injection of KLH-pulsed mRNA-transfected dendritic cells in a study participant. A 7-day CFSE dilution assay was performed on thawed PBMCs at the indicated time points prevaccination and postvaccination. The blue line indicates KLH was added to the cells on day 0; the red line shows the cells exposed to medium alone. The cells were gated on CD3<sup>+</sup>, CD4<sup>+</sup>, and CD25<sup>+</sup> lymphocytes.

responses to KLH, a neo-antigen, and CD4 proliferative responses to HIV-1 Nef, but these responses were transient. These results highlight that dendritic cell vaccination should be optimized to elicit stronger and long-lasting immune responses for this strategy to be effective as an HIV-1 therapeutic vaccine.

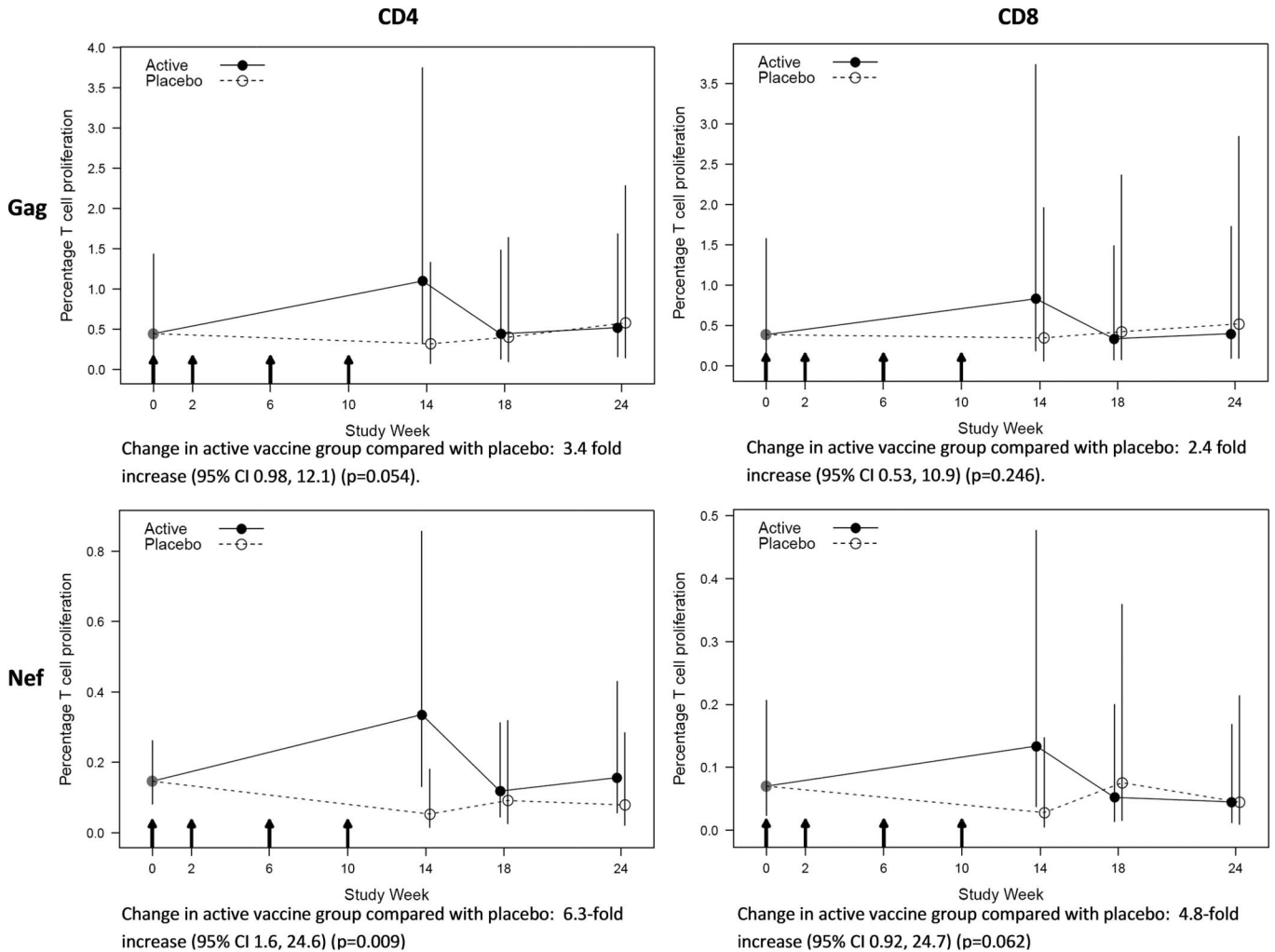
Why did we observe induction of T cell proliferative responses but not interferon-gamma ELISPOT responses? One possible explanation is that the vaccine elicited memory responses rather than immediate effector responses. In some HIV-1-infected patients who have low or undetectable ex vivo ELISPOT responses (as measured by interferon-gamma production), in vitro expansion of T cells results in detection of new antigen-specific responses; the cells that are induced to proliferate have a central memory phenotype.<sup>27</sup> We did not have adequate cells to assess this hypothesis but future studies of vaccine-induced responses should test this possibility.

