# Robust antibody and cellular responses induced by DNA-only vaccination for HIV

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**BACKGROUND.** HVTN 098, a randomized, double-blind, placebo-controlled trial, evaluated the safety, tolerability, and immunogenicity of PENNVAX-GP HIV DNA vaccine, administered with or without plasmid IL-12 (pIL-12), via intradermal (ID) or intramuscular (IM) electroporation (EP) in healthy, HIV-uninfected adults. The study tested whether PENNVAX-GP delivered via ID/EP at one-fifth the dose could elicit equivalent immune responses to delivery via IM/EP and whether inclusion of pIL-12 provided additional benefit.

**METHODS.** Participants received DNA encoding HIV-1 env/gag/pol in 3 groups: 1.6 mg ID (ID no IL-12 group, $n$ = 20), 1.6 mg ID + 0.4 mg pIL-12 (ID + IL-12 group, $n$ = 30), 8 mg IM + 1 mg pIL-12 (IM + IL-12 group, $n$ = 30), or placebo ($n$ = 9) via EP at 0, 1, 3, and 6 months. Results of cellular and humoral immunogenicity assessments are reported.

**RESULTS.** Following vaccination, the frequency of responders (response rate) to any HIV protein based on $CD4^+$ T cells expressing IFN-γ or IL-2 was 96% for both the ID + IL-12 and IM + IL-12 groups; $CD8^+$ T cell response rates were 64% and 44%, respectively. For ID delivery, the inclusion of pIL-12 increased $CD4^+$ T cell response rate from 56% to 96%. The frequency of responders was similar (≥90%) for IgG binding antibody to gp140 consensus Env across all groups, but the magnitude was higher in the ID + IL-12 group compared with the IM + IL-12 group.

**CONCLUSION.** PENNVAX-GP DNA induced robust cellular and humoral immune responses, demonstrating that immunogenicity of DNA vaccines can be enhanced by EP route and inclusion of pIL-12. ID/EP was dose sparing, inducing equivalent, or in some aspects superior, immune responses compared with IM/EP.

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## Introduction

DNA has been included as part of the vaccine regime for several candidate HIV vaccines contributing to robust immunogenicity (e.g., refs. 1–3). Although the immune response due to the DNA component was initially relatively poor, this has increased over time largely because of plasmid optimization and improved

**Conflict of interest:** ES, LP, and MP are employed by the National Institute of Allergy and Infectious Diseases (NIAID). MCW, JY, MPM, ASK, JDB, LH, SW, NYS, and MLB are/were employees of Inovio Pharmaceuticals, Inc. and own shares or have been awarded stock options in the company. DBW has received grant funding, participates in industry collaborations, and has received speaking honoraria and fees for consulting, including serving on scientific review committees and board services; remuneration includes direct payments or stock or stock options, and he notes potential conflicts associated with this work with Inovio, Pfizer, Merck, VGXI, MedImmune, and possibly others. DBW has a patent for DNA vaccine delivery pending to Inovio (European Patent Application 12187666.6, Improved Vaccines and Methods for using the same).

**Role of funding source:** Within the terms of the Grant Award of the Cooperative Agreement with the HVTN, the study sponsor, NIAID, NIH, reviewed and approved the study design, contributed to the review and analysis of data, concurred with the decision to publish, and assisted with the preparation of the manuscript. NIAID was not involved in the data collection and did not perform statistical analyses. The sponsors had full access to all the data in the study and shared final responsibility for the decision to submit for publication.



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DNA formulation (4). Perhaps the most marked increase in immunogenicity has resulted from delivery via in vivo electroporation (EP). Although there were initial concerns about the feasibility and tolerability of EP vaccination in vivo, several trials have shown acceptability of EP among study participants and enhanced immune responses when delivered by the intramuscular (IM) (5–8) and intradermal (ID) (9, 10) routes. The HIV Vaccine Trials Network (HVTN) 098 study, reported here, explored improvements in plasmid and construct design, formulation, as well as delivery in healthy, HIV-1–uninfected study participants in a multicenter clinical trial.

Previously, the HVTN evaluated a multigene HIV-1 DNA immunogen encoding HIV-1 clade B *gag*, *pol*, and *env* plasmids administered with plasmid IL-12 (pIL-12) by IM injection followed by EP (6). This early-generation adaptive IM/EP markedly increased both the $CD4^+$ and $CD8^+$ T cell responses when compared with a similar vaccine regimen in a prior study administered without EP (6). After the third vaccination, the $CD4^+$ T cell and $CD8^+$ T cell response rates increased from 19% to 81% and 7% to 52%, respectively.

In vivo EP was developed to improve the immunogenicity of DNA for therapeutic applications. The first DNA vaccine trial to demonstrate clinical efficacy included delivery via CELLECTRA IM/EP (8) using consensus synthetic DNA cassettes (encoding HPV 16/18 E6 and E7 proteins) for therapy of an HPV-associated cervical intraepithelial neoplasia (CIN). A significant difference in regression of CIN was noted among vaccine recipients compared with placebo, and complete clearance of virus was associated with $CD8^+$ T cells trafficking to the cervical tissue.

Skin is an attractive target tissue for delivering DNA vaccines for multiple reasons: (a) skin is the largest organ of the human body and readily accessible; (b) it harbors Langerhans and other antigen-presenting cells, so it is capable of developing a broad immune response to antigens; and (c) intradermal delivery is less invasive and avoids stimulation of muscle tissue (11, 12). Although delivery via IM/EP has been well tolerated, EP with ID administration (ID/EP) may improve tolerability as the electrodes do not penetrate as deeply. A recent clinical study demonstrated both cellular and humoral immunogenicity for a DNA vaccine targeting the Ebola virus glycoprotein that was administered via ID/EP (10).

Preclinical studies have demonstrated that the immunogenicity of DNA vaccines can be substantially increased by the use of cytokine adjuvants, such as IL-12 (13–15), although there are limited data demonstrating enhanced immunogenicity in clinical studies. The HVTN 087 study tested HIV-1 multiantigen DNA with increasing doses of pIL-12 administered via an IM/EP approach, followed by recombinant vesicular stomatitis virus (VSV) expressing HIV-1 Gag (16). High-dose pIL-12 increased the magnitude of $CD8^+$ T cell responses after the VSV boost compared with no pIL-12. In contrast, $CD4^+$ T cell responses following the DNA priming were higher in the group that did not receive the pIL-12. Two other HVTN studies tested DNA plus pIL-12 with EP (HVTN 080) and without EP (HVTN 070) (6). In HVTN 070, the addition of pIL-12 in the absence of EP did not provide any benefit. In HVTN 080, however, a study that used EP, $CD4^+$ T cell response rates increased from 44% to 81% after the third vaccination when pIL-12 was included, although this was not statistically different likely because of the small sample size for the group without pIL-12. $CD8^+$ T cell responses were also higher in the group with pIL-12 (52% compared with 33%). Responses were most often detected to Gag or Pol with few responses to Env.

The HVTN 098 trial, reported here, was designed to build on the promising induction of $CD4^+$ and $CD8^+$ T cell immune responses demonstrated in HVTN 080 (6). Constructs were further designed with the following important enhancements. We focused on increasing the magnitude and breadth of Env-specific immune responses in HVTN 098 by designing and including 2 *env* plasmids encoding both RNA- and codon-optimized clade A and C consensus Env immunogens (rather than the single *env* clade B plasmid in HVTN 080). The env plasmids included a substitution of an optimized IgE leader as well as the deletion of their cytoplasmic tail, improving surface expression. This change was added to further improve B cell engagement while maintaining T cell response induction. We also increased the dose of the HIV-1 *env A* and *env C* plasmids relative to the *gag* and *pol* plasmids. The *gag* and *pol* plasmids were modified to have an improved design as they encoded multiclade consensus immunogens versus the *gag* and *pol* plasmids that encoded a primary strain clade B immunogen in HVTN 080. In addition to obtaining safety and tolerability data, HVTN 098 was designed to address whether administration via ID/EP CELLECTRA is equivalent to IM/EP though a fraction of the antigen dose is used, as well as whether inclusion of an IL-12 plasmid as a genetic adjuvant provided additional benefit for induction of cellular and humoral responses.

