# Extended Evaluation of the Virologic, Immunologic, and Clinical Course of Volunteers Who Acquired HIV-1 Infection in a Phase III Vaccine Trial of ALVAC-HIV and AIDSVAX B/E

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(See the editorial commentary by Spetz and Chiodil on pages 1189–92.)

***Background.*** The Thai Phase III Trial of ALVAC-HIV and AIDSVAX B/E showed an estimated vaccine efficacy (VE) of 31% to prevent acquisition of human immunodeficiency virus (HIV). Here we evaluated the effect of vaccination on disease progression after infection.

***Methods.*** CD4$^+$ T-cell counts and HIV viral load (VL) were measured serially. The primary analysis evaluated vaccine efficacy (VE$_P$) as the percent reduction (vaccine vs placebo) in cumulative probability of a primary composite endpoint of clinical and CD4$^+$ count components at prespecified time points after infection. Secondary analyses of biomarker-based endpoints were assessed using marginal mean and linear mixed models.

***Results.*** There were 61 endpoints in the modified intent-to-treat cohort (mITT; $n = 114$). There was no evidence for efficacy at 30, 42, 54, and 60 months in the mITT and per protocol ($n = 90$) cohorts. Estimated VE$_P$ (mITT) was 15.8% (−21.9, 41.8) at 60 months postinfection. There was weak evidence of lower VL and higher CD4$^+$ count at 60 and 66 months in the vaccine group. Lower mucosal VL was observed among vaccine recipients, primarily in semen ($P = .04$).

***Conclusions.*** Vaccination did not affect the clinical course of HIV disease after infection. A potential vaccine effect on the genital mucosa warrants further study.

***Trial registration.*** Clinicaltrials.gov identifier: NCT00337181.

The RV144 Thai Phase III HIV vaccine study demonstrated modest (31.2%) but statistically significant efficacy in the prevention of human immunodeficiency virus type 1 (HIV-1) infection through 42 months after enrollment but failed to impact early pos-infection viral load or CD4$^+$ T-cell counts [1], endpoints that might presumably be subjected to vaccine-induced adaptive cellular immune responses more efficiently after vaccination than natural infection [2–4]. There are several lines of evidence suggesting these responses might play a role in the control of HIV-1 viremia. In studies of HIV-infected subjects, control of viremia is associated with CD8$^+$ cytotoxic T lymphocytes (CTLs)

directed primarily at Gag protein [5–10]. From animal studies, lower levels of viremia after intravenous challenge have been reported for several T-cell–based vaccine regimens, including canarypox HIV-1 candidates [11–13]. Although antibodies, and broadly neutralizing antibody (bNAb) responses in particular, are thought to be the primary correlate of immune protection for most licensed vaccines (reviewed in [14]) and essential for protection against HIV-1 acquisition (reviewed in [15]), humoral immunity does not appear to significantly impact the progression of natural HIV infection [16, 17].

Studies with the vaccine regimen employed in the RV144 study and similar canarypox-based regimens have demonstrated induction of CD4+ T cells, CD8+ CTLs, and neutralizing antibodies (NAb) against T-cell line adapted HIV-1 isolates [18–20], but no primary isolate neutralizing antibody (peripheral blood mononuclear cell [PBMC]) or Tier 2 NAb was detected. Despite the measurement of HIV-1 specific cytolytic T cells after 14-day in vitro stimulation with the chromium release assay, more recent indirect CTL evaluation by direct ex vivo measurement using flow-cytometry based techniques or interferon-γ enzyme-linked immunospot assay demonstrated minimal induction of CTLs by the prime-boost regimens with canarypox-vectored vaccines, including the RV144 prime-boost combination [18–23].

Given the absence of strong ex vivo CTL, bNAb responses, and the lack of a vaccine-associated effect on viremia or CD4+ T-cell count, it is less likely that differences in HIV disease outcomes would be seen in extended follow-up of RV144. This prediction assumes that viral load and CD4+ T-cell impact on longer-term clinical outcomes are similar between vaccine breakthrough infection and naturally occurring HIV infection. However, Letvin et al [24] showed that monkeys receiving DNA/recombinant adenovirus serotype 5 simian immunodeficiency virus (SIV) vaccines have a survival advantage over placebo despite equivalent viral load setpoint after intravenous SIVmac251 challenge. In contrast, declining naturally acquired antibody or vaccine-induced immune responses in humans have been associated with more severe disease in dengue infection and also after early killed measles and respiratory syncytial virus (RSV) vaccines [25]. Therefore, in this study, extended follow-up of infected volunteers in RV144 was important in order to evaluate possible late effects of vaccination on the course of both clinical and biomarker-based events reflecting HIV disease progression.

