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The F4/AS01$_B$ HIV-1 Vaccine Candidate Is Safe and Immunogenic, But Does Not Show Viral Efficacy in Antiretroviral Therapy-Naive, HIV-1-Infected Adults

A Randomized Controlled Trial

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Abstract: The impact of the investigational human immunodeficiency virus type 1 (HIV-1) F4/AS01$_B$ vaccine on HIV-1 viral load (VL) was evaluated in antiretroviral therapy (ART)-naive HIV-1 infected adults.

This phase IIb, observer-blind study (NCT01218113), included ART-naive HIV-1 infected adults aged 18 to 55 years. Participants were randomized to receive 2 (F4/AS01$_B$_2 group, N = 64) or 3 (F4/AS01$_B$_3 group, N = 62) doses of F4/AS01$_B$ or placebo (control group, N = 64) at weeks 0, 4, and 28. Efficacy (HIV-1 VL, CD4$^+$ T-cell count, ART initiation, and HIV-related clinical events), safety, and immunogenicity (antibody and T-cell responses) were evaluated during 48 weeks.

At week 48, based on a mixed model, no statistically significant difference in HIV-1 VL change from baseline was demonstrated between F4/AS01$_B$_2 and control group (0.073 log$_{10}$ copies/mL [97.5% confidence interval (CI): −0.088; 0.235]), or F4/AS01$_B$_3 and control group (−0.096 log$_{10}$ copies/mL [97.5% CI: −0.257; 0.065]). No differences between groups were observed in HIV-1 VL change, CD4$^+$ T-cell count, ART initiation, or HIV-related clinical events at intermediate timepoints. Among F4/AS01$_B$ recipients, the most frequent solicited symptoms were pain at injection site (252/300 doses), fatigue (137/300 doses), myalgia (105/300 doses), and headache (90/300 doses). Twelve serious adverse events were reported in 6 participants; 1 was considered vaccine-related (F4/AS01$_B$_2 group: angioedema). F4/AS01$_B$ induced polyfunctional F4-specific CD4$^+$ T-cells, but had no significant impact on F4-specific CD8$^+$ T-cell and anti-F4 antibody levels.

F4/AS01$_B$ had a clinically acceptable safety profile, induced F4-specific CD4$^+$ T-cell responses, but did not reduce HIV-1 VL, impact CD4$^+$ T-cells count, delay ART initiation, or prevent HIV-1 related clinical events.

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Abbreviations: AE = adverse event, ART = antiretroviral therapy, ATP = according-to-protocol, CD40L = CD40-ligand, CI = confidence interval, DAIDS = Diseases Division of AIDS, EU =

ELISA unit, GMC = geometric mean antibody concentration, HIV = human immunodeficiency virus, ICS = intracellular cytokine staining, IFN-γ = interferon-γ, IL-2 = interleukin-2, pIMD = potentially immune mediated disease, RT = reverse transcriptase, SAE = serious adverse event, SAS = Statistical Analysis System, SD = standard deviation, TNF-α = tumor necrosis factor-α, TVC = total vaccinated cohort, VL = viral load.

INTRODUCTION

Antiretroviral therapy (ART) has greatly enhanced viral control and improves the quality of life for human immunodeficiency virus (HIV)-infected individuals. However, ART is associated with significant side effects and cannot eliminate or decrease the latent reservoir of infected cells. So, there is a great need for the development of successful therapies that can decrease or eliminate these viral reservoirs and therefore reduce the need for lifelong ART.[1] Therapeutic vaccines inducing strong T-cell-mediated immune responses against HIV type 1 (HIV-1) are currently under development.[2,3] One investigational indication for these vaccines is to complement ART with the aim to control HIV-1 viral load (VL) and to potentially eradicate the virus.[4]

An HIV-1 investigational vaccine (F4/AS01$_B$), consisting of a recombinant fusion protein (F4) containing 4 HIV-1 clade B antigens combined with the AS01$_B$ adjuvant system, has recently been developed. In previous trials, F4/AS01$_B$ had a clinically acceptable safety profile and induced long-lasting F4-specific polyfunctional CD4$^+$ T-cell responses, but no CD8$^+$ T-cell responses.[3,5,6] In HIV-1 seronegative adults, similar magnitudes and qualities of CD4$^+$ T-cell responses were observed as those displayed by subjects who spontaneously control an HIV infection.[7] A post-hoc analysis of a pilot placebo controlled trial of F4/AS01$_B$ revealed continued suppression of the HIV-1 VL in treatment experienced participants, and a transient decrease in HIV-1 VL levels after the 2nd immunization in treatment naive participants, which was associated with higher polyfunctional CD4$^+$ T$^+$ cell responses.[8] Vaccine-induced F4-specific CD4$^+$ T-cell responses were lower and less persistent in ART-naive than in ART-experienced HIV-1 infected adults.

One of the 2 coprimary objectives of this study was to confirm the transient antiviral effect observed in the pilot trial. Although F4/AS01$_B$ essentially induced F4-specific CD4$^+$ T$^+$ cell responses and not functional CD8$^+$ T-cells (the latter playing an essential role in controlling HIV-1 replication), HIV-1-specific CD4$^+$ T-cells are also needed to generate effective immune responses and to maintain functional CD8$^+$ T-cells.[9–19] In addition, a 3rd dose of F4/AS01$_B$ could have a higher impact on HIV-1 VL in ART-naive HIV-1 infected patients by improving the magnitude and duration of F4-specific CD4$^+$ T-cell responses or any

other unknown immunological mechanism. Next to virological efficacy evaluations, this phase IIb, proof-of-concept study, was also designed to evaluate the safety and immunogenicity of 2 or 3 doses of F4/AS01$_B$ compared to placebo in this population.

METHODS

Study Design and Participants

This phase IIb, observer-blind, randomized study was conducted in 15 centers in the United States, 10 in France, 8 in Germany, and 7 in Spain between November 2010 and November 2012. Participants were ART-naive HIV-1 infected adults aged 18 to 55 years at the time of 1st vaccination, who were under the care of HIV physicians for ≥6 months (or ≥12 months if they initially presented with a clinical diagnosis of primary HIV-1 infection), with CD4$^+$ T-cell count >500 cells per mm^3 and HIV-1 VL level between 2000 and 80 000 copies/mL at screening, and with no planned ART initiation within the next 12 months. Standard eligibility and exclusion criteria were used for enrollment, as detailed in the ClinicalTrials.gov registry (NCT01218113).