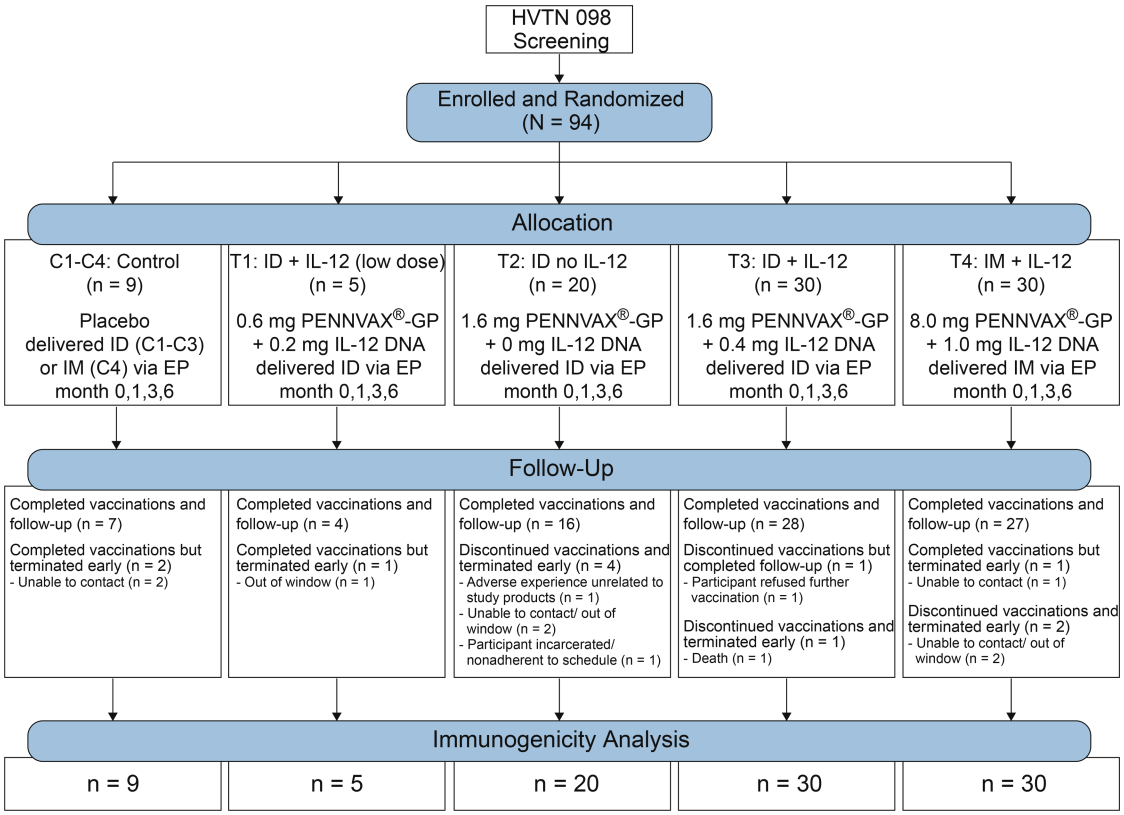


**Figure 1. HVTN 098 CONSORT diagram.**

## Results

*Safety and tolerability*. A total of 94 participants were enrolled; 9 were placebo recipients and 85 were vaccine recipients (Figure 1). Details on the safety, tolerability, and acceptability of these vaccinations by either the ID or IM route with EP are reported separately (Edupuganti et al., unpublished observations). Overall, vaccinations were safe and well tolerated. IM/EP was associated with more pain as compared with ID/EP. Most participants in the ID/EP group had skin lesions, including scars and hypo- or hyperpigmentation, and these resolved within 6 months in half of these participants. The majority (82%) of both IM/EP and ID/EP participants reported that the level of discomfort was acceptable, and all but 2 said they would be willing to undergo EP vaccination for a serious disease.

*T cell responses*. $CD4^+$ and $CD8^+$ T cells expressing IFN-γ or IL-2 were detected postimmunization following ex vivo stimulation with any HIV-1 Env, Gag, and Pol peptide pool (Figure 2A and Supplemental Figure 1; supplemental material available online with this article; https://doi.org/10.1172/jci.insight.137079DS1). $CD4^+$ T cell response rates after the fourth ID and IM immunization were highest in the groups receiving pIL-12 (96%) and significantly lower ($P = 0.002$, $q = 0.04$) for the ID no IL-12 group (56%) compared with the ID + IL-12 group (96%). However, the response magnitudes were not different between the ID groups after either the third or fourth vaccination. Comparing the IM + IL-12 group and the ID + IL-12 group, the response magnitude after the third vaccination was higher for the IM group (median of 0.14% of $CD4^+$ T cells for ID + IL-12 vs. 0.26% for IM + IL-12, $P = 0.006$, $q = 0.07$), but the magnitudes of the 2 groups were not significantly different 2 weeks after the fourth vaccination (0.19% vs. 0.23%). The fourth vaccination did not significantly increase the $CD4^+$ T cell response rate to any HIV antigen over the response to the third vaccination; however, the response magnitude after the fourth vaccination was significantly increased in the ID + IL-12 group as compared with after the third vaccination (from median of 0.14% to 0.19%, $P = 0.004$, $q = 0.03$).

HIV-specific $CD8^+$ T cell response rates were similar across treatment groups, with the highest response rates in the ID + IL-12 group (64% as compared with 56% for ID no IL-12 and 44% for IM + IL-12; $P \geq 0.18$, $q \geq 0.7$) after the fourth immunization. The response magnitudes were generally higher for $CD8^+$ T

