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Protein Dose-Sparing Effect of $AS01_B$ Adjuvant in a Randomized Preventive HIV Vaccine Trial of ALVAC-HIV (vCP2438) and Adjuvanted Bivalent Subtype C gp120

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Background. HVTN 120 is a phase 1/2a randomized double-blind placebo-controlled human immunodeficiency virus (HIV) vaccine trial that evaluated the safety and immunogenicity of ALVAC-HIV (vCP2438) and MF59- or $AS01_B$-adjuvanted bivalent subtype C gp120 Env protein at 2 dose levels in healthy HIV-uninfected adults.

Methods. Participants received ALVAC-HIV (vCP2438) alone or placebo at months 0 and 1. At months 3 and 6, participants received either placebo, ALVAC-HIV (vCP2438) with 200 μg of bivalent subtype C gp120 adjuvanted with MF59 or $AS01_B$, or ALVAC-HIV (vCP2438) with 40 μg of bivalent subtype C gp120 adjuvanted with $AS01_B$. Primary outcomes were safety and immune responses.

Results. We enrolled 160 participants, 55% women, 18–40 years old (median age 24 years) of whom 150 received vaccine and 10 placebo. Vaccines were generally safe and well tolerated. At months 6.5 and 12, $CD4^+$ T-cell response rates and magnitudes were higher in the $AS01_B$-adjuvanted groups than in the MF59-adjuvanted group. At month 12, HIV-specific Env-gp120 binding antibody response magnitudes in the 40 μg gp120/$AS01_B$ group were higher than in either of the 200 μg gp120 groups.

Conclusions. The 40 μg dose gp120/$AS01_B$ regimen elicited the highest $CD4^+$ T-cell and binding antibody responses.

Clinical Trials Registration. NCT03122223.

Keywords. HIV; vaccine; dose; adjuvant.

In 2009, the RV144 trial concluded in a modified intention to treat analysis that there was modest efficacy of a preventive human immunodeficiency virus (HIV) vaccine regimen (vaccine efficacy 31.2%, 95% confidence interval [CI], 1.1–52.1; $P = .04$). The regimen comprised a canarypox vector vaccine plus an adjuvanted protein vaccine: ALVAC-HIV (vCP1521) plus subtype B/E glycoprotein 120 (gp120) Env protein (AIDSVAX B/E) formulated with aluminum hydroxide adjuvant [1]. Among the correlates of protection were binding antibodies to V1V2 antigens [2]. Thereafter, vaccine candidates were manufactured to match more closely the world's most prevalent HIV subtype, subtype C. One of those vaccine concepts—a canarypox vector vaccine with subtype C inserts and a subtype

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C protein vaccine adjuvanted with MF59—was tested in the HVTN 702 trial. In 2020, this trial did not demonstrate efficacy in the South African population where HIV subtype C dominates [3].

In parallel, the post-RV144 subtype C vaccine research program investigated the immunological profiles elicited by various combinations and doses of subtype C vaccine candidates and different adjuvants to optimize the magnitude and duration of immune responses [4]. RV135 evaluated immune responses to a regimen identical to the one used in RV144 but randomized participants to a higher or lower dose of the Env protein vaccine: 200 μg or 600 μg total (100 μg or 300 μg each of MN and A244 proteins). When compared to participants who received the higher Env protein dose, those who received the lower dose had lower anti-MN and anti-A244 antibody response rates, lower geometric mean titers of antibodies to MN and A244, and lower neutralization antibody response rates [5].

Adjuvants are known modifiers of the potency, quality, and longevity of antigen-specific immune responses [6]. The MF59 adjuvant, which was used in the HVTN 702 trial, is an oil-in-water emulsion licensed for influenza vaccines in certain countries. MF59 has demonstrated recruitment of antigen-presenting cells in preclinical models; upregulation of cytokines, chemokines, and receptors [7]; improvement of antibody affinity maturation epitope breadth and binding affinity [8]; and balancing of the T-helper 1 and T-helper 2 responses and proliferation of T cells [9].

$AS01_B$ belongs to a liposome-based class of adjuvants and contains 2 immunostimulants. The first is 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL), a nontoxic derivative of the lipopolysaccharide from *Salmonella minnesota*, a Toll-like receptor 4 (TLR4) agonist, and a stimulant of nuclear factor-κB (NF-κB) transcriptional activity and subsequent cytokine production [10]. MPL directly activates antigen-presenting cells such as dendritic cells to produce cytokines and express elevated levels of costimulatory molecules [11–13]. The second is QS-21, a natural saponin molecule extracted from the bark of the South American tree *Quillaja saponaria* Molina [14–16], which elicits high antigen-specific antibody responses in humans [16, 17]. $AS01_B$ is an MPL, QS-21, and liposome based adjuvant system (50 mg MPL and 50 μg QS-21) that is also part of the licensed herpes zoster vaccine (Shingrix; GSK) and $AS01_E$ is also part of the licensed RSV vaccine (Arexvy; GSK). $AS01_E$ (containing 25 μg MPL, 25 μg QS-21, and liposome) is part of the candidate M72 tuberculosis vaccine that demonstrated partial efficacy [18] and is part of the RTS,S malaria vaccine given to children in Kenya, Malawi, and Ghana [19].

Currently, no studies have evaluated immune responses with varying doses of Env proteins in the context of ALVAC prime-boost and protein adjuvanted with MF59 or $AS01_B$. Here we describe the outcome of HIV Vaccine Trials Network 120 (HVTN 120), which compared the human safety profiles and immune responses to the vaccine products that did not demonstrate efficacy in HVTN 702—ALVAC-HIV (vCP2438) and MF59-adjuvanted bivalent subtype C gp120—with 2 corresponding regimens containing the $AS01_B$ adjuvant, one at the same protein dose (200 μg) as HVTN 702, the other at a lower dose (40 μg).