Participants were randomized (1:1:1) to receive 3 doses of F4/AS01$_B$ at weeks 0, 4, and 28 (F4/AS01$_B$_3 group); 2 doses of F4/AS01$_B$ at weeks 0 and 4, and 1 dose of placebo at week 28 (F4/AS01$_B$_2 group); or 3 doses of placebo at weeks 0, 4, and 28 (control group). Blood samples were collected from the participants at weeks 0, 4, 6, 16, 28, 30, 38, and 48. This study was observer-blind, since the vaccine recipients and those responsible for the evaluation of any study endpoint were all blinded to the treatment. Vaccine preparation and administration were done by authorized medical personnel who did not participate in any of the study clinical evaluation or assays.

The randomization was performed at GSK Vaccines (Rixensart, Belgium) using a standard Statistical Analysis System (SAS; Institute Inc., Cary, NC) program. The randomization algorithm used a minimization procedure accounting for country, gender, CD4$^+$ T-cells count, and HIV-1 VL at screening.

The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki. The protocol and associated documents were reviewed and approved by the investigational independent ethics committee. All participants provided written informed consent prior to study entry. This study has been registered at http://www.clinicaltrials.gov NCT01218113.

Study Vaccine

The F4/AS01$_B$ investigational vaccine contained 10 µg of F4, a recombinant fusion protein encoding 4 HIV-1 clade B antigens (p24, reverse transcriptase [RT], Nef, and p17), and was adjuvanted with AS01$_B$, containing 3-*O*-desacyl-4'-monophosphoryl lipid A, QS-21 (*Quillaja saponaria* Molina, fraction 21; licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation), and liposomes.[6] The associated placebo was saline (NaCl 0.9%). F4/AS01$_B$ (0.5 mL) and the placebo (0.7 mL) were injected intramuscularly into the deltoid muscle of the participant's nondominant arm.

Study Objectives

The primary objectives were to evaluate differences in HIV-1 VL change from baseline at week 48 between participants who received F4/AS01$_B$ or the placebo, and to assess the reactogenicity and safety of F4/AS01$_B$. Secondary objectives were to evaluate differences in HIV-1 VL change from baseline at week 48 between the F4/AS01$_B$_2 and the F4/AS01$_B$_3 groups, and to compare the following endpoints at all timepoints between the 3 groups: absolute HIV-1 VL and CD4$^+$ T-cell counts, and their changes from baseline; incidence of, and time to ART initiation or occurrence of HIV-related clinical events; and HIV-specific T-cell and antibody immune responses.

Safety Assessments

Occurrence, intensity, and relationship to vaccination of local (injection site pain, redness, and swelling) and general (fever, fatigue, headache, sweating, myalgia, and gastrointestinal symptoms) solicited adverse events (AEs) were recorded for 7 days, and of unsolicited AEs for 28 days, after each vaccination. During the entire study period, occurrence and relationship to vaccination of serious adverse events (SAEs) and potentially immune mediated diseases (pIMDs) were reported, and hematological and biochemical parameters were evaluated. The National Institute of Allergy and Infectious Diseases Division of AIDS (DAIDS) scale was used to grade AEs, and hematological and biochemical parameters.[20] If a solicited local or general AE was scored as grade 4 in accordance with the DAIDS scale, it was considered as a SAE.

In order to monitor carefully a potential ophthalmological toxicity of F4/AS01$_B$, with lens opacities described in a minipig model during preclinical assessments (not confirmed in rabbits during a repeated toxicological study in New Zealand), an ophthalmologic examination with slit-lamp was performed at baseline and at the end of the study.

Efficacy Assessment

At a validated central laboratory designated by GSK Vaccines, HIV-1 VL was tested in plasma samples by an ultrasensitive RT-polymerase chain reaction using the Abbott RealTime HIV-1 assay (cut-off: 50 copies/mL). CD4$^+$ T-cell counts were performed on blood samples by flow cytometry. Time to ART initiation or occurrence of HIV-related clinical events was computed in days following the 1st vaccination during the entire study period. HIV-related clinical events were defined as confirmed CD4$^+$ T-cell count <350 cells/mm^3, confirmed HIV-1 VL >100,000 copies/mL or clinical disease progression.

Immunogenicity Assessments

F4-specific CD4$^+$ and CD8$^+$ T-cell responses were measured by flow cytometry using intracellular cytokine staining (ICS) after 2 hous in-vitro stimulation with p17, p24, RT, and Nef peptide pools in presence of anti-CD48/anti-CD49d antibodies followed by ~18 hours incubation with Brefeldin A to assess the expression of interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and CD40-ligand (CD40L; T-cell activation marker). ICS was performed on peripheral blood mononuclear cells isolated from venous blood, using an adaptation of a previously described method (Supplement 1, http://links.lww.com/MD/A671).[6]

Standard in-house enzyme-linked immunosorbent assays were used to measure antibody levels in enzyme-linked immunosorbent assays units (EU) against p17 (cut-off: 187 mEU/mL), p24 (119 mEU/mL), RT (125 mEU/mL), Nef (232 mEU/mL), and F4 (42 mEU/mL), as previously described.[6]

Statistical Analyses

The target sample size was 150 evaluable participants (50 participants in each group), in order to observe the primary efficacy objective with a power of ≥90% for a 0.5 true group difference in terms of HIV-1 VL (corresponding to an approximate 3-fold decrease) and for a 0.7 standard deviation (SD) of the change from baseline in $\log_{10}$-transformed HIV-1 VL (copies/mL) using a Bonferroni adjustment to account for the 2 planned comparisons (2-sided 0.025 level used for each comparison). Considering a drop-out rate of approximately 20%, 189 participants were planned to be enrolled.

Safety analyses were performed on the total vaccinated cohort (TVC). Numbers and percentages of patients reporting AEs were calculated with exact 95% confidence intervals (CIs). SAEs and pIMDs were described in detail.