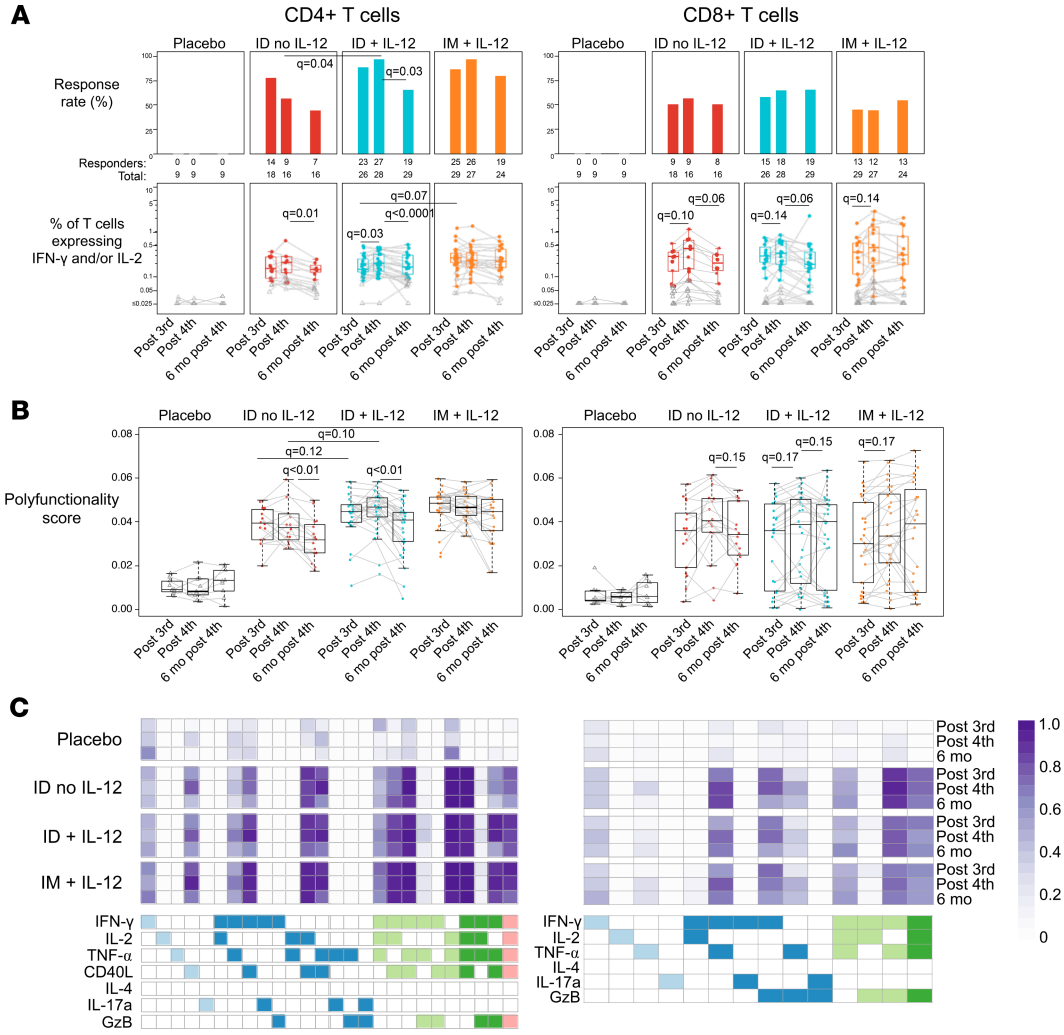


**Figure 2. T cell responses measured by ICS.** (**A**) CD4⁺ and CD8⁺ T cells expressing IFN-γ or IL-2 to any HIV peptide pool. Positive responses are shown in filled circles in color; negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only (the upper and lower quartiles and the median). Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants. Assays were performed 2 weeks after the third and fourth vaccinations and 6 months after the fourth vaccination. (**B**) COMPASS CD4⁺ and CD8⁺ T cell polyfunctionality scores (PFSs) to any HIV Env peptide pool. (**C**) Heatmaps for CD4⁺ and CD8⁺ T cell responses to Any Env peptide pool showing the mean posterior probabilities of antigen-specific responses from COMPASS. Columns correspond to the different subsets of cytokines being considered grouped from left to right by increasing number of functions in the key below the heatmap; a filled box indicates cells in that column are expressing that function, with different colors indicating cells with 1, 2, 3, 4, or 5 functions. Rows correspond to mean across the individual participants in each treatment group at each time point. Each cell shows the probability that the corresponding antigen-specific subset (column) is being expressed in the corresponding treatment group in average (row) and is color-coded ranging from 0 (white) to 1 (dark purple). Positive response rates were compared using the Fisher exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All *P* values are 2 sided. False discovery rate–adjusted *q* values were calculated to account for multiple antigens, multiple time points, or treatment groups.

cells as compared with CD4⁺ T cells (medians ranging from 0.33% to 0.43% for CD8⁺ as compared with 0.19% to 0.23% for CD4⁺ T cells after the fourth vaccination) but were not significantly different between the groups. The fourth vaccination boosted the magnitude of the CD8⁺ T cell responses in all 3 groups ($P \leq 0.05$, $q < 0.14$).

At 6 months after the fourth vaccination, response rates remained high for both CD4⁺ and CD8⁺ T cells, with a significant decrease only for CD4⁺ T cells in the ID + IL-12 group (from 96% to 66%, $P = 0.004$, $q = 0.03$). Remarkably, the CD8⁺ T cell response rates remained similar at 6 months. Response mag-

**Table 1. Response rates for T cells expressing IFN-γ or IL-2 at 2 weeks after the fourth vaccination**

| | T cell | Placebo | ID no IL-12 | ID + IL-12 | IM + IL-12 |
|---|---|---|---|---|---|
| Any HIV | $CD4^+$ | 0%<br>0/9 | 56%<br>9/16 | 96%<br>27/28 | 96%<br>26/27 |
| | $CD8^+$ | 0%<br>0/9 | 56%<br>9/16 | 64%<br>18/28 | 44%<br>12/27 |
| Any Env | $CD4^+$ | 0%<br>0/9 | 50%<br>8/16 | 86%<br>24/28 | 93%<br>25/27 |
| | $CD8^+$ | 0%<br>0/9 | 50%<br>8/16 | 57%<br>16/28 | 44%<br>12/27 |
| Any Gag | $CD4^+$ | 0%<br>0/9 | 31%<br>5/16 | 50%<br>14/28 | 56%<br>15/27 |
| | $CD8^+$ | 0%<br>0/9 | 0%<br>0/16 | 7%<br>2/28 | 0%<br>0/27 |
| Any Pol | $CD4^+$ | 0%<br>0/9 | 0%<br>0/16 | 0%<br>0/28 | 7%<br>2/27 |
| | $CD8^+$ | 0%<br>0/9 | 6%<br>1/16 | 14%<br>4/28 | 7%<br>2/27 |

nitudes for $CD4^+$ and $CD8^+$ T cells were significantly reduced at the later time point for both the ID groups ($q \leq 0.06$) but not for the IM group.

The immunogenicity of the *env*, *gag*, and *pol* components of the vaccine differed. $CD4^+$ T cell response rates were highest for Env (50%, 86%, and 93% for the ID no IL-12, ID + IL-12, and IM + IL-12 groups, respectively), followed by Gag (31%, 50%, and 56% for the ID no IL-12, ID + IL-12, and IM + IL-12 groups, respectively), and nearly absent for Pol (Table 1). $CD8^+$ T cell responses were primarily induced in response to Env (50%, 57%, and 44% for the ID no IL-12, ID + IL-12, and IM + IL-12 groups, respectively), with only a few responding individuals for Gag and Pol.

Polyfunctionality analysis using Combinatorial Polyfunctionality Analysis of Single Cells (COMPASS) demonstrates a highly diverse functional profile among the responding $CD4^+$ T cells to Env (Figure 2, B and C). Among the 7 functional markers examined, many cells expressed combinations including up to 5 of these markers. Only 2 of the markers, IL-4 and IL-17, were not detected among the responding cells. IFN-γ, IL-2, TNF-α, and CD40L were the functions most commonly detected, and granzyme B was coexpressed in some cases. There was a less diverse functional profile for the responding $CD8^+$ T cells as compared with $CD4^+$ T cells, including cells producing IFN-γ, IL-2, TNF-α, and granzyme B or subsets of these markers. The distribution of functional profiles was similar for all treatment groups. The Env-specific $CD4^+$ T cell PFS derived from COMPASS was higher for the ID + IL-12 group compared with the ID no IL-12 group at 2 weeks after the third ($P = 0.04$, $q = 0.12$) and fourth vaccinations ($P = 0.02$, $q = 0.10$). The $CD8^+$ T cell PFS was not significantly different between treatment groups for Env, but for Gag, it was higher for the ID + IL-12 group compared with the IM + IL-12 group at 2 weeks after the fourth vaccination ($P = 0.006$, $q = 0.06$, data not shown), although the magnitudes of the scores were much lower than for Env.

In summary, the cellular immunogenicity revealed similar response rates and magnitudes for both the ID and IM routes (with pIL-12) after the fourth vaccination. At 6 months after the fourth vaccination, these responses were maintained for $CD8^+$ T cells and only modestly decreased for $CD4^+$ T cells. There was a benefit of including pIL-12 with the ID delivery as reflected in increased $CD4^+$ T cell response rate and increased $CD4^+$ T cell polyfunctionality.

*Antibody responses*. Serum IgG antibodies binding Env gp140, gp120, gp41, and Gag p24 proteins were induced in all treatment groups (Figure 3). Antibody response rates to consensus gp140 were uniformly high and similar for all treatment groups (up to 100%) after the fourth vaccination, but the response magnitude was higher for the ID + IL-12 group versus the IM + IL-12 group (median MFIs of 9690 vs. 1967, $P = 0.008$, $q = 0.08$). Response rates and magnitudes were markedly lower for gp120 compared with gp140, with response rates up to 100% for gp140 and only up to 57% for gp120. This difference is likely due to additional epitope specificities (e.g., conformational) present in the gp140 antigens and not in the gp120 antigens. The gp120 response rates were not different between the groups, but response magnitude was higher for