METHODS

Study Design

This was a multicenter, randomized, placebo-controlled, double-blinded clinical trial conducted from February 2018 to January 2020 at 9 sites in the United States and 1 site each in Tanzania, Zambia, and Zimbabwe (Clinical Trials Registration at https://clinicaltrials.gov/ct2/show/NCT03122223).

Study Population

Eligible participants were healthy adults aged 18 to 40 years who provided written informed consent, demonstrated understanding of the study, were deemed low risk for HIV acquisition, agreed not to enroll in other studies of investigational products, had normal hematology and chemistry panels, and were not infected with HIV-1, HIV-2, hepatitis B, nor hepatitis C. Pregnant women were excluded.

Study Products

Four products were administered in various combinations, all required intramuscular injection. First, ALVAC-HIV (vCP2438) expressed the gene products 96ZM651 gp120 (subtype C strain) linked to the sequences encoding the HIV-1 transmembrane anchor sequence of gp41 (28 amino acids subtype B LAI strain) and gag and pro (subtype B LAI strain). Second, bivalent subtype C gp120, combining subtype C TV1.C gp120 Env and subtype C 1086.C gp120 Env, each at a dose of 100 μg, mixed with MF59 adjuvant (200 μg Pr + MF59). Third, bivalent subtype C gp120, where subtype C TV1.C gp120 Env and subtype C 1086.C gp120 Env are each included at a dose of 20 μg or 100 μg, mixed with $AS01_B$ adjuvant. Fourth, there was a placebo of 0.9% sodium chloride. See Supplementary Methods for further information.

Study Procedures

Participants gave written informed consent in their preferred language. At screening, participants underwent safety assessments through medical history, physical examination, and laboratory tests, which included complete blood count, chemistry, pap smear and urinalysis, as well as tests for pregnancy, HIV, syphilis, and hepatitis B and C. HIV antibody testing outside of the study was actively discouraged during participation to avoid potential negative impacts of vaccine-induced positive serology. Participants also underwent risk reduction

counseling, pregnancy prevention assessment, and behavioral risk assessment.

Randomization

After confirmation of eligibility, participants were randomized. The randomization sequence was obtained by computer-generated random numbers. Allocation to vaccine or placebo was provided to sites through a web-based randomization system (see Supplementary Methods).

Safety Measures

Standard safety laboratory testing included hematology, serum chemistry, and urinalysis, which were obtained at baseline (during screening) and at each 2-week postvaccination visit plus a month 15 visit (except urinalysis, collected at 2 weeks after the first, fourth, and fifth vaccination). Participants were observed for 30 minutes after vaccinations and recorded solicited local and systemic symptoms (reactogenicity) for 3 days after each vaccination. Adverse events (AEs) were recorded until 30 days after each vaccination, except for AEs leading to early participant withdrawal or early product discontinuation and serious AEs, which were recorded throughout the trial. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 21.1, and severity was graded using version 2.0 of the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (November 2014). Safety reviews were conducted by the protocol safety review team and the National Institute of Allergy and Infectious Diseases (NIAID) Data and Safety Monitoring Board.

Immunogenicity Assays

All laboratory assays were performed blinded to treatment group with validated and/or qualified methods detailed below and in Supplementary Methods. Measurements included HIV-specific binding antibody, antibody-dependent cell-mediated cytotoxicity, neutralizing antibody in serum, and T-cell responses 2 weeks after the final vaccination (assessing peak immunogenicity, month 6.5) and at 12 months (6 months after the final vaccination, assessing durability, month 12). A list of the specific antigens used in all immunogenicity assays is in Supplementary Table 1.

Binding Antibody Multiplex Assay

HIV-1–specific immunoglobulin G (IgG) binding antibody responses were measured by binding antibody multiplex assay (BAMA) [20–22]. The area under the titration curve (AUTC) was calculated using the trapezoidal rule based on the raw mean fluorescence intensity (MFI) values truncated at zero across log base 10 dilution or as a 1:50 dilution when the linear range could be captured. Tested antigens and assay reagents are in Supplementary Table 1.

Intracellular Cytokine Staining Assay

Peripheral blood mononuclear cells were isolated and cryopreserved from whole blood, as previously described [23]. T-cell responses to vaccine-matched antigens (ENV ZM96.C gp140, 1086.C gp120, TV1.C gp120, and LAI-Gag) were measured by intracellular cytokine staining as described previously [24, 25] (see Supplementary Methods and antibodies listed in Supplementary Table 2).

Antibody-Dependent Cell-Mediated Cytotoxicity

GranToxiLux antibody-dependent cellmediated cytotoxicity (ADCC-GTL) [26] and the ADCC-Luc [27] assays were performed as previously described (see Supplementary Methods).

Approvals

The study was approved by the institutional review boards of Atlanta-Hope Clinic/Emory University, Boston-Brigham/Partners, Boston-Fenway, Case Western University, Vanderbilt University, University of Pennsylvania, University of Rochester, University of California San Francisco, and Fred Hutch Cancer Center in the United States; and Medical Research Council of Zimbabwe, University of Zambia Biomedical Research Ethics Committee, and Mbeya Medical Research and Ethics Committee in Africa.

RESULTS

Study Population

In total, 160 participants enrolled in HVTN 120 between 22 February 2018 and 14 August 2018. Of these, 50 were randomized to each of the 3 groups with active study product: 200 μg protein + MF59 group (200 μg Pr + MF59), 200 μg Env protein with $AS01_B$ (200 μg Pr + $AS01_B$), and 40 μg Env protein with $AS01_B$ (40 μg Pr + $AS01_B$) (Supplementary Table 3). Ten were randomized to the placebo group. Median age was 24 years (interquartile range, 21–29 years), and 88 (55%) were women (Table 1). Demographics were similar across the 4 groups with overrepresentation of men in the placebo group.