## METHODS

### Study Design and Population

This is a prospective, extended follow-up of volunteers enrolled in the RV144 Thai Phase III HIV vaccine study conducted at Thai Ministry of Public Health facilities in Rayong

and Chonburi provinces, who received at least 1 vaccination and became HIV infected. There were 132 infections in RV144, 7 of which occurred prior to the first vaccination, yielding 125 eligible for evaluation in study protocol RV152. The details of the original RV144 study can be found in Rerks-Ngarm et al [1, 26]. Participants in RV144 were eligible for extended follow-up in RV152 if they received at least 1 vaccination, became HIV infected prior to completion of the final RV144 study visit, and provided written, informed consent.

The study was designed to test differences between subjects, who received either vaccine or placebo in RV144, from the estimated date of infection to the time when a prespecified primary composite endpoint was reached. The primary composite endpoint was defined as the first occurrence of 1 of the 3 endpoints: (1) CD4+ T-cell count confirmed <350 cells/μL on at least 2 measurements 2 weeks apart; (2) AIDS-defining illness according to US Centers for Disease Control 1993 and Thai Ministry of Public Health case definitions; (3) initiation of highly active antiretroviral therapy (HAART). Secondary objectives presented in the prespecified analysis plan evaluated the vaccine effect on (1) long-term clinical outcomes, AIDS-defining illnesses, and death; (2) longitudinal trajectory of pre-HAART viral loads and pre-HAART CD4+ T-cell counts; (3) mucosal viral load at the first RV152 study visit. Additional secondary objectives that were not part of this analysis included (1) cellular and humoral immune responses; (2) postinfection CD4+ and CD8+ T-cell responses, lymphoproliferation, and neutralizing antibody responses pre- and post-HAART; (3) host genetics and HIV-1 viral sequences.

Volunteers and treating physicians remained blinded to RV144 primary treatment allocation (vaccine/placebo). After enrollment in RV152, follow-up visits were scheduled at 0, 1, 3, and 6 months, and every 3 months thereafter. After month 12, CD4+ T-cell counts and viral load were obtained at 6-month intervals until the CD4+ T-cell count declined to <350/μL or HAART was initiated, at which time CD4+ T-cell counts and viral load were obtained every 3 months. Peripartum antiretroviral drugs given for prevention of mother-to-child-transmission was not considered a study endpoint; however, HAART initiated during pregnancy and continued postpartum was counted. After a single CD4+ T-cell count <350/μL a second sample was requested about 2 weeks later, and if the confirmatory measurement was >350/μL, a study endpoint was not registered and the volunteer resumed a normal visit schedule. A single genital fluid collection for viral load was obtained at the first RV152 visit. Clinical and laboratory data from RV144, including CD4+ T-cell ccount and HIV-1 plasma viral load measurements, were linked to RV152 to inform primary and secondary protocol analyses as well as volunteer care and treatment. An independent, blinded Endpoints Adjudication Committee composed of US and Thai

HIV/AIDS experts confirmed each study endpoint. Volunteers received HAART according to World Health Organization (WHO) and Thai Ministry of Public Health guidelines, whether they enrolled in RV152 or not. Those with asymptomatic HIV infection received HAART when the $CD4^+$ T-cell count was ≤200 cells/μL, which changed to 350 cells/μL in 2009. The ethics committees of the Ministry of Public Health, Mahidol University, the Royal Thai Army, and the Walter Reed Army Institute of Research approved the study. The manufacturers were full trial collaborators and were a part of the trial steering committee. Both RV144 (NCT00223 080) and RV152 (NCT00337181) are registered with Clinical Trials.gov.

### Statistical Analysis

Time-to-event analyses evaluated the time between the estimated date of HIV-1 infection and endpoints measuring HIV-1 progression. The date of HIV-1 infection was estimated as described elsewhere [1]. The primary analysis evaluated a vaccine efficacy parameter, $VE_P$, defined as the percent reduction (vaccine vs placebo) in the cumulative probability of the primary composite endpoint at prespecified time points ($T_A$) of 30, 42, 54, and 66 months after the estimated date of infection. Wald statistics based on Kaplan-Meier and Greenwood estimates were used to test the null hypothesis of $VE_P = 0\%$ and to construct 95% point-wise confidence intervals. The cause-specific cumulative incidence curves for each of the 3 components of the primary composite endpoint were estimated by nonparametric maximum likelihood [27]. Because RV144 showed evidence for a vaccine effect on HIV-1 infection, the analysis of postinfection vaccine effects is susceptible to postrandomization selection bias. Therefore, sensitivity analyses were also performed using the semiparametric maximum likelihood method of Shepherd, Gilbert, and Lumley [28].