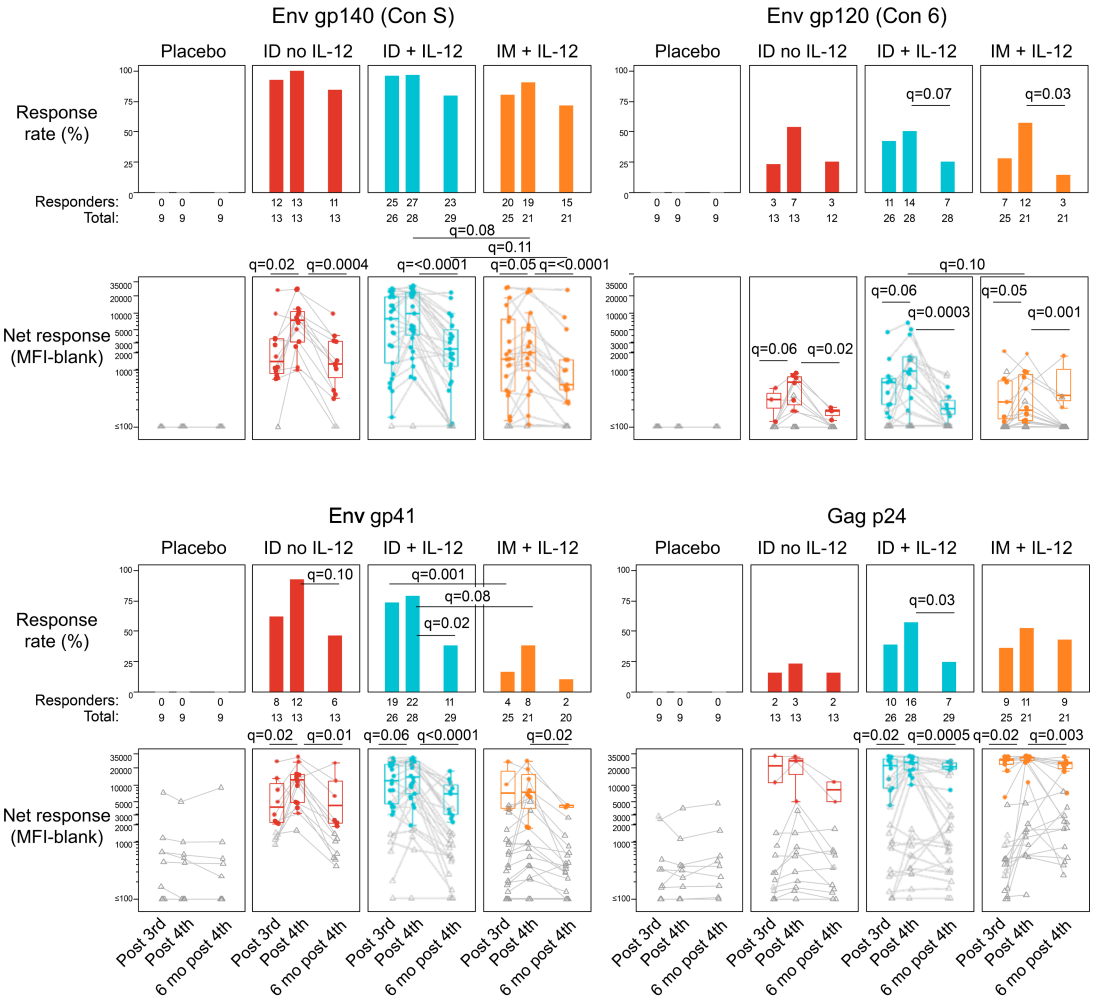


**Figure 3. IgG binding antibody responses, as measured by binding antibody multiplex assay against consensus Env gp140 and gp120 antigens, Env gp41, and Gag p24.** Assays were performed 2 weeks after the third and fourth vaccinations and 6 months after the fourth vaccination. Positive responses are shown in filled circles in color; negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only (the upper and lower quartiles and the median). Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants. Positive response rates were compared using the Fisher exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All *P* values are 2 sided. False discovery rate–adjusted *q* values were calculated to account for multiple antigens, multiple time points, or treatment groups.

the ID + IL-12 group compared with IM + IL-12 2 weeks after the fourth vaccination ($P = 0.03$, $q = 0.10$). For gp41, IgG response rates were high for both ID groups compared with the IM + IL-12 group; the IM + IL-12 response rate was significantly lower than that for the ID + IL-12 group after the third vaccination ($P < 0.0001$, $q = 0.001$) and after the fourth vaccination ($P = 0.007$, $q = 0.08$), although response magnitudes were not different.

There was a boosting effect of the fourth dose, as reflected in significantly higher magnitudes between the third and the fourth vaccinations for gp140 in the ID no IL-12 group ($P = 0.006$, $q = 0.02$) and the IM group ($P = 0.03$, $q = 0.05$), as well as for gp41 in both ID groups ($P \leq 0.04$, $q \leq 0.06$) and for gp120 in all 3 groups ($P \leq 0.04$, $q \leq 0.06$). Response rates 6 months after the fourth vaccination remained high for gp140 but were significantly reduced for gp41 and gp120 among nearly all the treatment groups (*P* from 0.13 to 0.001, *q* from 0.20 to 0.02), suggesting that the most durable antibody responses were to conformational epitopes present in gp140 Env antigens and not in gp120 and gp41. The magnitudes of responses were significantly reduced at 6 months after the fourth vaccination for all 3 Env antigens in all 3 groups ($P \leq 0.02$, $q \leq 0.02$). Response rates for Gag p24 were lower than for the Env antigens, with a maximum of 57% in the ID + IL-12 group after the fourth vaccination. Response rates and magnitudes were not significantly different between groups.

Response rates and magnitudes for gp140 Env proteins representing clades A, B, and C were high and similar to the consensus gp140 responses, even though there was no clade B–specific immunogen administered as part of the vaccine (Supplemental Figure 2). Similar to the consensus, there were no significant differences in response rates between treatment groups. Response magnitudes were lower for the IM + IL-12 group at all 3 time points as compared with the ID + IL-12 group, and the differences were significant for clade B for all these time points ($P \leq 0.03$, $q = 0.1$). The fourth vaccination boosted the magnitude of the response for all treatment groups against clades A and B and for the ID no IL-12 group against clade C ($P \leq 0.04$, $q \leq 0.06$). Response rates against these gp140 antigens remained high at 6 months after the fourth vaccination, with significant drop in the response rate only in the ID + IL-12 group for clade A ($P = 0.02$, $q = 0.07$) and clade C ($P = 0.03$, $q = 0.1$). Response magnitudes at this later time point were significantly reduced for all treatment groups and all gp140 antigens ($P \leq 0.002$, $q \leq 0.002$). Response magnitude after the third vaccination was higher for the ID + IL-12 group versus the ID no IL-12 group for all 3 antigens ($P \leq 0.04$, $q \leq 0.3$), showing a potential benefit of IL-12 in enhancing the antibody response to near maximum with only 3 doses of vaccine.

To examine IgG anti-Envelope binding antibody breadth against gp120 and specifically against clade C envelope proteins, responses to 2 panels of gp120 antigens were examined 2 weeks after the fourth vaccination (Supplemental Figure 3). One panel included 10 antigens from clade C viruses isolated in southern Africa and India. Another panel included 8 antigens from clades A, B, AE, and BC viruses (17). There were no significant differences in the comparison of the area under the curve (AUC) measures between the ID groups with and without pIL-12 or between the ID and IM groups; however, for both antigen panels, the AUC for the ID + IL-12 group was generally greater than that for the other groups, especially at the higher magnitudes. Statistical comparisons performed for each antigen separately revealed 3 clade C antigens with significantly higher response rates for ID compared with IM (96ZM651.D11gp120.avi, CAP210_D11gp120.avi/293F, TV1c8_D11gp120.avi/293F) and 2 antigens with significantly higher magnitude for ID compared with IM (clade AE A244 D11gp120_avi, clade B TT31P.2792_D11gp120.avi/293F, $P \leq 0.04$, $q \leq 0.15$).

The RV144 HIV vaccine trial is the only study to show efficacy against HIV infection (18). Binding IgG antibody levels to the variable regions 1 and 2 (V1V2) of HIV-1 Env were inversely correlated with risk of HIV infection (19). We therefore measured the levels of V1V2-specific antibodies at 2 weeks and 6 months after the fourth vaccination (Figure 4). IgG binding antibodies to V1V2 Env antigens were detected in up to 56% of participants 2 weeks after the fourth vaccination (clade AE shown in Figure 4). Response rates were lower for the IM + IL-12 group compared with the ID + IL-12 group for all 3 antigens tested, but this difference was significant for only the clade AE antigen (14% vs. 56%, $P = 0.006$, $q = 0.04$). Response rates markedly decreased by 6 months after the fourth vaccination in all groups.