Safety and Tolerability

All 160 participants received the first vaccination, 157 received the second, 156 received the third, and 154 received the fourth (Figure 1). Vaccines were generally safe and well tolerated. Five participants discontinued vaccinations: 4 relocated and 1 because of mild to moderate reactogenicity symptoms (grade 1 headache, malaise/fatigue, chills and local pain, grade 2 local tenderness after the third vaccination). We observed a higher trend of severity in maximum local reactogenicity among vaccine recipients in the 200 μg Pr + $AS01_B$ arm (Figure 2*A* and 2*B*). We also observed a higher trend of severity for maximum systemic reactogenicity among vaccine recipients who received the $AS01_B$ adjuvant regardless of protein dose (Figure 2*C* and 2*D*). We detected significant differences in reactogenicity

Table 1. Demographic Information of the 4 Trial Groups

Characteristic	200 μg Pr + MF59 (n = 50)	200 μg Pr + AS01$_B$ (n = 50)	40 μg Pr + AS01$_B$ (n = 50)	Placebo (n = 10)	Total (n = 160)
Age, y					
Median (IQR)	24 (21–26)	23.5 (21–28)	25.5 (22–30)	24 (22–30)	24 (21–29)
18–20	9 (18)	9 (18)	6 (12)	2 (20)	26 (16)
21–30	30 (60)	32 (64)	32 (64)	6 (60)	100 (63)
31–40	11 (22)	9 (18)	12 (24)	2 (20)	34 (21)
Sex					
Male	19 (38)	20 (40)	25 (50)	8 (80)	72 (45)
Female	31 (62)	30 (60)	25 (50)	2 (20)	88 (55)
Body mass index, kg/m^2, median (IQR)	22.6 (21–24)	22.3 (21–26)	22 (22–26)	23 (21–26)	22.8 (21–26)
Ethnicity					
Hispanic or Latino/a	2 (4)	1 (2)	1 (2)	1 (10)	5 (3)
Not Hispanic or Latino/a	48 (96)	49 (98)	49 (98)	9 (90)	155 (97)
Race					
United States, Asian	1 (2)	0 (0)	1 (2)	0 (0)	2 (1)
United States, Black	0 (0)	1 (2)	1 (2)	1 (10)	3 (2)
United States, White	18 (36)	16 (32)	17 (34)	3 (30)	54 (34)
SSA, Black	30 (60)	30 (60)	30 (60)	6 (60)	96 (60)
Mixed	1 (2)	3 (6)	1 (2)	0 (0)	5 (3)
Vaccination frequencies					
Day 0	50 (100)	50 (100)	50 (100)	10 (100)	160 (100)
Day 28	49 (98)	49 (98)	50 (100)	9 (90)	157 (98)
Day 84	49 (98)	49 (98)	49 (98)	9 (90)	156 (98)
Day 168	49 (98)	48 (96)	48 (96)	9 (90)	154 (96)

Data are No. (%) except where indicated.

Abbreviations: IQR, interquartile range; Pr, protein; SSA, sub-Saharan Africa.

between the placebo group and pooled treatment groups for pain, tenderness, and chills (Supplementary Figures 1*A* and 1*C* and Supplementary Table 4). We did not observe any significant difference in temperature. We also looked for a difference in reactogenicity symptoms across all vaccinations among the 3 treatment groups and found significant differences in chills and myalgia, which occurred more often and with greater severity in the AS01$_B$ arms compared to MF59 (Supplementary Figure 1*C*). To further investigate differences in reactogenicity, we looked for differences between treatment groups in a pairwise fashion after vaccinations 3 and 4 where adjuvant differed between groups (Supplementary Figures 1*B* and 1*D* "boost only"). We found significant differences in chills, headache, and myalgia, which occurred more often in the AS01$_B$ adjuvanted groups compared to MF59 but were not significantly different between low- and high-dose protein adjuvanted with AS01$_B$ (Supplementary Figure 1*D* and Supplementary Table 4).

A total of 222 AEs among 92 participants were reported; 216 (97%) were mild or moderate in severity. Five participants reported 6 grade 3 (severe) AEs, none of which were deemed related to vaccination. Of these, 3 participants experienced 4 episodes of decreased neutrophil count (1 had an episode 2 weeks after vaccination 2, and another 2 weeks after vaccination 3), 1 participant had an increased serum creatinine (while taking creatinine supplements), and 1 participant experienced a migraine (nearly 4 weeks after vaccination 1). Eleven participants had 13 AEs deemed related to vaccination, all resolved within 2 weeks. Seven experienced mild injection site pruritis (1 of these also had corresponding ipsilateral lymph node pain), 1 participant experienced mild pruritis of the medial aspect of the same arm as grade 3 injection site erythema, 1 experienced mild diarrhea 2 days after vaccination 1, and 1 experienced mild insomnia and night sweats 1 day after vaccination 4. One additional participant experienced grade 2 (moderate) shoulder and wrist tenderness of the vaccinated arm. No serious AEs or deaths were reported among vaccine recipients.

Vaccine-Induced Seropositivity

Vaccine-induced seropositivity assessed by commercially available HIV serology kits occurred in 1 vaccine recipient (0.6%). This individual tested reactive with only the Alere Determine HIV-1/2 Ag/Ab Combo test.