Pre-HAART mean viral loads and $CD4^+$ T-cell counts were modeled across the planned postinfection diagnosis visit time points <1, 1, 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 54, 60, and 66 months with marginal mean models, estimated via generalized estimating equations with multiple imputation (MIGEE) to handle missing data due to HAART initiation or missed visits. Parametric linear models and nonparametric Wilcoxon rank sum tests were used to assess vaccine effect on mucosal viral load stratified by specimen type (seminal or cervicovaginal). Logistic regression was used to assess vaccine effect on the proportion of subjects with undetectable viral load. Spearman rank correlation coefficients and the Kappa statistic were used to assess the correlation or concordance of viral loads measured for paired plasma and mucosal specimens. Further details about the statistical analysis can be found in the supplementary methods.

**Table 1.** Baseline Characteristics of Subjects in the Modified Intent-to-Treat Cohort Who Are Infected With Human Immunodeficiency Virus

| Characteristic | Total, n = 114 (%) | Vaccine, n = 49 (%) | Placebo, n = 65 (%) | P |
|---|---|---|---|---|
| Sex at birth | | | | |
| Male | 67 (58.8) | 30 (61.2) | 37 (56.9) | .64 |
| Female | 47 (41.2) | 19 (38.8) | 28 (43.1) | |
| Age | | | | |
| ≤20 | 22 (19.3) | 12 (24.5) | 10 (15.4) | .27 |
| 21–25 | 51 (44.7) | 18 (36.7) | 33 (55.8) | |
| ≥26 | 41 (36.0) | 19 (38.9) | 22 (33.9) | |
| Risk group | | | | |
| Low | 45 (39.5) | 17 (34.7) | 28 (43.1) | .41 |
| Medium | 28 (24.6) | 11 (22.4) | 17 (26.2) | |
| High | 41 (36.0) | 21 (42.9) | 20 (30.8) | |
| Calendar year of infection diagnosis | | | | |
| 2004–2005 | 30 (26.3) | 13 (26.5) | 17 (26.2) | .09 |
| 2006 | 39 (34.2) | 13 (26.5) | 26 (40.0) | |
| 2007 | 27 (23.7) | 17 (34.7) | 10 (15.4) | |
| 2008–2009 | 18 (15.8) | 6 (12.2) | 12 (18.5) | |
| Received treatment during pregnancy | | | | |
| Yes | 13 (27.7) | 7 (36.8) | 6 (21.4) | .25 |
| No | 34 (72.3) | 12 (63.2) | 59 (78.6) | |

## RESULTS

### Study Population

A total of 120 HIV-1–infected RV144 volunteers enrolled in RV152, including 6 of the 7 subjects infected prior to vaccination in RV144 [1] and were not included in the mITT analysis, yielding a total of 114 participants (49 vaccine and 65 placebo recipients). The per-protocol analysis includes 90 infected volunteers, 39 vaccine and 51 placebo recipients, who completed all 4 vaccinations and were HIV negative at 24 weeks. This definition differs from RV144 where per-protocol subjects received all vaccinations within a protocol-defined study window. There were no significant differences in baseline characteristics between the 49 vaccine and 65 placebo recipients in the RV152 mITT analysis (Table 1).