Antibodies with the same epitope specificity but of different subclasses can have different functional attributes. In particular there is precedence for the role of IgG3 antibodies in immune-mediated pathogen control, and in fact, we have shown that vaccine-induced Env V1V2 IgG3 correlates with lower HIV-1 infection risk in the RV144 vaccine efficacy trial (20). In our study, response rates were high for IgG3 subclass antibodies binding to consensus gp140 and gp41 (Figure 5) and low for consensus gp120 (Supplemental Figure 4). For both gp140 and gp41, response rates were significantly higher for the ID + IL-12 group compared with the IM + IL-12 group ($P \leq 0.01$, $q \leq 0.05$), although response magnitudes were not different. Response rates and magnitudes dropped significantly at 6 months after the fourth vaccination ($P \leq 0.02$, $q \leq 0.02$). IgG3 responses to gp120 and Gag p24 were detected in only a few individuals (Supplemental Figure 4). IgG3 responses to the V1V2 antigens were very low, with the highest response rate (18%) for the ID + IL-12 group after the fourth vaccination and nearly all responses not detectable by 6 months.

The functionality of the antibody responses was also determined by exploring the serum neutralization capacity, antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cellular cytotoxicity (ADCC). Neutralizing antibodies were mainly detected only to the tier 1A viral isolate, MW965.26, with response rates as high as 75% for the ID + IL-12 group 2 weeks after the fourth vaccination (Figure 6A). Only one individual in the ID + IL-12 group had a response to a tier 1B virus (clade C DU156.12, titer 20.7) and to 3 tier 2 viruses (clade C 25710-2.43, titer 20.9; clade C Cell76_A3, titer 22.7; and clade G X1632-S2-B10, titer 12.4). The ADCP response rate to the ConS gp140 antigen after the fourth vaccination was significantly higher in the ID + IL-12 group than in the IM + IL-12 group (82% vs. 50%, $P = 0.015$), although the magnitude among responders did not differ between the groups (Figure 6B). ADCC as measured using target cells infected with a clade C infectious molecular clone revealed few responses

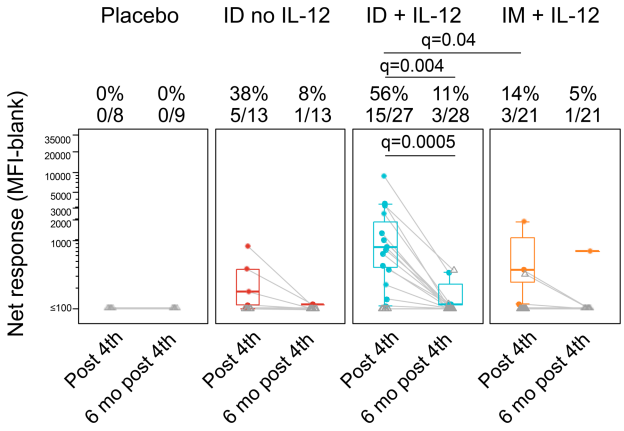


**Figure 4. IgG binding antibody responses as measured by binding antibody multiplex assay against clade AE (A244) Env V1V2.** Positive responses are shown in filled circles in color; negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only (the upper and lower quartiles and the median). Response rates are listed above each graph along with numbers of positive responders and total participants. Positive response rates were compared using the Fisher exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All *P* values are 2 sided. False discovery rate–adjusted *q* values were calculated to account for multiple antigens, multiple time points, or treatment groups.

(Figure 6C). In the ID + IL-12 group, responses were detected in 8% and 12% of participants after the third and fourth vaccinations, respectively. Two individuals had a response after the third vaccination, but no responses were detectable after the fourth vaccination. Results were similar for the assay using gp120-coated target cells with detectable responses in 16% of participants after both the third and fourth vaccinations for the ID + IL-12 group, and for the IM + IL-12 group, one individual had a response; however, this response was also present at baseline (Supplemental Figure 5).

*Correlations between immunogenicity measures*. Correlation analyses were performed across the main immune measures for IgG binding antibodies, T cell intracellular cytokine staining (ICS), and ADCP. ADCC was not included because few responses were detected. Scatter plots are shown in Supplemental Figure 6, including Spearman's correlation coefficients (*r*) displayed on a gradient color scale. The most significant correlations with the largest correlation coefficients are between different measures within the same class of response, such as different antibody measures or different $CD4^+$ or $CD8^+$ T cell measures. There was a relatively poor correlation between $CD4^+$ and $CD8^+$ T cells (highest *r* value is for $CD4^+$ vs. $CD8^+$ for any HIV protein, $r = 0.44$). There was also not a strong correlation between the antibody measures and the T cell measures (highest *r* value of 0.51). ADCP correlated well with IgG binding antibody responses, especially for Env gp120 (Con 6) and Env gp140 (Con S), with *r* values of 0.84 and 0.87, respectively. Thus, the antibody and T cell measures appear to be relatively independent and provide unique information regarding immunogenicity, although the ADCP results may be predicted by the binding antibody data.

## Discussion

The HVTN 098 study demonstrated excellent cellular and humoral immunogenicity for DNA vaccination delivered via CELLECTRA EP. Although prior DNA vaccination studies have induced $CD4^+$ T cells (4), the concomitant induction of HIV-specific $CD8^+$ T cells in HVTN 098 in more than half of the vaccine recipients has been observed infrequently as in our prior study that administered DNA via IM and with pIL-12 and EP, HVTN 080 (6). Furthermore, the generation of Env-specific antibodies in nearly all vaccine recipients has not been seen in previous HIV vaccine studies using DNA to our knowledge. The ID route of administration was equivalent or superior to administration by IM even at one-fifth the IM dose, when both were administered via EP and with pIL-12.

The effect of pIL-12 was evaluated only in the groups that received ID/EP (with and without pIL-12). Although the effect of pIL-12 was not as marked as the comparisons between route of administration, pIL-12 significantly improved the $CD4^+$ T cell response rate and accelerated the antibody response by achieving

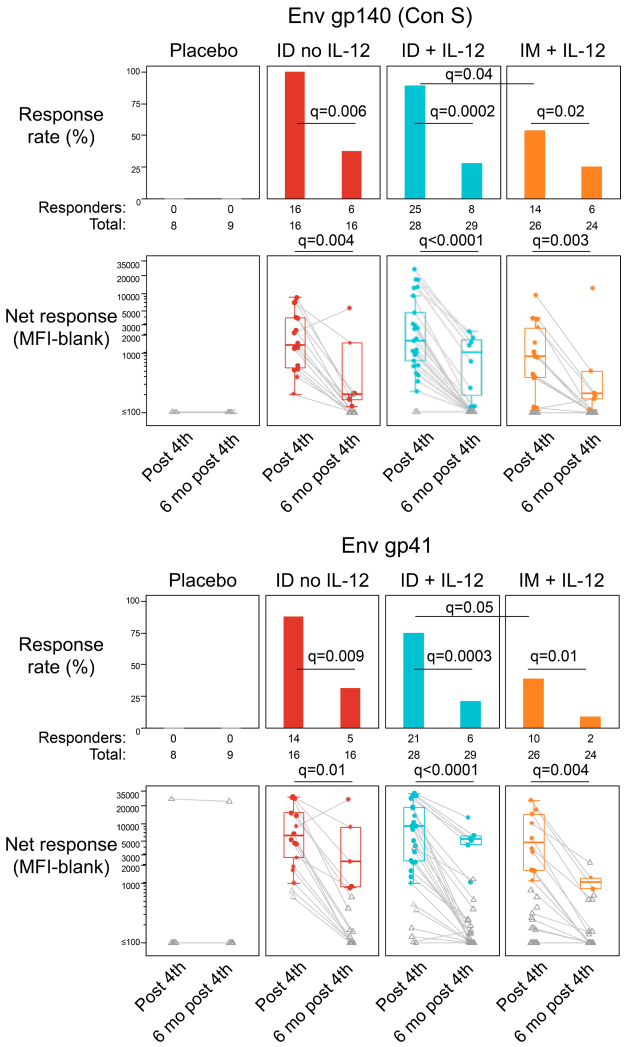


**Figure 5. IgG3 binding antibody responses as measured by binding antibody multiplex assay against consensus Env gp140 and Env gp41.** Positive responses are shown in filled circles in color; negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only (the upper and lower quartiles and the median). Bar plots show response rates. Numbers below the bars indicate numbers of positive responders and total participants. Positive response rates were compared using the Fisher exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All *P* values are 2 sided. False discovery rate–adjusted *q* values were calculated to account for multiple antigens, multiple time points, or treatment groups.