Higher Magnitude Antibody Response to Env Following Vaccine Adjuvanted With AS01$_B$ Compared to MF59

Antibody responses and magnitudes to gp120/gp140 envelope (Figure 3*A* and 3*B*) and V1V2 proteins (Figure 3*C* and 3*D*) were assessed at 2 weeks (months 6.5) and 6 months (month 12) after the fourth vaccination. At month 6.5 the response

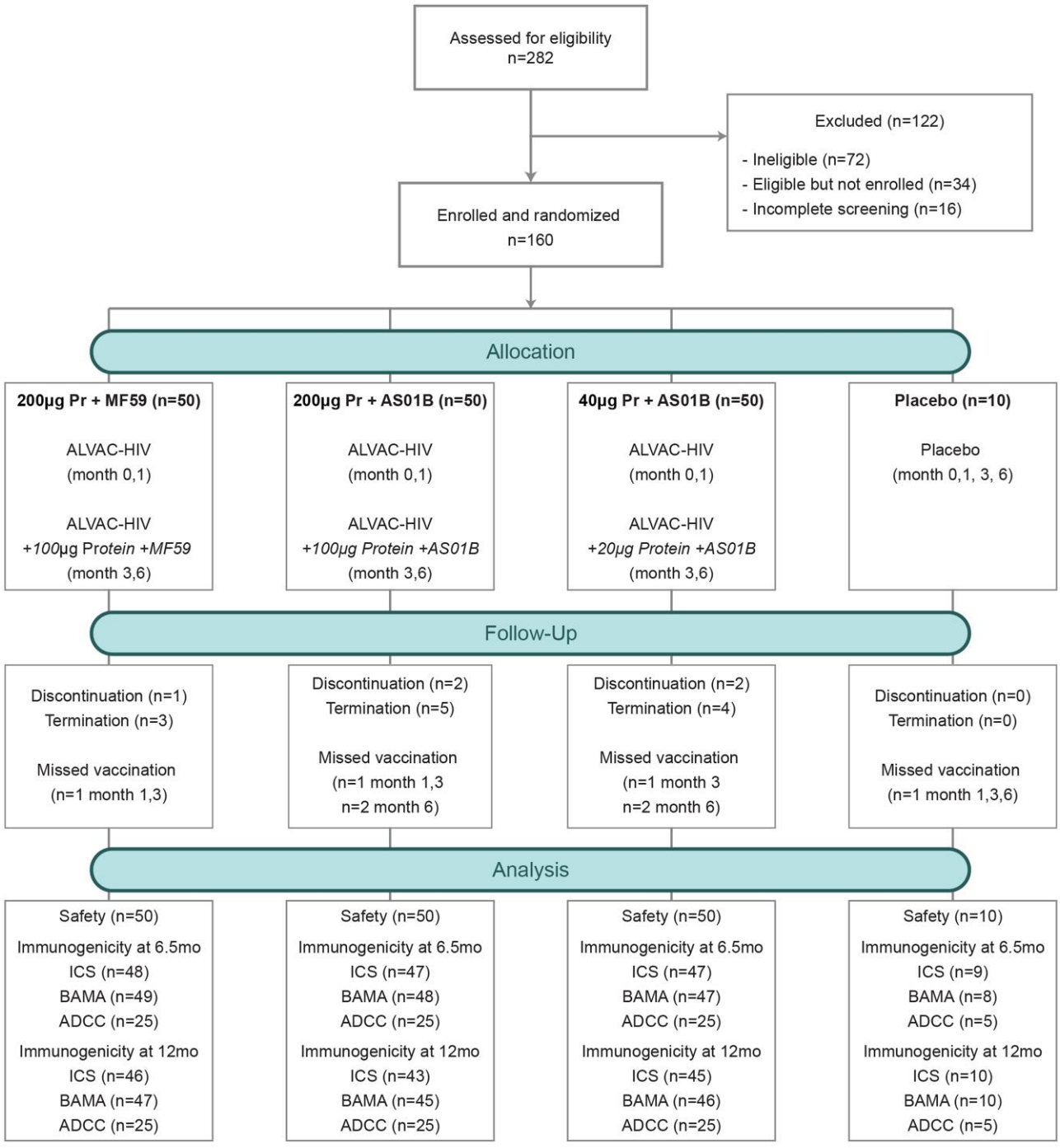

Figure 1. CONSORT flow diagram of the HVTN 120 trial. Abbreviations: ADCC, antibody-dependent cellmediated cytotoxicity; BAMA, binding antibody multiplex assay; ICS, intracellular cytokine staining; Pr, protein.

magnitude of gp120 IgG binding measured by AUTC among all participants was higher in both the 200 µg Pr + $AS01_B$ ($P = .002$ for antigen 1086.C; $P < .001$ for antigen ZM96; $P < .001$ for antigen TV1.C) and 40 µg Pr + $AS01_B$ ($P = .015$ for antigen 1086.C; $P = .617$ for antigen ZM96; $P = .015$ for antigen TV1.C) groups compared to that in the 200 µg Pr + MF59 group. At month 12, the binding antibody response net MFI magnitude of the 200 µg Pr + $AS01_B$ ($P < .001$ for all antigens 1086.C, ZM96, antigen TV1.C) and 40 µg Pr + $AS01_B$ group ($P < .001$ for antigen 1086.C; $P = .002$ for antigen ZM96; $P < .001$ for antigen TV1.C) remained higher than that of the 200 µg Pr + MF59 group.