Efficacy analyses were performed on the modified TVC that was predefined by protocol to include all eligible participants, who received at least 1 vaccine dose and with sufficient data to perform the efficacy analysis. For study participants who initiated ART treatment during the study period, $CD4^+$ T-cell count and HIV-1 VL data were only considered if they were collected before ART initiation and were censored after that timepoint. Moreover, $CD4^+$ T-cell count, HIV-1 VL, and ART initiation data were censored if they were recorded after an active study vaccine administration that was missed (not for placebo), not performed according-to-protocol (ATP), or performed after a medication or concomitant vaccination that led to discontinuation of the participant. Changes from baseline in terms of mean $CD4^+$ T-cell count and median HIV-1 VL were evaluated for each group and at each timepoint. The antiviral impact of $F4/AS01_B$ was demonstrated if the upper limit of the 2-sided 97.5% CI for the difference in HIV-1 VL change from baseline at week 48 between the $F4/AS01_B\_2$ and the control group, or between the $F4/AS01_B\_3$ and the control group, was <0. To control the global type I error below 2.5% (one-sided), a Bonferroni adjustment was used for the 2 comparisons. For HIV-1 VL and $CD4^+$ T-cell count, a mixed model for repeated measurements was used to detect differences between groups.[21,22] The model included baseline HIV-1 VL ($\log_{10}$-transformed), baseline $CD4^+$ T-cells count (crude value), gender, and country as covariates, and used an unstructured variance−covariance matrix, time, and time∗group as fixed categorical effects.

Immunogenicity analyses were performed on the ATP immunogenicity cohort that included all participants from the TVC, who did not receive any vaccine or medication not specified or forbidden in the protocol, complied with protocol defined procedures and intervals, had no elimination criteria during the study, and for whom immunogenicity results were available against at least 1 study vaccine antigen after vaccination. Only data collected before ART initiation were considered for immunogenicity analyses, and data collected after that timepoint were censored. Immunogenicity results were summarized using descriptive statistics for continuous variables and percentages with 95% CIs for categorical variables. ICS results were expressed as the percentage of the total $CD40L^+CD4^+$ and $CD8^+$ T-cells expressing IL-2 and/or IFN-γ and/or TNF-α in response to stimulation with p17, p24, RT, or Nef antigen minus the response measured upon in-vitro stimulation with medium only. F4-specific $CD4^+$ or $CD8^+$ T-cell responses were estimated from the sum of specific $CD4^+$ or $CD8^+$ T-cell frequencies in response to each individual antigen. F4-specific $CD4^+$ T-cell measures were characterized based on their magnitude (frequencies of $CD40L^+CD4^+$ T-cells expressing at least IL-2 and at least 1 cytokine), cytokine coexpression profiles, and breadth (percentage of responders after in-vitro stimulation with each individual antigen and with at least 1, 2, 3, or 4 antigens). For any postvaccination time point, participants with undetectable cytokine secretion at prevaccination were defined as responders if they had ≥0.08% of $CD40L^+CD4^+$ T-cells expressing at least 1 cytokine; this cut-off was selected based on the prevaccination 95th percentiles for the percentage of $CD40L^+$ $CD4^+$ T-cells expressing at least 1 cytokine, and was computed to correspond to 300 cells per million $CD4^+$ T-cells for stimulation by each separate antigen, and to 800 cells per million $CD4^+$ T-cells for stimulation by F4 in 2 previous studies.[6,8] In participants with detectable cytokine secretion at prevaccination, response was defined as at least 2-fold increase from baseline in $CD40L^+CD4^+$ T-cells expressing at least 1 cytokine. F4-specific $CD8^+$ T-cell responses were characterized based on their magnitudes (frequencies of $CD8^+$ T-cells expressing at least 1 cytokine [IL-2, TNF-α, or IFN-γ]). Exploratory analyses were performed to characterize differences between groups in T-cell responses; any difference detected should be interpreted with caution considering that there was no adjustment for multiplicity of endpoints and that the clinical relevance of the difference was not accounted for. Percentages of participants with anti-F4 antibody concentrations above cut-offs were calculated with 95% CIs using the exact method for binomial variables. Geometric mean antibody concentrations (GMCs) for anti-F4 antibodies were calculated with 95% CIs using antilogs of the 95% CIs of mean $\log_{10}$-transformed antibody concentrations. Antibody concentrations below assay cut-offs were given an arbitrary value of half the cut-off for GMC calculations.

Analyses were performed using the SAS software version 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Study Participants

Of the 320 screened participants, 190 were included in the TVC (Figure 1). Of these, 185 participants were included in the modified TVC and 129 in the ATP immunogenicity cohort. Twelve participants withdrew from the study, none due to an AE. The groups were adequately balanced in terms of age, male to female ratio, ethnicity, and HIV-related parameters (Table 1 and Supplement 2, http://links.lww.com/MD/A671).

Safety Results

Pain at injection site was the most common solicited local symptom and was reported following a given dose by up to 89.6% of $F4/AS01_B$ recipients (9.2% for pain with grade ≥3 intensity) compared to 19% of participants in the control group (no pain with grade ≥3 intensity) (Figure 2). The most common solicited general symptom was fatigue, which was reported following a given dose by up to 46.7% of $F4/AS01_B$ recipients compared to 25.4% of participants in the control group. Headache and myalgia were also frequently reported following administration of $F4/AS01_B$. General symptoms with grade ≥3 intensity were reported in ≤5.4% of participants.