nearly maximal responses by the third vaccination, rather than the fourth vaccination. The increase in T cell response is consistent with our prior study (HVTN 080) also showing increase in $CD4^+$ T cell responses (although not statistically significant) when pIL-12 was included when the vaccine was administered EP (6). However, another prior study (HVTN 087) that used DNA manufactured by a different company revealed the opposite effect, i.e., decreased $CD4^+$ T cell responses when pIL-12 was included, perhaps suggesting that the specific design of the DNA plasmids is a critical factor for any beneficial effect of pIL-12 (16). Any beneficial effects on antibody response could not be evaluated because of the lack of induction of these responses in the prior studies. In light of the limited benefit observed in our current study and somewhat conflicting results in prior studies, further testing is needed to definitively document utility of pIL-12.

The remarkable binding antibody response to Env in a DNA-only vaccine regime is unique to our current study (HVTN 098) and was not observed in our prior study (HVTN 080) that administered lower dose *env* DNA via IM/EP with pIL-12 (6). In that prior study, the $CD4^+$ and $CD8^+$ T cell responses to Gag were similar to those in HVTN 098, but there was minimal cellular or antibody response to Env. For that reason,

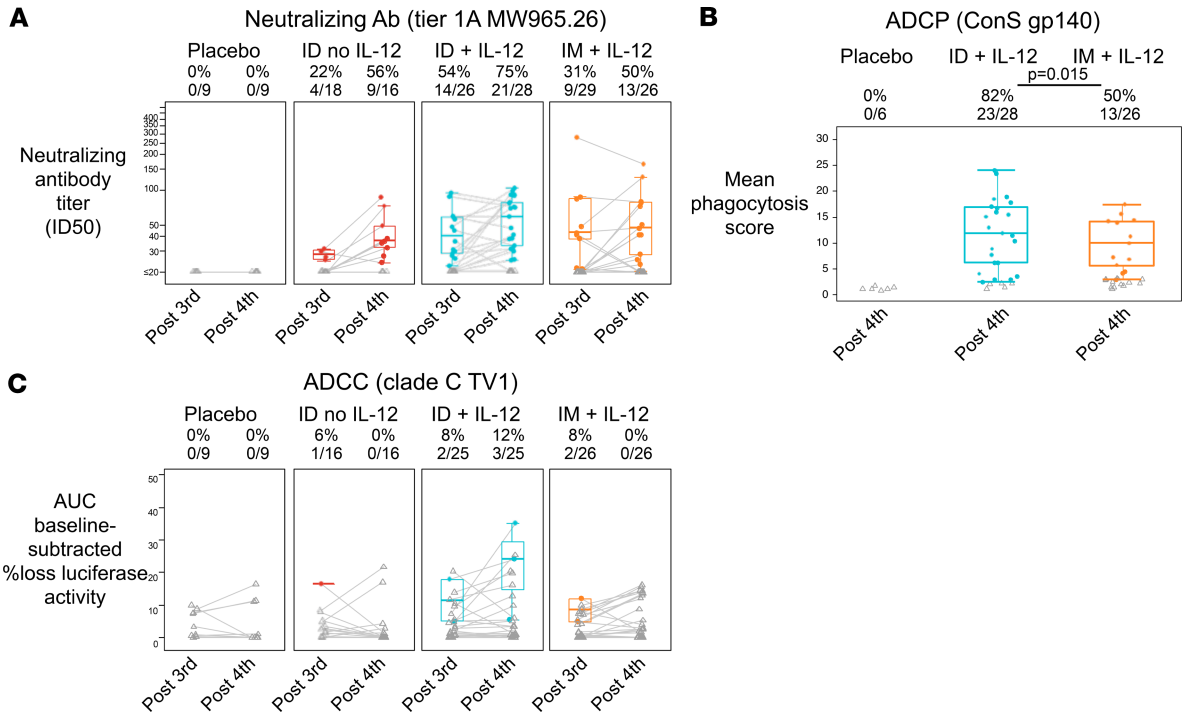


**Figure 6. Antibody functional responses.** (**A**) Neutralizing antibody responses to tier 1A Env-pseudotyped virus MW965.26 as determined by the TZM-bl neutralization assay 2 weeks after the third and fourth vaccinations. (**B**) ADCP 2 weeks after the fourth vaccination. (**C**) ADCC as determined by the infected cell target assay for a clade C viral isolate (TV1). For all plots, positive responses are shown in filled circles in color; negative responses are shown in open gray triangles. Box plots represent the distribution for the positive responders only (the upper and lower quartiles and the median). Response rates are listed above each graph along with numbers of positive responders and total participants. Response magnitudes among positive responders were compared using the Wilcoxon signed rank test; *P* values are 2 sided.

the novel *env* vaccine including 2 plasmids encoding clade A and C consensus immunogens, respectively, was designed and evaluated in this study. The *env* vaccine dose was also markedly increased from 1 mg to 6 mg in the IM group (3 mg for *env A* and 3 mg for *env C*). Since the IM + IL-12 group in HVTN 098 is otherwise comparable to the prior study (i.e., same EP device), the enhanced humoral immunogenicity observed must be due to the *env* design or increased dose, or both. Because of the recognized importance of inducing antibody responses for any candidate HIV vaccine, whether neutralizing or nonneutralizing, our study demonstrates the potential of DNA to serve as a component of a prime-boost regimen or even as a single-agent vaccine if functionality of induced antibodies can be further improved, such as for ADCC activity.

We explored the antibody profiles induced to evaluate responses that may be protective based on prior immune correlate studies. Similar to other HIV vaccine candidates tested to date, we detected neutralizing antibodies only against tier 1 viruses and little or no activity against the more difficult-to-neutralize tier 2 viruses. Antibodies against Env V1V2 and certain Fc-mediated antibody effector functions were shown to be a protective correlate against HIV acquisition in the RV144 vaccine trial (19–24). Though initially not a focus when the Env plasmids were designed, in HVTN 098, similar to RV144, V1V2-specific antibodies were induced in up to 56% of the ID + IL-12 vaccine recipients, but the durability of these responses by 6 months following the last vaccination was poor and requires improvement. ADCC was detected infrequently in vaccine recipients in this study, and the magnitudes were in a similar range of what we detected in other clinical trials. Therefore, future regimens could be modified to improve antibody functionality and binding specificity, perhaps by DNA design or by including a protein or viral vector boost to the DNA. ADCP was detected in the majority of the ID + IL-12 and also in the IM + IL-12 vaccine recipients. Although the potential clinical benefit of this function cannot be determined in this phase I study, ADCP was one of the antibody Fc effector functions associated with immune correlates in the RV144 trial and recently shown by our group to be a correlate of decreased HIV-1 risk in HVTN 505 (25). Further studies are needed with larger sample sizes to determine whether ID delivery improved antibody Fc effector functions over IM delivery.