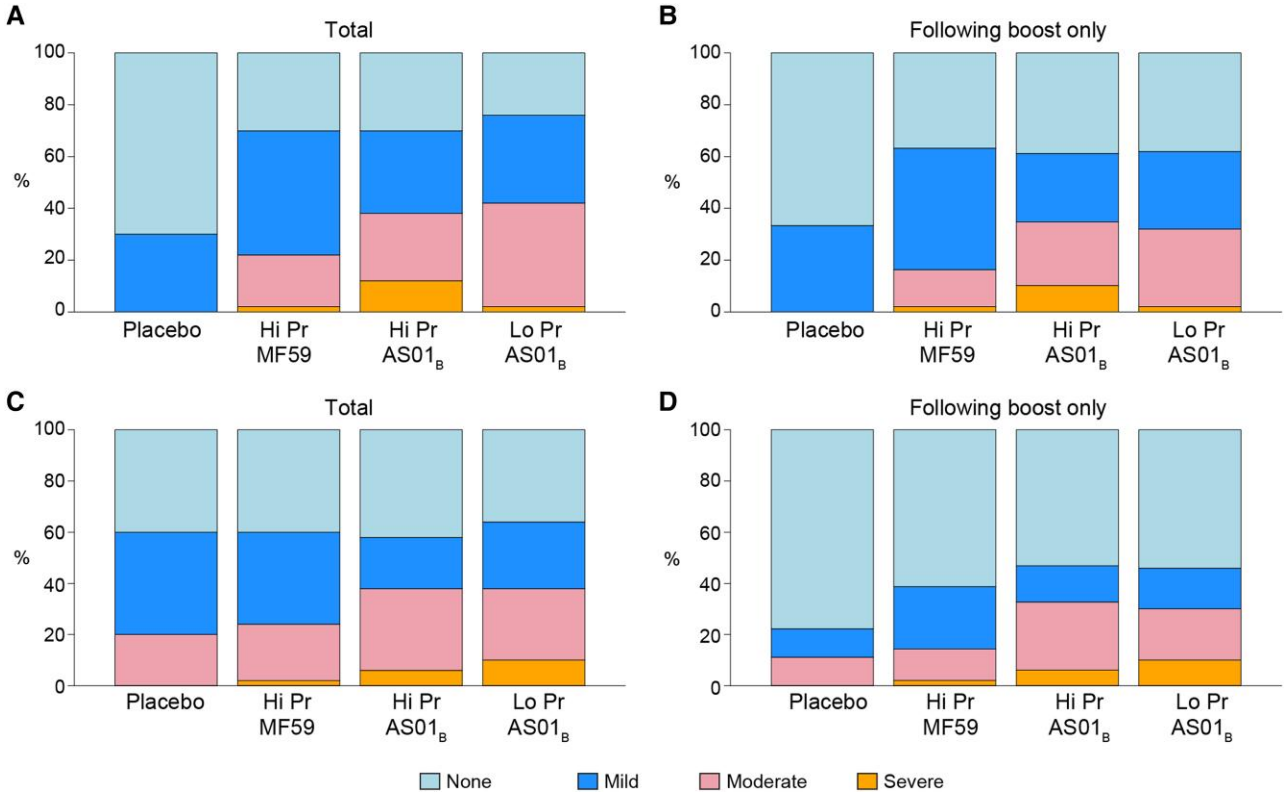

Figure 2. Maximum local and systemic reactogenicity. Stacked bar charts of maximum (*A* and *B*) combined local (pain, tenderness, erythema, induration) and (*C* and *D*) combined systemic (malaise/fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia, temperature) reactogenicity. *A* and *C*, Reactogenicity over all vaccinations among placebo (n = 10), ALVAC-HIV with 200 μg Env protein + MF59 adjuvant boost (Hi Pr + MF59, n = 50), ALVAC-HIV with 200 μg Env protein + $AS01_B$ adjuvant boost (Hi Pr + $AS01_B$, n = 50), and ALVAC-HIV with 40 μg Env protein + $AS01_B$ adjuvant boost (Lo Pr + $AS01_B$, n = 50). *B* and *D*, Reactogenicity over participants receiving 1 or more boost (third and fourth) vaccinations among placebo (n = 9), Hi Pr + MF59 (n = 49), Hi Pr + $AS01_B$ (n = 49), and Lo Pr + $AS01_B$ (n = 50). Grade 3 (severe), grade 2 (moderate), grade 1 (mild), none.

Overall, irrespective of vaccine formulation, low V1V2 responses were observed. Similar to the gp120 response, the V1V2 IgG magnitude was highest in the 200 μg Pr + $AS01_B$ group at month 6.5 (Figure 3*C*). While nearly 100% of vaccine recipients produced gp120/gp140 responses at month 6.5, only 68.9% of vaccine recipients produced V1V2 IgG responses. By month 12, the response to V1V2 had waned to <16%, whereas 100% of responders still had gp120/gp140 IgG responses regardless of treatment group.

Env Adjuvanted 40 μg With $AS01_B$ Induces Durable $CD4^+$ T-Cell Responses

We next assessed the $CD4^+$ T-cell response to the vaccine-matched Env peptide pools (TV1.C gp120, 1086.C gp120, and ZM96 gp140) by intracellular cytokine staining (Supplementary Figure 2). Vaccine-specific responses were assessed by enumerating the frequency of T cells expressing interferon-γ (IFN-γ) and/or interleukin 2 (IL-2) and /or CD40L (also known as CD154). The response rates and magnitudes to all 3 Env proteins were significantly higher in the $AS01_B$-adjuvanted groups compared to the MF59-adjuvanted group at month 6.5 and month 12 ($P < .05$; Figure 4). In addition, the 40 μg Pr + $AS01_B$ group had comparable response rates to the 200 μg Pr + $AS01_B$ regimen at both month 6.5 and 12, but significantly higher response magnitudes to all the Env peptide pools at month 12 ($P < .05$). Overall, the 40 μg Pr + $AS01_B$ consistently had the highest response magnitude compared to the other regimens. Neither adjuvant was able to enhance the response to poorly immunogenic antigens LAI Gag or LAI gp41 TM peptide pools at both time points (<10%, data not shown). Generally, the $CD4^+$ T-cell response rates in all 3 groups were durable and were maintained at similar levels at month 12 to those observed at month 6.5 for all 3 envelope peptide pools. Of note, the response magnitudes decreased significantly from month 6.5 to month 12 in all the groups, for all the antigens tested ($P < .05$; Supplementary Figure 3).