Unsolicited symptoms were reported in 48.4%, 65.6%, and 53.1% of participants in the $F4/AS01_B\_3$, $F4/AS01_B\_2$, and control group, respectively (unsolicited symptoms with grade ≥3 intensity in 4.8%, 10.9%, and 4.7% of participants). The most frequently reported unsolicited symptoms in the 3 groups
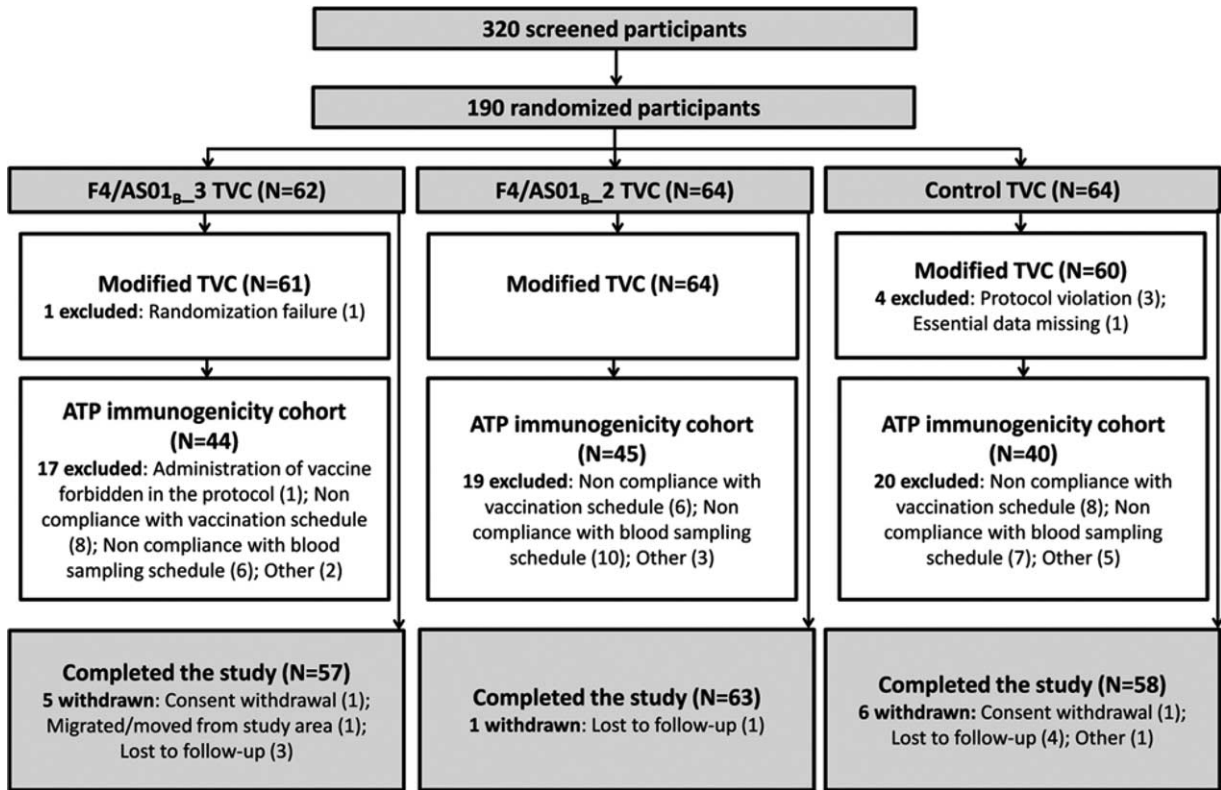
FIGURE 1. Flow of participants. $F4/AS01_B\_3$ = participants randomized to receive three doses of $F4/AS01_B$ at weeks 0, 4, and 28; $F4/AS01_B\_2$ = participants randomized to receive 2 doses of $F4/AS01_B$ at weeks 0 and 4, and 1 dose of placebo at week 28; control = participants randomized to receive 3 doses of placebo at weeks 0, 4, and 28; TVC = total vaccinated cohort; ATP = according-to-protocol; N = number of participants.

largely involved upper respiratory tract infections. The percentages of participants reporting solicited and unsolicited symptoms did not markedly increase between the 1st, 2nd, and 3rd doses.

Twelve SAEs were reported by 6 participants: 1 participant in the $F4/AS01_B\_3$ group, 3 in the $F4/AS01_B\_2$ group, and 2 in the control group (Supplement 3, http://links.lww.com/MD/A671). No fatal SAEs were reported. One SAE (angioedema) in the $F4/AS01_B\_2$ group was considered by the investigator as related to vaccination. This vaccine-related SAE was reported on the day of 2nd dose administration by a participant who had pain at injection site that did not resolve, took a pain medication (tramadol and paracetamol) in the evening, and experienced angioedema symptoms (lip and eye lid edema) that resolved after cetirizine treatment. The participant recovered 5 days after onset of the event, but was withdrawn from further vaccination in the study. One pIMD, which was not considered as related to vaccination, was reported by a participant in the $F4/AS01_B\_2$ group (psoriasis occurring 77 days after the second dose).

The vast majority of participants had no or grade 1 hematological and biochemical parameters, and only 12 events graded DAIDS category ≥3 were reported. Very few hematological and biochemical results worsened after vaccination.

Between initial visit and the end of the study, 10 participants had differences in lens opacity results (7 had improved lens opacity). No ophthalmologic observations were considered

clinically relevant (Supplement 4, http://links.lww.com/MD/A671).

Efficacy Results

HIV-1 VL

The data failed to demonstrate a statistically significant reduction of HIV-1 VL from baseline to week 48 in the $F4/AS01_B\_3$ or $F4/AS01_B\_2$ group compared to the control group (Table 2).

At week 48, mean (SD) HIV-1 VL values were 4.1 (0.4), 4.2 (0.6), and 4.2 (0.6) $\log_{10}$ copies/mL in the $F4/AS01_B\_3$, $F4/AS01_B\_2$, and control group, respectively. The median HIV-1 VL change from baseline to week 48, in terms of differences of each value at week 48 minus baseline value, was 0, 0.1, and 0.1 $\log_{10}$ copies/mL in the $F4/AS01_B\_3$, $F4/AS01_B\_2$, and control group, respectively (Supplement 5, http://links.lww.com/MD/A671). No differences between groups in terms of HIV-1 VL change from baseline were observed at intermediate timepoints, when the mixed model was used (Supplement 6, http://links.lww.com/MD/A671).

CD4$^+$ T-Cell Counts

At week 48, the median (minimum–maximum) absolute number of CD4$^+$ T-cells were 622.5 (294–1119), 597 (329–1331), and 580.5 (371–1310) in the $F4/AS01_B\_3$, $F4/AS01_B\_2$, and control group, respectively. No differences between groups

TABLE 1. Demographic and Baseline HIV Characteristics of the Study Participants (Modified Total Vaccinated Cohort)