Why was our vaccine not more immunogenic? One possibility is that the dendritic cells from HIV-infected patients are not able to effectively elicit HIV-specific T cell responses when injected in vivo, despite their ability to do so in vitro. This hypothesis is supported by data showing the monocyte-derived DCs from HIV-infected patients are dysfunctional as a result of multiple mechanisms (reviewed by Miller and Bhardwaj<sup>28</sup>), including the presence of plasma factors that impair IL-12 production.<sup>29</sup> Another possibility is that viral antigens were not expressed after mRNA transfection of DCs. This explanation is unlikely, however, because using the same methods in vitro led to efficient transfection of DCs and expression of HIV-1 antigens. In addition, T cell proliferative responses to HIV-1 antigens were observed in vivo after vaccination, albeit of transient

duration, suggesting antigen was expressed by the transfected DCs. A third potential explanation is that the method used to mature the DCs did not result in optimal immunogenicity; this possibility is supported by evidence that DCs treated with the cytokine cocktail used in this study (IL-1beta, IL-6, TNF-alpha, and prostaglandin E2) may not produce adequate amounts of IL-12p70.<sup>27</sup> Finally, it is possible that there was induction of T regulatory cells; we did not have adequate samples to assess this possibility.

How do the results of our clinical trial compare with other studies of DC vaccination in HIV-infected patients? As summarized in recent reviews,<sup>7,30</sup> other trials of mRNA-transfected DCs have shown stronger induction of T cell proliferation or ELISPOT responses than the responses seen in our study. In one strategy,<sup>31</sup> DCs transfected with autologous mRNA sequences encoding HIV-1 Gag, Vpr, Nef, and Rev were matured with CD40 ligand, which may improve their immunogenicity by inducing IL-12 production. Although a recent report<sup>32</sup> indicates that in a phase 2b placebo-controlled study of this personalized vaccine, called AGS-004, the primary endpoint of significant HIV-1 RNA reduction after ART interruption was not achieved, the vaccine reportedly induced memory T cell responses in most participants. In another important trial,<sup>33</sup> participants who underwent vaccination with DCs pulsed with heat-inactivated HIV-1 were more likely to achieve significant HIV-1 RNA decreases after ART interruption than those who received unpulsed DCs, but this effect was transitory, again arguing that a strategy that leads to stronger and long-lasting immune responses is needed.

The results of these previous studies—and our current trial—suggest that new approaches are needed to develop more immunogenic vaccines. What then is the way forward?



**FIGURE 4.** CD4 and CD8 T cell proliferative responses against HIV-1 Gag and Nef measured by CFSE dilution in HIV-infected recipients of mRNA-transfected (active) or mock-transfected (placebo) autologous DCs. Immunization was performed at weeks 0, 2, 6, and 10 (indicated by the arrows). The change in T cell proliferative response from baseline to week 14 was compared between the active vaccine and placebo groups (change in active vaccine group compared with placebo). The percent T cell proliferation was calculated as the percent of CD4 or CD8 T cells that were CFSE-low and CD25<sup>+</sup>; values shown are derived from a shared-baseline, random-slope linear mixed effect model (see Materials and Methods section). Column headings indicate type of T cell (CD4 or CD8); row headings indicate the HIV-1 antigen (Gag or Nef). Error bars indicate the 95% confidence interval.

Based on previous data suggesting that the maturation stimuli used in our trial may lead to inadequate IL-12p70 production by DCs, new approaches to mature DCs that improve IL-12p70 production have been developed (reviewed by Anguille et al<sup>34</sup>), such as the toll-like receptor 3 agonist, poly I:C. In addition, DC vaccination is now being combined with other immunotherapeutic strategies, such as antibodies against CTLA-4 or PD-1/PDL-1, starting first in cancer trials; the results of such combination trials are eagerly awaited. Third, new approaches to target immunogens to DCs in vivo are being developed. Finally, novel studies of latency-reversing agents, such as histone deacetylase inhibitors, with DC vaccines in HIV-1-infected patients are being pursued to assess whether the combination will lead to clearance of infected cells. The results of our study indicate that these novel approaches to optimizing DC

vaccination are needed to improve the magnitude and duration of HIV-specific immune responses, which is critical to developing an effective HIV therapeutic vaccine.

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