We then examined CD4 T-cell polyfunctionality scores (PFS) using the COMPASS method [28] and found they were higher in the $AS01_B$-adjuvanted groups versus the MF59 group for all Env antigens assayed at month 6.5 and 12 ($P < .05$; Supplementary Figure 4). No significant differences were

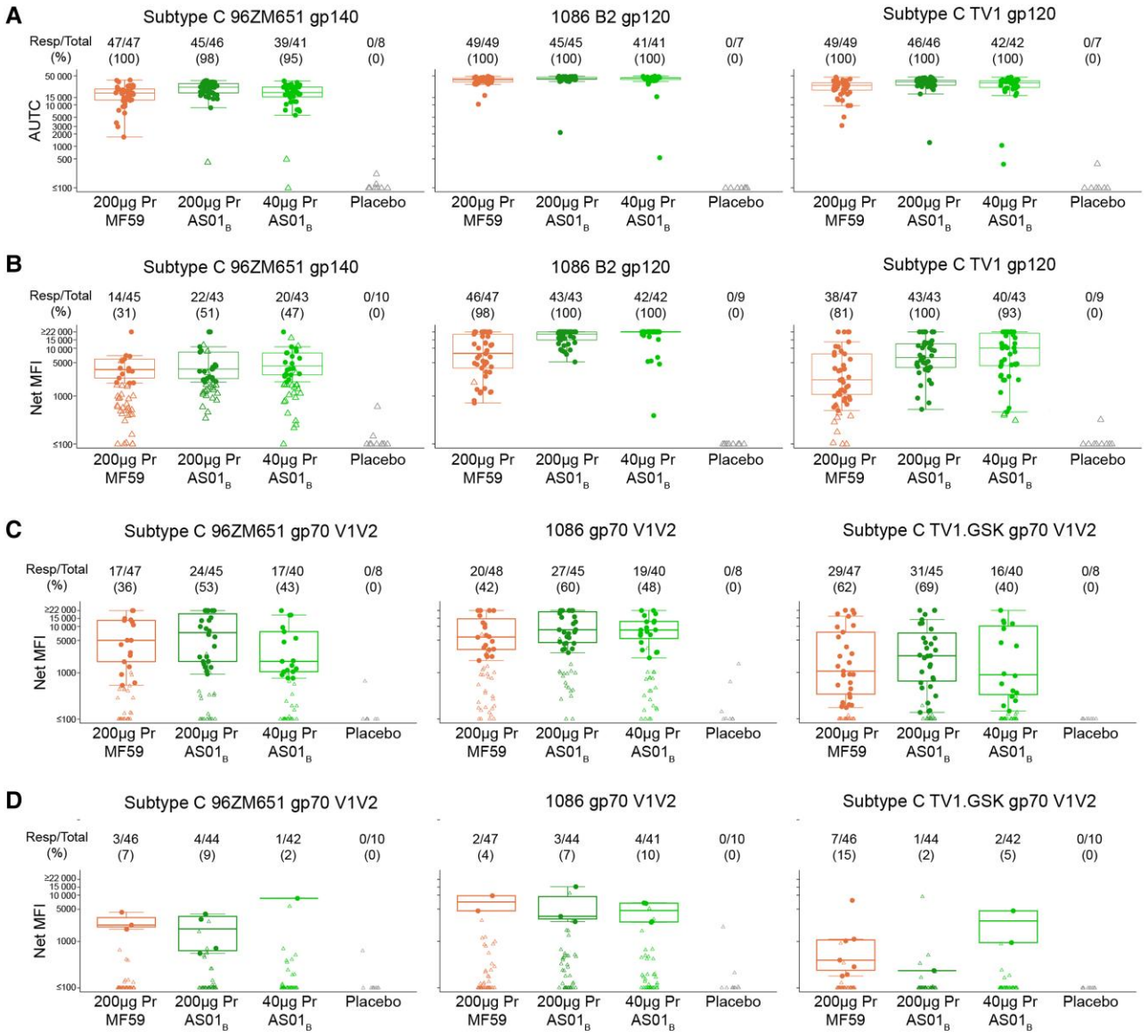

Figure 3. Binding antibody response against gp120 and V1V2. Samples from the participants collected at (*A*) 2 weeks after the last vaccination (month 6.5) and (*B*) 6 months after the last vaccination (month 12) were tested against HIV1 subtype C 96ZM651 gp120, 1086 B2 gp120, and subtype C TV1 gp120. *C*, Month 6.5 and (*D*) month 12 samples were also tested against HIV1 subtype C 96ZM651 gp70 V1V2, 1086 B2 gp70, and subtype C TV1 GSK gp70 V1V2. Graphs show (*A*) nonparametric AUTC and (*B–D*) net MFI. Numbers above indicate responders/total and percent. The median and boxplots (which display the first and third quartiles, whiskers indicate variability) are based on positive responders only (shown as filled circles); negative responders (below background) are shown as open symbols. Treatment groups are 200 µg Pr + MF59 (orange), 200 µg Pr + $AS01_B$, 40 µg Pr + $AS01_B$, and placebo. Abbreviations: AUTC, area under the baseline subtracted curve; gp, glycoprotein; MFI, mean fluorescence intensity; Pr, protein.

observed between the low- and high-dose protein groups. The PFS decreased from month 6.5 to 12 in the $AS01_B$ adjuvanted groups to all the antigens, whereas in the MF59 adjuvanted group, a temporal decrease was only observed to 1086 gp120 ($P < .05$) (Supplementary Figure 4). Heatmaps of PFS show that the highest posterior probabilities at months 6.5 and 12 were found in cells coexpressing 2 markers (IFN-γ or IL-2 and CD40L), 3 markers (IL-2, tumor necrosis factor [TNF], and CD40L), and 4 markers (IFN-g, IL-2, TNF, and CD40L) (Supplementary Figure 5).

Potent ADCC Response Following Low Protein Env Adjuvanted With $AS01_B$

Lastly, we assessed the ADCC response following vaccination (Figure 5 and Supplementary Figure 6). We observed that response rates and magnitude-breadth of the ADCC responses were overall higher in the participants who received the $AS01_B$ adjuvant compared to MF59, although the response rates were not significantly different between groups (Supplementary Figure 6). The magnitude-breadth was significantly higher in the vaccinees who received the $AS01_B$ adjuvant regardless of protein dose (Figure 5).