| Characteristic	Parameter or Category	F4/AS01$_B$_3 (N = 61)	F4/AS01$_B$_2 (N = 64)	Control (N = 60)
Demographic characteristics				
Age, years	Mean (range)	34.8 (18, 51)	37.0 (22, 53)	36.5 (24, 55)
Gender	Male, n, %	52 (85.2)	54 (84.4)	51 (85.0)
Geographic ancestry	White-Caucasian/European, n, %	42 (68.9)	51 (79.7)	38 (63.3)
	Other, n, %	19 (31.1)	13 (20.3)	22 (36.7)
Mode of transmission	Homosexual contact, n, %	47 (77.0)	49 (76.6)	45 (75.0)
	Heterosexual contact, n, %	13 (21.3)	16 (25.0)	12 (20.0)
	Injectable drug use, n, %	2 (3.3)	0 (0.0)	2 (3.3)
	Transfusion, n, %	1 (1.6)	2 (3.1)	0 (0.0)
	Occupational exposure, n, %	1 (1.6)	0 (0.0)	1 (1.7)
	Other risk, n, %	1 (1.6)	1 (1.6)	1 (1.7)
Baseline HIV characteristics				
Time from diagnosis, years	Median (range)	2.17 (0.50, 11.33)	2.13 (0.50, 12.92)	2.50 (0.58, 24.17)
CD4$^+$ T-cells count nadir	Median (range)	566.0 (339, 834)	546.5 (136, 1228)	563.0 (323, 1121)
Time from CD4$^+$ T-cell nadir, years	Median (range)	0.58 (0.00, 5.92)	0.71 (0.00, 7.75)	0.54 (0.00, 9.08)
HIV clade	B, n, %	24 (39.3)	21 (32.8)	18 (30.0)
	Other, n, %	5 (8.2)	9 (14.1)	8 (13.3)
	Unknown, n, %	32 (52.5)	34 (53.1)	34 (56.7)

F4/AS01$_B$_3 = participants randomized to receive three doses of F4/AS01$_B$ at weeks 0, 4, and 28; F4/AS01$_B$_2 = participants randomized to receive 2 doses of F4/AS01$_B$ at weeks 0 and 4, and 1 dose of placebo at week 28; control = participants randomized to receive 3 doses of placebo at weeks 0, 4, and 28; N = total number of participants; n (%) = number (percentage) of participants in a given category. HIV = human immunodeficiency virus, SD = standard deviation.
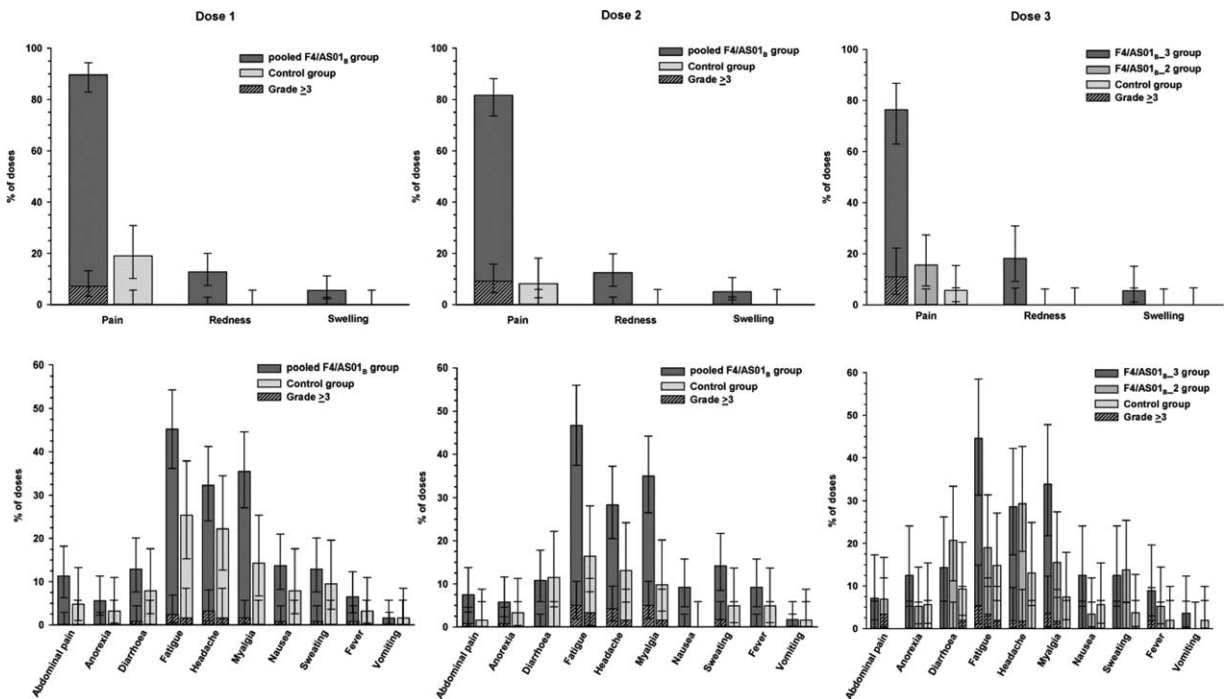
FIGURE 2. Percentage of participants reporting solicited local and general symptoms during the 7-day postvaccination period after each dose (total vaccinated cohort). F4/AS01$_B$_3 = participants randomized to receive 3 doses of F4/AS01$_B$ at weeks 0, 4, and 28; F4/AS01$_B$_2 = participants randomized to receive 2 doses of F4/AS01$_B$ at weeks 0 and 4, and 1 dose of placebo at week 28; control = participants randomized to receive three doses of placebo at weeks 0, 4, and 28; pooled F4/AS01$_B$ = pooled F4/AS01$_B$_3 and F4/AS01$_B$_2 groups; errors bars represent exact 95% confidence intervals.

TABLE 2. Between Group Comparisons in Terms of Change in HIV-1 Viral Load From Baseline to Week 48 (Modified Total Vaccinated Cohort)

		F4/AS01$_B$ Group			Control Group	Estimated Difference (F4/AS01$_B$ Minus Placebo) in HIV-1 VL, log$_{10}$ copies/mL
	N	Estimated Mean (95% CI)		N	Estimated Mean (95% CI)	Value (97.5% CI)
F4/AS01$_B$_3	61	0.037 (−0.071, 0.146)	Control	60	0.133 (0.033, 0.234)	−0.096 (−0.257, 0.065)
F4/AS01$_B$_2	64	0.207 (0.099, 0.315)	Control	60	0.133 (0.033, 0.234)	0.073 (−0.088, 0.235)

F4/AS01$_B$_3 = participants randomized to receive 3 doses of F4/AS01$_B$ at weeks 0, 4, and 28; F4/AS01$_B$_2 = participants randomized to receive 2 doses of F4/AS01$_B$ at weeks 0 and 4, and 1 dose of placebo at week 28; control = participants randomized to receive 3 doses of placebo at weeks 0, 4, and 28; N = number of participants used in the model; mean = geometric mean based on least squares means from the repeated-measures mixed model. The differences (log$_{10}$-transformed HIV-1 viral load) were based on a repeated-measures mixed model. CI = confidence interval, HIV = human immunodeficiency virus, VL = viral load.

in terms of changes in CD4$^+$ T-cell count from baseline were observed at each timepoint, when the mixed model was used (Supplement 7, http://links.lww.com/MD/A671).