Unlike the antibody responses, both the $CD4^+$ and $CD8^+$ T cell responses were maintained or slightly reduced 6 months after final vaccination. Although many of the antibody measurements were improved with ID delivery compared with IM, T cell responses were similar between both routes. Comparing to other HIV vaccine regimens, the $CD4^+$ T cell response rates in RV144 were under 50% with very few $CD8^+$ responses detected (19), and for adenovirus-based (Ad-based) vaccines the $CD8^+$ T cell responses were dominant, such as for the Ad5 vaccine in the Step Study ($CD4^+$ T cells were induced in 41% and $CD8^+$ T cells were induced in 73% of vaccine recipients) (26) and for the DNA/Ad5 prime-boost used in HVTN 505 (62% and 64% $CD4^+$ and $CD8^+$ T cell responses, respectively) (27). Thus, $CD4^+$ T cell response rates were the highest for the DNA-only vaccines we tested, but $CD8^+$ T cell response rates were somewhat lower than the Ad5-containing regimens. Both the $CD4^+$ and $CD8^+$ T cells included high proportions of polyfunctional T cells, although cells expressing IL-4 were rarely detected. Since polyfunctional $CD4^+$ T cells expressing IL-4 were a protective correlate in RV144, increased induction of these cells may be beneficial and may contribute to a more durable antibody response (28). The cellular immunogenicity differed markedly between Env, Gag, and Pol, with the dominant $CD4^+$ and $CD8^+$ T cell responses to Env. Gag-induced $CD4^+$ T cell responses in about half of vaccine recipients, but few, if any, $CD8^+$ T cell responses. Pol was poorly immunogenic in contrast to the high response in our prior HVTN 080 study (79% response rate for $CD4^+$ T cells and 58% for $CD8^+$ T cells) (6). The lower responses for Gag and Pol could simply be a dose effect since the combined dose for the *env* plasmids was 6 times the dose for the *gag* and *pol* plasmids or due to the design of the *pol* plasmid. Specifically, the removal of integrase from the *pol* vaccine in HVTN 098 for a potential safety concern may result in some epitope loss, thus leading to lower Pol-specific cellular responses. Antigenic competition is also a possibility considering the dominant Env response (29). Since the ID route is dose sparing, it would be feasible to increase the *gag* and *pol* dose in future studies. Further optimization of the design of the *gag* and *pol* plasmids should also be considered.

Overall, HIV DNA vaccines have previously demonstrated limited immunogenicity, lacking $CD8^+$ T cell and antibody induction, and were initially considered only as one component of a combination vaccine regimen. In contrast, our study demonstrates that DNA alone (with adaptive EP and pIL-12) can induce broad immunity across both $CD4^+$ and $CD8^+$ T cells as well as antibody production. The ID/EP route is equivalent or superior to the IM/EP route, and the ID/EP route was shown to be less painful and thus more tolerable in this study (Edupuganti, unpublished observations). Given that the ID route is dose sparing, this increases the feasibility of using higher doses (although relatively low compared with what would be used in IM). Higher antigen doses may be necessary to improve durability of antibody responses and increase ADCC function in order to enhance potential efficacy, and further optimization of the plasmids and/or combination vaccination, including a boost using protein and/or viral vectors, could possibly achieve this goal. DNA has a number of logistical advantages in terms of design, manufacture, and production as compared with other vaccine approaches for genomic delivery, such as viral vectors, and as compared with protein/adjuvant approaches. Given the broad cellular and humoral immunogenicity now demonstrated, DNA has potential for use as a single-agent vaccine modality in HIV as well as for a rapid response to emerging infections, such as coronavirus, Zika, Ebola, and new strains of influenza.

## Methods

*Study design*. The study schema is included as Supplemental Table 1 and Supplemental Methods. Participants were healthy, HIV-uninfected adults, ages 18–55 years. The study schedule consisted of 4 groups each receiving 4 vaccinations at months 0, 1, 3, and 6 administered via EP. Group 1 was a pilot safety group testing low-dose PENNVAX-GP with pIL-12 DNA ($n = 5$) or placebo ($n = 1$) delivered via ID/EP. Due to the small size of this group, immunogenicity data for this group are not included in the analyses presented here. Groups 2 and 3 received vaccinations via ID/EP: group 2 without pIL-12 (referred to as "ID no IL-12," $n = 20$) or placebo ($n = 2$) and group 3 with pIL-12 (referred to as "ID + IL-12," $n = 30$) or placebo ($n = 3$). Group 4 received vaccinations via IM/EP, with pIL-12 (referred to as "IM + IL-12," $n = 30$), or placebo ($n = 3$). For this IM group, the doses of pIL-12 and the *gag* and *pol* plasmids were 1 mg each, and the dose of the clade A and C *env* plasmids was 3 mg each. For the ID administration in groups 2 and 3, one-fifth the IM dose of all HIV plasmids was used (0.2 mg each for *gag* and *pol*, and 0.6 mg for each *env* A and *env* C), and half the vaccine dose was administered at each of 2 injection sites (opposite deltoids). In addition, group 3 received 0.4 mg of pIL-12, split and administered across 2 injection sites.

The PENNVAX-GP *env* consensus clade A and clade C plasmids included an optimized IgE leader sequence, designed to express and traffic protein to the surface of transfected cells. The *gag* and *pol* plasmids were consensus multiclade (A, B, C, and D). IL-12 DNA consisted of a single plasmid containing a dual promoter system for expression of both the IL-12 p35 and p40 genes necessary for the production of the active heterodimeric IL-12 protein. The placebo consisted of sterile water. Two electroporation devices were used; the CELLECTRA 3P EP system was used for ID delivery, and the CELLECTRA 5P EP system was used for IM delivery (30, 31).

*Sample processing*. Serum for humoral assays was obtained from serum-separating tubes and frozen at –80°C. Peripheral blood mononuclear cells (PBMCs) for cellular assays were isolated and cryopreserved from heparin-anticoagulated whole blood within 6 hours of venipuncture, as described previously (32). Samples were collected at 2 weeks after the third vaccination, as well as 2 weeks and 6 months after the fourth vaccination for the immunogenicity assessments described below.

*ICS assay*. Flow cytometry was used to examine HIV-1-specific $CD4^+$ and $CD8^+$ T cell responses using a validated ICS assay. The assay was similar to a published report and the details of the staining panel are included in Supplemental Table 2 (33). The peptide pools evaluated were global potential T cell epitopes pooled by frequency with 3 pools for Env, 1 pool for Gag, and 2 pools for Pol (34). Previously cryopreserved PBMCs were stimulated for 6 hours with the 6 synthetic peptide pools. As a negative control, cells were not stimulated. As a positive control, cells were stimulated with a polyclonal stimulant, staphylococcal enterotoxin B. Two replicates were tested for the negative control. $CD4^+$ and $CD8^+$ T cells expressing IFN-γ or IL-2 were the primary immunogenicity endpoints. Since the multiple pools for each HIV protein can include variant peptides covering the same protein region, the overall response for Env and Pol (referred to as Any Env and Any Pol) was represented as the maximum of each of the pools for each protein (see Supplemental Table 3). The overall response to "Any HIV protein" was defined as the sum of the response to Any Env, Any Pol, and Gag.

Several criteria were used to determine whether assay data were acceptable and could be statistically analyzed. The blood draw date must have been within the allowable visit window as determined by the protocol. On the second day after sample thawing, the viability must have been 66% or greater. If not, the sample for that specimen at that time point was retested. If upon retesting, the viability remained below this threshold, the ICS assay was not performed, and no data were reported to the statistical center for the time point. For the negative control acceptance criteria, if the average cytokine response for the negative control wells was above 0.1% for either the $CD4^+$ or $CD8^+$ T cells, then the sample was retested, and the response for the retested sample was analyzed. Furthermore, the total number of $CD4^+$ and $CD8^+$ T cells must have exceeded certain thresholds. If the number of $CD4^+$ or $CD8^+$ T cells was less than 5000 for any of the HIV-1 peptide pools or one of the negative control replicates for a particular sample, data for that stimulation were filtered. If both negative control replicates were fewer than 5000 cells, the sample was retested. If upon retesting, one negative control replicate was fewer than 5000, the negative control replicate with more than 5000 cells was used. If both negative control replicates from the retest for a T cell subset were fewer than 5000, then data for the T cell subset were not included in the analysis.

Positivity was determined using a 1-sided Fisher exact test (35). A multiplicity adjustment was made across all tested HIV antigens using the discrete Bonferroni's adjustment method. If the adjusted *P* value for an antigen was no more than $10^5$, the response to the antigen for the T cell subset was considered positive. If any peptide pool was positive for a T cell subset, then the overall response for that T cell subset was considered positive.