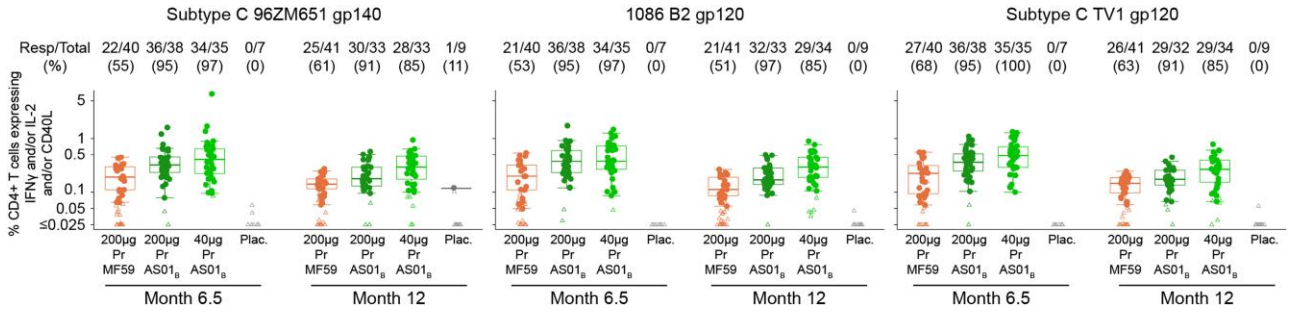

Figure 4. $CD4^+$ T-cell responses as measured by intracellular cytokine staining. The $CD4^+$ T-cell responses rate (numbers above graph) and magnitude (boxplots) 2 weeks after (month 6.5) and 6 months after (month 12) the final immunization for each treatment arm for the following vaccine-matched antigens: subtype C 96ZM651 gp120, 1086 B2 gp120, and subtype C TV1 gp120. Numbers above indicate responders/total and percent. The median and boxplots (which display the first and third quartiles, whiskers indicate variability) are based on positive responders only (shown as filled circles), negative responders (below background) are shown as open symbols. Treatment groups are 200 µg Pr + MF59, 200 µg Pr + $AS01_B$, 40 µg Pr + $AS01_B$, and placebo. Abbreviations: gp, glycoprotein; IFN-γ, interferon-γ; IL-2, interleukin 2; Pr, protein.

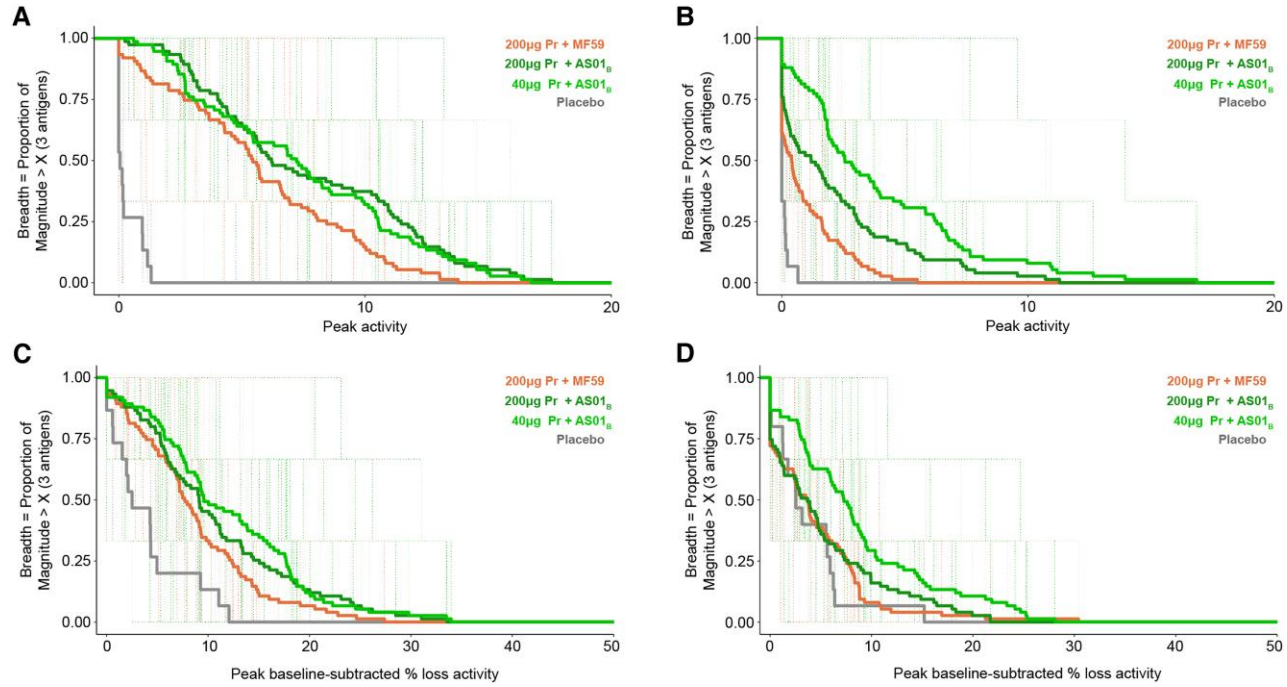

Figure 5. Higher magnitude-breadth in vaccinees who received the $AS01_B$ adjuvant. Magnitude-breadth of antibody-dependent T-cell-mediated cytotoxicity responses measured by (*A* and *B*) GranToxiLuc and (*C* and *D*) luciferase. Samples from the participants collected at 2 weeks after the last vaccination (month 6.5; *A* and *C*) and 6 months after the last vaccination (month 12; *B* and *D*) were tested against HIV1 subtype C 96ZM651 gp120, 1086 B2 gp120, and subtype C TV1 gp120. Treatment groups are 200 µg Pr + MF59, 200 µg Pr + $AS01_B$, 40 µg Pr + $AS01_B$, and placebo. Abbreviations: gp, glycoprotein; Pr, protein.