ART Initiation and HIV-Related Clinical Events

ART initiation was reported in 8.2%, 6.3%, and 8.3% of participants, and mean time to ART initiation was 261.4, 253.5, and 179.8 days after the 1st vaccination in the F4/AS01$_B$_3, F4/AS01$_B$_2, and control group, respectively. HIV-related clinical events were reported in 9.8%, 10.9%, and 10.0% of participants in the F4/AS01$_B$_3, F4/AS01$_B$_2, and control group, respectively. No difference between groups was observed in terms of ART initiation or HIV-related clinical events (Supplements and 9, http://links.lww.com/MD/A671).

Immunogenicity Results

CD4$^+$ T-Cell Response

At baseline, high preexisting F4-specific CD40L$^+$CD4$^+$ T-cells expressing at least 1 cytokine were detected in the 3 groups, but levels of CD40L$^+$CD4$^+$ T-cells expressing at least IL-2 (alone or together with other cytokines) were low. Following administration of 2 or 3 doses of F4/AS01$_B$, percentages of F4-specific CD40L$^+$CD4$^+$ T-cells expressing at least IL-2 increased to similar levels (Figure 3A). Vaccine-induced F4-specific CD40L$^+$CD4$^+$ T-cell levels declined overtime, but the responder rate for F4-specific CD40L$^+$CD4$^+$ T-cells expressing at least IL-2 was still 51% of participants in the F4/AS01$_B$_3 group at week 48 (Supplement 10, http://links.lww.com/MD/A671). In the control group, no increases in F4-specific CD40L$^+$CD4$^+$ T-cell responses were observed after administration of the placebo (data not shown).

Differences between F4/AS01$_B$ and control groups in terms of number of CD40L$^+$ CD4$^+$ T-cell expressing at least IL-2 were for each antigen is described in Supplement 11, http://links.lww.com/MD/A671. Vaccine-induced CD40L$^+$ CD4$^+$ T-cells had a polyfunctional cytokine profile, and mainly expressed IL-2 alone or in combination with TFN-α and/or IFN-γ (Figure 3B). In the control group, CD40L$^+$CD4$^+$ T-cells mainly expressed IFN-γ or TNF-α alone or in combination, and only few CD40L$^+$CD4$^+$ T cells expressed IL-2. The proportion of polyfunctional CD40L$^+$CD4$^+$ T-cells induced by 2 doses of F4/AS01$_B$ further increased following the 3rd dose administration and were maintained up to the study end (Figure 3C).

CD8$^+$ T-Cell Response

High levels of F4-specific CD8$^+$ T-cells were observed over the whole study period, without any impact of vaccination (Supplement 12, http://links.lww.com/MD/A671). F4-specific CD8$^+$ T-cells mainly expressed IFN-γ with or without TNF-α.

Humoral Immune Responses

The observed anti-F4, -p17, -p24, and -RT antibody levels were not impacted by F4/AS01$_B$ vaccination (Supplement 13, http://links.lww.com/MD/A671). An increase in anti-Nef GMCs was observed after the 3rd F4/AS01$_B$ dose administration.

Post-Hoc Exploratory Analyses

Post-hoc observations of the individual kinetics of VL or CD4$^+$ T-cells count did not allow to detect any indication of vaccine effect in different subsets of subjects who showed an increase or decrease at week 6 or 48 compared to baseline. No correlation was found between HIV-specific CD4$^+$ T-cells immune response and change in VL (Supplement 14, http://links.lww.com/MD/A671).

DISCUSSION

This study confirmed that 2 or 3 doses of the F4/AS01$_B$ investigational vaccine had a clinically acceptable safety and reactogenicity profile in ART-naive HIV-1 infected adults. No safety concerns were raised in the study population, which is in line with results of previous studies conducted in healthy adults and in HIV-1 infected patients.[6,8]