*T cell polyfunctionality analyses*. COMPASS is a computational framework for unbiased polyfunctionality analysis of antigen-specific T cell subsets (28). Participant-level responses were quantified by 2 summary statistics: (a) the functionality score is defined as the estimated proportion of antigen-specific subsets detected among all possible T cell subsets; (b) the PFS is similar but weighs the different subsets by their degree of functionality. For this analysis, expression of IFN-γ, IL-2, IL-4, IL-17a, TNF-α, CD40L, and granzyme B was included for the $CD4^+$ T cells, and the same markers, except for CD40L, were included for $CD8^+$ T cells. Scores were compared between treatment groups using the Wilcoxon rank sum test. For Env-specific scores, overall responses across pools were computed by summing the cell counts, both for the number of expressing cells and the total cell count (numerator and denominator), in fitting a COMPASS model. A heatmap for each stimulation and T cell subset shows the mean posterior probabilities of antigen-specific responses from COMPASS.

*Binding antibody multiplex assays*. Serum HIV-1–specific IgG and IgG3 responses were measured on a Bio-Plex instrument (Bio-Rad) using a standardized custom HIV-1 Luminex assay (20, 36) that uses gp120 and gp140 proteins and V1V2 antigens described previously (17, 37). Details of the antigens included are listed in

Supplemental Table 3. The positive control was purified polyclonal IgG from HIV subjects (HIVIG) using a 10-point standard curve (4PL fit). The negative controls were HIV-1–seronegative human sera and blank beads.

Several criteria were used to determine whether data from an assay were acceptable and could be statistically analyzed. First, the blood draw date must have been within the allowable visit window as determined by the protocol. Second, if the blank bead negative control exceeded 5000 MFI, the sample was repeated. If the repeat value exceeded 5000 MFI, the sample was excluded from analysis because of high background. Samples from postenrollment visits were declared to have positive responses if they met 3 conditions: (a) the MFI – blank values were ≥ antigen-specific cutoff at the 1:50 dilution level (based on the 95th percentile of the baseline visit serum samples and at least 100 MFI), (b) the MFI – blank values were > 3 times the baseline (day 0) MFI – blank values, and (c) the MFI values were > 3 times the baseline MFI values. The MFI – blank responses (MFI*) at the 1:50 dilution level was used to summarize the magnitude at a given time point. For the expanded gp120 panels shown in Supplemental Figure 3, simultaneous evaluation of magnitude and breadth was used as previously described (38).

*HIV-1–specific neutralizing antibody assays*. Neutralizing antibodies against HIV-1 were measured as a function of reductions in Tat-regulated luciferase reporter gene expression in TZM-bl cells (39). The assay measured neutralization titers against Env-pseudotyped viruses that exhibit a tier 1A, tier 1B, or tier 2 neutralization phenotype (see Supplemental Table 3 for additional details on the antigens used). Response to a virus/isolate in the TZM-bl assay was considered positive if the neutralization titer was above a prespecified cutoff (one-half the lowest dilution tested). A titer was defined as the serum dilution that reduces relative luminescence units (RLUs) by 50% compared with the RLUs in virus control wells (cells + virus only) after subtraction of background RLUs (cells only). The prespecified cutoff was 10 for TZM-bl cells.

*ADCC*. ADCC-mediated antibody responses were measured by 2 assays. The luciferase assay used clade A and clade C infectious molecular clone–infected (IMC-infected) target cells (see Supplemental Table 3 for details on the antigens used). Participant sera in addition to control sera were incubated with IMC-infected cells and tested in a 96-well plate. ADCC was detected through the use of Viviren luminescence. One positive control in duplicate and 1 standardized negative control in duplicate were used per plate. The readout is reduction in RLUs, referred to as percentage specific killing (40, 41). For each sample, percentage of specific killing was measured in 2 wells at 6 dilution levels: 1:50, 1:250, 1:1250, 1:6250, 1:31,250, and 1:156,250. For each subject at each time point, percentage loss of luciferase activity was calculated relative to control wells for each experimental well and averaged over wells within subject, time point, and dilution. The analyses in the data focused on the following readouts based on the baseline-subtracted percent loss of activity: (a) peak percentage loss of luciferase activity defined as the maximum activity across the 6 dilution levels ("peak activity") and (b) nonparametric area under the percentage loss of activity versus $\log_{10}$(dilution) curve ("AUC"), calculated using the trapezoidal rule, with activity less than 0% set to 0%. A positive response was defined as peak baseline-subtracted activity greater than or equal to 10% and baseline-subtracted activity greater than or equal to 10% for either the 1:50 or 1:250 dilution.

A second ADCC assay, the GranToxiLux (GTL) assay, tested against clade A, B, and C Env using gp120-coated cells (percentage granzyme B readout) (see Supplemental Table 3 for details on the antigens used) (42). Participant sera were incubated with effector cells and gp120-coated target cells, and ADCC was quantified as net percentage granzyme B activity, which is the percentage of target cells positive for GTL detected by flow cytometry. For each participant at each time point, percentage granzyme B activity was measured at 6 dilution levels: 1:50, 1:250, 1:1250, 1:6250, 1:31,250, and 1:156,250 for each antigen. The analyses in the data focused on the following readouts: (a) peak net percentage granzyme B activity defined as the maximum activity across the 6 dilution levels ("peak activity"), and (b) nonparametric area under the net percentage granzyme B activity versus $\log_{10}$ (dilution) curve ("AUC"), calculated using the trapezoidal rule. Peak activity less than 0% was set to 0%. A positive response was defined as peak activity greater than or equal to 8%.

*ADCP*. To assess the ability of vaccine-induced antibodies to engage Fc receptors, ADCP was measured in serum specimens obtained at baseline and 2 weeks after the fourth vaccination in the ID + IL-12 and IM + IL-12 treatment groups, the 2 groups with the better immunogenicity in the other assays, using methods previously described (43). Briefly, neutravidin fluorescent beads (Thermo Fisher Scientific) were coated with the ConS gp140 biotinylated HIV-1 antigen, then incubated with monoclonal antibodies (positive control CH31 and negative control CH65, Catalent) or diluted participant serum (1:50 dilution). For the ADCP assay, THP-1 cells (ATCC), pretreated with anti-human CD4 (BioLegend) to reduce CD4-Env–mediated virus internalization, were incubated with the antibody/bead mixture, then paraformaldehyde-fixed before

analysis by flow cytometry. A phagocytic score was determined based on the ratio of experimental sample to the no-antibody control. The mean phagocytosis score was defined as: (% bead positive for participant × MFI bead positive for participant) / (% bead positive for the no-antibody control × MFI bead positive for the no-antibody control). Samples were assayed in duplicate and the average scores of the replicates were reported. This ADCP assay has been standardized but has not been qualified or validated.

Samples from postbaseline visits were declared to have positive responses if both of the following criteria were met: mean phagocytosis score ≥ 1.6 and mean phagocytosis score ≥ 3 times the mean phagocytosis score at baseline. The positivity threshold, 1.6, is defined separately for each protocol and antigen as the 95th percentile of the mean phagocytosis score of 60 baseline samples.

*Statistics*. Positive response rates were compared using the Fisher exact test for unpaired data (between treatment groups) and the McNemar test for paired data (between visits). Response magnitudes among positive responders were compared using the Wilcoxon rank sum test for unpaired data and the Wilcoxon signed rank test for paired data. All $P$ values are 2 sided. False discovery rate–adjusted $q$ values were calculated to account for multiple antigens, multiple time points, or treatment groups (when appropriate) (44). A $q \leq 0.2$ with $P < 0.05$ was considered significant for the purpose of hypothesis generating. The $q$ values (i.e., adjusted $P$ values) are listed in the figures for all comparisons for which the unadjusted $P$ value was less than 0.05.

*Study approval*. The study was reviewed and approved by the Institutional Review Boards at the Fred Hutchinson Cancer Research Center, University of Rochester, Vanderbilt University, and Emory University. Written informed consent was received from participants prior to inclusion in the study.

## Author contributions

SCDR, SE, ME, YH, ES, LP, SAK, MP, ASK, NYS, MLB, DBW, LC, and MJM contributed to the concept, design, and implementation of this clinical trial. SAK, MCK, JM, and MJM contributed to the clinical conduct of this trial. MCW, JY, MPM, ASK, JDB, LH, SW, NYS, MLB, and DBW contributed to the design and preliminary evaluation of the study products. SCDR, GF, GDT, DCM, and MJM contributed to the immunogenicity testing. YH, XH, and YL contributed to the statistical analysis. All authors contributed to the writing and review of the manuscript.

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