Using the ADCC-GTL assay, we observed that at month 6.5, the magnitude-breadth of the responses were similar between the different doses administered with $AS01_B$, but the 200 µg Pr + $AS01_B$ group developed significantly higher magnitude-breadth than the group administered 200 µg Pr + MF59 ($P = .022$; Figure 5*A*). Of note, at month 12, the magnitude-breadth of the responses in the 40 µg Pr + $AS01_B$ group was significantly higher than that of the 200 µg Pr + $AS01_B$ group ($P = .007$), and both high and low Pr + $AS01_B$ groups developed significantly higher magnitude-breadth than the 200 µg Pr + MF59 group ($P = .013$ and $P < .001$, respectively; Figure 5*B*). For the responses detected with the ADCC-Luc assay, at month 6.5, 40 µg Pr + $AS01_B$ induced a higher magnitude-breadth compared to 200 µg Pr + MF59, and this was statistically significant, albeit only marginally ($P = .052$; Figure 5*C*). At month 12, both 40 µg Pr + $AS01_B$ and 200 µg Pr + $AS01_B$ induced

significantly higher magnitude-breadth compared to 200 μg Pr + MF59 (200 μg Pr + $AS01_B$ vs 40 μg Pr + $AS01_B$, $P = .010$; 200 μg Pr + MF59 vs 40 μg Pr + $AS01_B$, $P = .0017$; Figure 5*D*).

DISCUSSION

Our study has four major findings. First, all regimens were generally safe and well tolerated in healthy volunteers in the United States and sub-Saharan Africa. Second, we found that $AS01_B$-adjuvanted groups, regardless of protein dose, induced higher $CD4^+$ T-cell and binding antibody responses compared to the MF59-adjuvanted group. Of significance, the lower protein dose also tended to elicit stronger responses than the higher dose when both were adjuvanted with $AS01_B$. Third, the low-protein $AS01_B$-adjuvanted dose elicited higher HIV-specific Env-gp120 binding antibody response magnitudes and higher $CD4^+$ T-cell response rates and magnitudes to all 3 Env proteins than the MF59-adjuvanted dose. Fourth, none of the regimens elicited persistent high-level responses to V1V2 envelope, a major correlate in the RV144 study, and, additionally, was associated with HIV-1 risk in the HVTN 702 study when combined with $CD4^+$ T-cell responses [29].

HVTN 702 demonstrated that 64% of vaccine recipients made Env-specific $CD4^+$ T-cell responses, which was a significantly higher proportion than the 40% observed in RV144 ($P = .03$) [29] and similar to the 73% observed here. We found that the $CD4^+$ T-cell responses were durable up to 6 months after the completion of the vaccine regimen (month 12), but that the response magnitudes and polyfunctionality had decreased significantly by then. At month 6.5, the response rates and magnitudes of gp120 IgG binding antibody to gp120/gp140 envelope and V1V2 proteins were higher in both $AS01_B$-adjuvanted groups compared to the MF59-adjuvanted group. However, we observed only 69.9% response rate to V1V2 compared to nearly 100% against gp120/gp140. At month 12, the antibody response magnitude of the $AS01_B$ adjuvanted low-dose group remained higher than either the $AS01_B$ or MF59 high-dose groups.

Three decades of HIV vaccine efficacy trials suggest that simply demonstrating that a vaccine antigen can bind to certain antibodies or elicit specific cellular responses does not necessarily signify protection against HIV infection. Recently, the 4-dose primary regimen adjuvanted with MF59 presented here was also tested with a month 12 booster in another early-phase trial (HVTN 100), which demonstrated its humoral and cellular immunogenicity [30]. However, the advanced phase trial (HVTN 702) found no HIV preventive efficacy, even with month 12 and 18 boosters [3]. HVTN 120 adapted the previous regimen with $AS01_B$ adjuvant and found a significant boosted in both $CD4^+$ T-cell responses and IgG responses to V1V2, even at a protein dose that was one-fifth of that used in the HVTN 100 and HVTN 702 studies. The decision to include $AS01_B$ as an adjuvant in this regimen was driven by data showing that it had the ability to enhance and contribute to the induction of durable immune responses, both humoral and cellular, and in some studies this correlated with protection [31].

The only trial to show partial efficacy, RV144, showed the inverse correlation between HIV incidence and IgG bound to V1V2 and the direct correlation between HIV incidence and plasma Env-specific binding IgA [2]. IgG antibodies to vaccine-matched V1V2 at 2 weeks after the fourth vaccination were observed in 100% of RV144 vaccine recipients compared to 67% of HVTN 702 vaccine recipients and 69% of participants receiving the low dose of gp120/$AS01_B$ in our study [29]. It is unclear if such comparisons are valid because they extrapolate the IgG correlate from RV144 to other vaccine regimens tested in populations with different races, ethnicity, and genetics.

One limitation of our study is the assessment of positive responders only to estimate the magnitude of B- and T-cell responses. This is a helpful metric to understand the scale of positive responses, although this introduces bias into the interpretation as it excludes negative data.

Protein vaccines have been studied in 6 of the 9 HIV-1 vaccine efficacy trials conducted; none were adjuvanted by $AS01_B$ [32]. Our findings about the increased CD4 T-cell and binding antibody responses as well as dose-sparing effect of $AS01_B$ suggest that adjuvants may have beneficial effects for a gp120 vaccine. However, the V1V2 envelope responses, that were associated with a lower risk of HIV acquisition during RV144, remain low and not persistent, supporting the requirement for an immunologically effective antigen. In the quest for an effective prophylactic HIV vaccine, these data warrant further studies, including adjuvant comparisons together with a better understanding of correlate of protection for HIV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. S. G. is an employee of Sanofi Pasteur and C. A. D. G. was employed by Sanofi Pasteur at the time of the study. M. Ko. and O. V. D. M. are employees of GSK and hold shares in GSK. All other authors report no potential conflicts. Funding to pay the Open Access publication charges for this article was provided by the NIH.

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