The primary efficacy objective of the study was not met, since no statistically significant reduction of HIV-1 VL was detected in F4/AS01$_B$ vaccine recipients as compared to placebo at 48 weeks after the 1st vaccination. This study did not confirm the transient reduction in HIV-1 VL compared to placebo, which was previously observed in a smaller study.[8] Moreover, 2 or 3 doses of the F4/AS01$_B$ vaccine did not significantly impact the HIV-1 VL, CD4$^+$ T-cell counts, rates of ART initiation or HIV-1 related clinical events, or the already
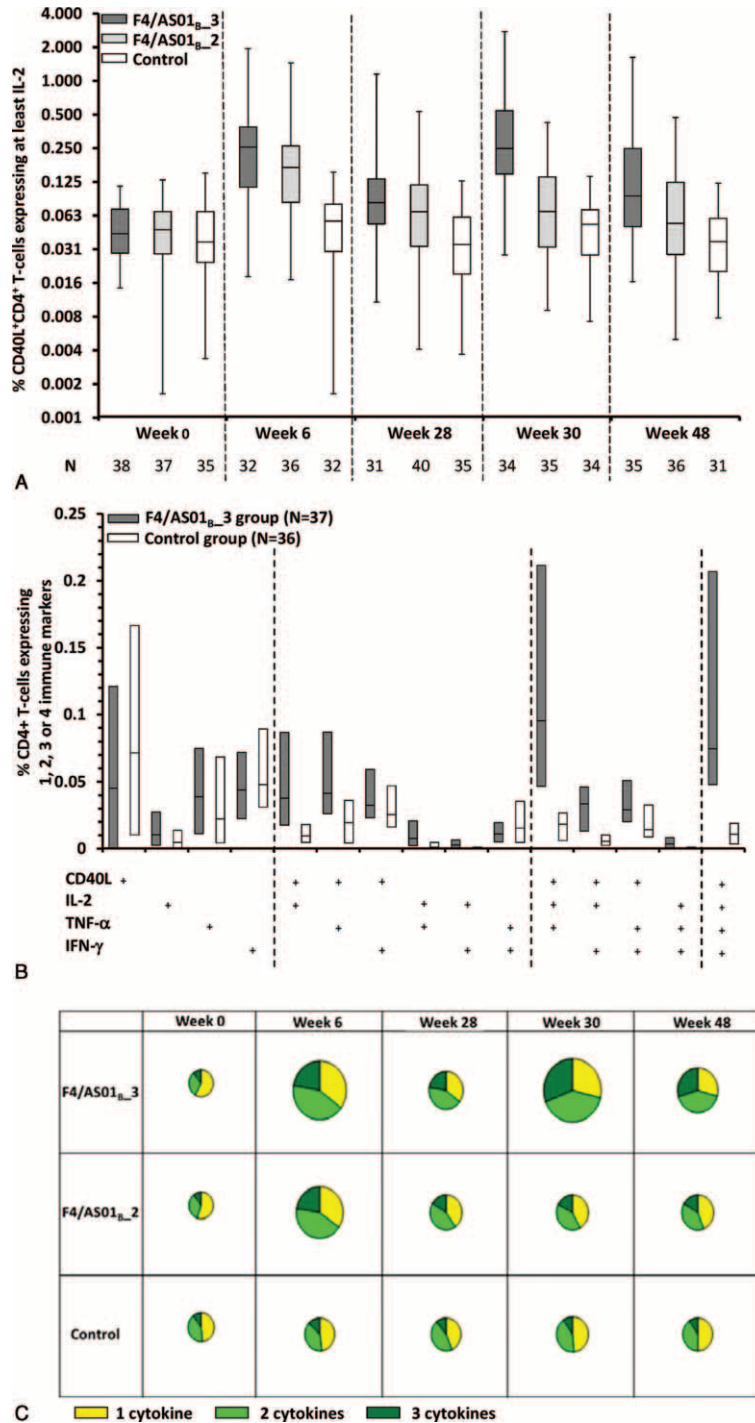
FIGURE 3. (A) Percentage of F4-specific CD40L⁺CD4⁺ T-cells expressing at least IL-2 (alone or together with other cytokines) at each timepoint, (B) cytokine coexpression profile of F4-specific CD4⁺ T-cells in the F4/AS01ᵦ_3 group and the control group at week 30, and (C) pie charts of the cytokine coexpression of F4-specific CD40L⁺CD4⁺ T-cells at each timepoint in the three groups (according-to-protocol cohort for immunogenicity). F4/AS01ᵦ_3 = participants randomized to receive 3 doses of F4/AS01ᵦ at weeks 0, 4, and 28; F4/AS01ᵦ_2 = participants randomized to receive 2 doses of F4/AS01ᵦ at weeks 0 and 4, and 1 dose of placebo at week 28; control = participants randomized to receive 3 doses of placebo at weeks 0, 4, and 28. The box plot: the central box shows the interquartile range (Q1–Q3), with the thick horizontal line representing the median (Q2), and the whiskers (above and below the box), the maximum and the minimum. The percentage of CD40L⁺CD4⁺ T-cells expressing cytokines in response to the fusion protein F4 was determined by adding the individual frequencies of the CD40L⁺CD4⁺ T-cell response to each of the 4 individual antigens. Whiskers were not added to Figure 3B for clarity. The sizes of the pie charts represent the proportions of total CD40L⁺ CD4⁺ T-cells producing at least 1 cytokine. CD40L = CD40-ligand, IFN-γ = interferon-γ, IL-2 = interleukin-2, TNF-α = tumor necrosis factor-α.

high baseline levels of F4-specific $CD8^+$ T-cell responses or anti-F4-antibody levels in our experimental conditions. However, it induced significant polyfunctional F4-specific $CD40L^+CD4^+$ T-cell responses in ART-naive HIV-1 infected patients. Contrary to past observation made in ART-treated patients after administration of an HIV-1 recombinant canarypox vaccine followed by analytical treatment interruption,[23] no exacerbation of virus replication was observed after F4/$AS01_B$ vaccination, confirming preliminary observations made in a previous phase I study.[8]

The absence of enhanced viral control despite the vaccine-induced $CD4^+$ T-cell response may be explained by the challenging conditions imposed by viral replication in ART-naive HIV-1 infected patients (incomplete immune response due to the immunosuppressive effects of HIV-1, direct killing of activated HIV-1-specific $CD4^+$ T-cell by HIV-1, and high preexisting immune responses induced by HIV-1 infection). Alternatively, it may be due to a selection bias, since most participants were immunological controllers and had high baseline F4-specific $CD8^+$ T-cell responses and anti-F4 antibody levels in our experimental conditions. A beneficial effect might possibly be observed in immunodiscordant patients, who show viral suppression during ART treatment, but fail to recover $CD4^+$ T-cell response. Another explanation could be that $CD4^+$ T-cell responses on their own are not sufficient to exert an antiviral effect, although direct antiviral effects of $CD4^+$ T-cells have been previously shown.[11,24] Of note, the differences between the F4/$AS01_B$ investigational vaccine and the placebo in terms of F4-specific $CD4^+$ T-cell responses could have been overestimated in our study since the participants censored at ART initiation might be those with the lowest vaccine-induced immune response. Nevertheless, the significant $CD4^+$ T-cell responses induced by this vaccine may still be valuable, and further evaluations are needed to determine if F4/$AS01_B$ vaccination could contribute to an antiviral effect, especially when combined with other interventions. When combined with ART, the F4/$AS01_B$ vaccine could be used as therapeutic vaccine to restimulate the HIV-specific immune effectors as part of a combined shock and kill strategy, a hypothesis that still remains to be demonstrated.[25] Sequential or coadministration of this vaccine and other vaccines that induce specific $CD8^+$ T-cell responses could induce a complementary $CD4^+$ and $CD8^+$ T-cell response, which could deal with viral escape and inhibit viral activity across clades.[26,27] This has been demonstrated in a phase I trial in healthy subjects were vaccination with the F4/$AS01_B$ vaccine after priming or coadministered with an Adenovirus 35 Gag-RT-Int-Nef Vaccine resulted in strong, multifunctional, and complementary HIV-specific immune responses.[28]

The polyfunctionality level of F4-specific $CD4^+$ T-cells was increased following the 3rd F4/$AS01_B$ vaccine dose administration, confirming that immunological responses may be improved by the use of a prime-boost vaccination strategy.[29] Vaccine induced F4-specific $CD4^+$ T-cells were mainly directed against Nef and RT, and mainly produced IL-2 alone or in combination with TNF-α and/or IFN-γ.[2,4–7]

This study showed that 2 or 3 doses of the F4/$AS01_B$ vaccine, administered to ART-naive HIV-1 infected adults did not raise any safety concerns, induced polyfunctional F4-specific $CD4^+$ T-cell responses, but had no significant impact on the already high baseline levels of F4-specific $CD8^+$ T-cell responses and anti-F4-antibody levels. Vaccine-induced F4-specific $CD4^+$ T-cell responses did not reduce HIV-1 VL, impact $CD4^+$ T-cells count, delay ART initiation, or prevent HIV-1 related clinical events. We feel that the evaluation of strategies employing this vaccine in conjunction with other treatments may be warranted.

Data Availability

The results summary for this study (GSK study number 111679-NCT01218113) is currently available on the GSK Clinical Study Register and can be accessed at http://www.gsk-clinicalstudyregister.com/study/111679. Upon authorization or termination of development of this medicine, anonymized patient-level data underlying this study will be made available to independent researchers, subject to review by an independent panel, at www.clinicalstudydatarequest.com. To further protect the privacy of patients and individuals involved in our studies, GSK does not publically disclose subject level data.

REFERENCES

1. Mylvaganam GH, Silvestri G, Amara RR. HIV therapeutic vaccines: moving towards a functional cure. *Curr Opin Immunol.* 2015;35:1–8.

2. Berkley SF, Koff WC. Scientific and policy challenges to development of an AIDS vaccine. *Lancet.* 2007;370:94–101.

3. Leroux-Roels I, Koutsoukos M, Clement F, et al. Strong and persistent CD4 + T-cell response in healthy adults immunized with a candidate HIV-1 vaccine containing gp120, Nef and Tat antigens formulated in three adjuvant systems. *Vaccine.* 2010;28:7016–7024.

4. Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity.* 2012;36:491–501.

5. Goepfert PA, Tomaras GD, Horton H, et al. Durable HIV-1 antibody and T-cell responses elicited by an adjuvanted multi-protein recombinant vaccine in uninfected human volunteers. *Vaccine.* 2007;25:510–518.

6. Van Braeckel E, Bourguignon P, Koutsoukos M, et al. An adjuvanted polyprotein HIV-1 vaccine induces polyfunctional cross-reactive $CD4^+$ T cell responses in seronegative volunteers. *Clin Infect Dis.* 2011;52:522–531.

7. Van Braeckel E, Desombere I, Clement F, et al. Polyfunctional CD4(+) T cell responses in HIV-1-infected viral controllers compared with those in healthy recipients of an adjuvanted polyprotein HIV-1 vaccine. *Vaccine.* 2013;31:3739–3746.

8. Harrer T, Plettenberg A, Arastéh K, et al. Safety and immunogenicity of an adjuvanted protein therapeutic HIV-1 vaccine in subjects with HIV-1 infection: a randomised placebo-controlled study. *Vaccine.* 2014;32:2657–2665.

9. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific $CD8^+$ T cells. *Blood.* 2006;107:4781–4789.

10. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. *J Virol.* 2004;78:8844–8851.

11. Soghoian DZ, Jessen H, Flanders M, et al. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. *Sci Transl Med.* 2012;4:123ra25.

12. Bourgeois C, Veiga-Fernandes H, Joret AM, et al. CD8 lethargy in the absence of CD4 help. *Eur J Immunol.* 2002;32:2199–2207.

13. Janssen EM, Lemmens EE, Wolfe T, et al. CD4$^+$ T cells are required for secondary expansion and memory in CD8$^+$ T lymphocytes. *Nature.* 2003;421:852–856.

14. Letvin NL, Mascola JR, Sun Y, et al. Preserved CD4$^+$ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science.* 2006;312:1530–1533.

15. Lichterfeld M, Kaufmann DE, Yu XG, et al. Loss of HIV-1-specific CD8$^+$ T cell proliferation after acute HIV-1 infection and restoration by vaccine-induced HIV-1-specific CD4$^+$ T cells. *J Exp Med.* 2004;200:701–712.

16. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science.* 2003;300:337–339.

17. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science.* 2003;300:339–342.

18. Sun JC, Williams MA, Bevan MJ. CD4$^+$ T cells are required for the maintenance, not programming, of memory CD8$^+$ T cells after acute infection. *Nat Immunol.* 2004;5:927–933.

19. Yang TC, Millar J, Groves T, et al. On the role of CD4$^+$ T cells in the CD8$^+$ T-cell response elicited by recombinant adenovirus vaccines. *Mol Ther.* 2007;15:997–1006.

20. National Institute of Allergy and Infectious Diseases DoHHS, National Institutes for Health. Division of AIDS table forgrading the severity of adult and pediatric adverse events. 2004; http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/daidsaegradingtable.pdf. [Accessed January 20, 2014].

21. Verbeke G, Molenberghs G. Linear Mixed Models in Practice: A SAS-Oriented Approach New York, NY: ; 1997.

22. Verbeke G, Molenberghs G. Linear Mixed Models for Longitudinal Data. New York, NY: ; 2000.

23. Papagno L, Alter G, Assoumou L, et al. Comprehensive analysis of virus-specific T-cells provides clues for the failure of therapeutic immunization with ALVAC-HIV vaccine. *AIDS.* 2011;25:27–36.

24. Soghoian DZ, Streeck H. Cytolytic CD4(+) T cells in viral immunity. *Expert Rev Vaccines.* 2010;9:1453–1463.

25. Carcelain G, Autran B. Immune interventions in HIV infection. *Immunol Rev.* 2013;254:355–371.

26. Keefer MC, Gilmour J, Hayes P, et al. A phase I double blind, placebo-controlled, randomized study of a multigenic HIV-1 adenovirus subtype 35 vector vaccine in healthy uninfected adults. *PloS One.* 2012;7:e41936.

27. McDermott AB, Koup RA. CD8(+) T cells in preventing HIV infection and disease. *AIDS.* 2012;26:1281–1292.

28. A Phase I Double Blind, Placebo-Controlled, Randomized Study of the Omosa-Manyonyi GMpendo J, Ruzagira E, Kilembe W, et al. Safety and immunogenicity of an adjuvanted HIV-1 Gag-Pol-Nef fusion protein and adenovirus 35 Gag-RT-Int-Nef vaccine in healthy HIV-uninfected African adults. *PLoS One.* 2015;10:e0125954.

29. Kim JH, Rerks-Ngarm S, Excler JL, et al. HIV vaccines: lessons learned and the way forward. *Curr Opin HIV AIDS.* 2010;5:428–434.