### Primary Analysis

The primary analysis (Figure 1A) assesses the vaccine effect on the primary composite endpoint by the prespecified fixed time points 30, 42, 54, and 66 months after the estimated date of HIV-1 infection, with 95% simultaneous confidence intervals (*bold vertical segments*). In the mITT cohort, $VE_P$ ($T_A$) estimates (95% confidence intervals) at $T_A$ = 30, 42, 54, and 66 months were 2.2% (−64.4, 41.9), −10.1 (−70.3, 28.8), −3.6 (−51.7, 29.2), and 15.8 (−21.9, 41.8). In the per-protocol
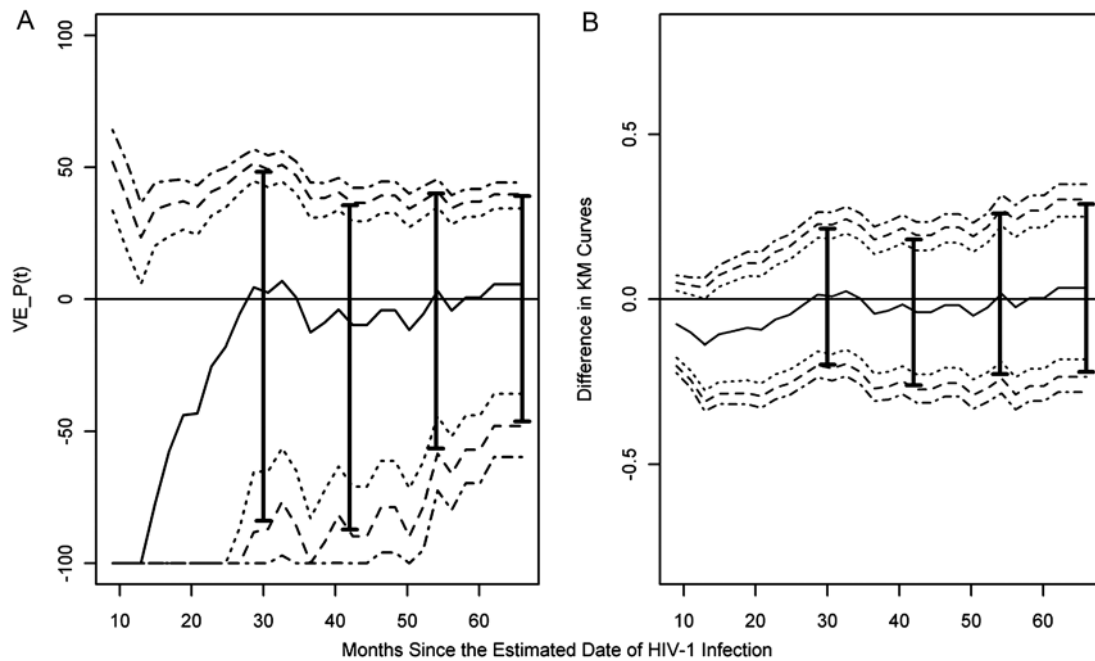
**Figure 1.** Primary analysis of the vaccine effect on the time from estimated date of infection with human immunodeficiency virus type 1 (HIV-1) until the composite endpoint. *A*, Estimated $VE_P(t)$ at the prespecified time points: 30, 42, 54, 66 months. *B*, Estimated survival curve difference (vaccine minus placebo) at all time points through 66 months (modified intent-to-treat [mITT] cohort). Point estimates (*solid lines*), 95% pointwise confidence intervals (*dotted lines*), and 80% and 95% simultaneous confidence intervals (*dashed lines*) over all follow-up times since the estimated date of infection with human immunodeficiency virus type 1 (HIV-1), and 95% simultaneous confidence intervals for the 4 prespecified time points: 30, 42, 54, and 66 months (bold vertical segments).

cohort, $VE_P$ $(T_A)$ estimates were 13.8 (−49.4, 50.3), −1.0 (−60.2, 36.3), −8.4 (−62.8, 27.8), and −7.8% (−58.9, 26.9). Time to the primary composite endpoint evaluated over all times 0 through 66 months also showed no difference between vaccine and placebo (Figure 1B).Univariate analyses of age, sex, baseline behavioral risk level (low, medium, high), calendar year of HIV-1 infection diagnosis, and plasma viral load at infection diagnosis were examined, and only plasma viral load at diagnosis significantly predicted the primary composite endpoint. The hazard ratio (HR) estimate was $1.83/\log_{10}$ increase, $P = .0004$ for the mITT cohort and $1.84/\log_{10}$ increase, $P = .001$ for the per-protocol cohort. Assessment of the cumulative incidence of each of the 3 component endpoints of the primary composite endpoint (as the first occurring endpoint) showed no difference with respect to vaccine or placebo (Figure 2A, $P > .20$). $CD4^+$ T-cell count $<350/\mu L$ was the dominant endpoint trigger (89% of the 61 endpoints, Supplementary Table 1). There is a descriptive increase in the rate of the $CD4^+$ T-cell endpoint in the vaccine group, and a corresponding increase in the primary composite endpoint rate that disappears just prior to month 30. Although for most of the study HAART was initiated at or below a $CD4^+$ T-cell count of $200/\mu L$, there again was evidence of a nonsignificant increase in HAART use among vaccine recipients early after

infection and resolving by 52 months (Figure 2B). Importantly, there was no evidence for a vaccine causal effect for accelerated time-to-HAART using the prespecified sensitivity analysis method of Shepherd et al [28] (see supplementary results for further details).

**Impact of Vaccination on Pre-HAART Viral Load**

Figure 3 shows individual pre-HAART viral load and $CD4^+$ T-cell count trajectories for subcohorts defined by calendar year of infection diagnosis. There was no difference in mean pre-HAART viral loads between vaccine and placebo at 12 (estimated means, 4.38 and $4.34 \log_{10}/mL$ in vaccine and placebo, respectively, $P = .90$) or 18 months postinfection diagnosis (4.32 and 4.44 $\log_{10}$ copies/mL in vaccine and placebo, respectively, $P = .69$), extending the initial findings in RV144. Analysis of the vaccine effect on mean pre-HAART log10 viral load using the MIGEE method shows no difference between the vaccine and placebo groups through month 48 (Figure 4A). At the last 2 visit time points (months 60 and 66), the mean pre-HAART log10 viral load was significantly lower in the vaccine group than the placebo group. However, the statistical inference is unstable at these late time points, depending on a few data points, suggesting that the evidence for a vaccine effect is weak. Accounting for all time points, an
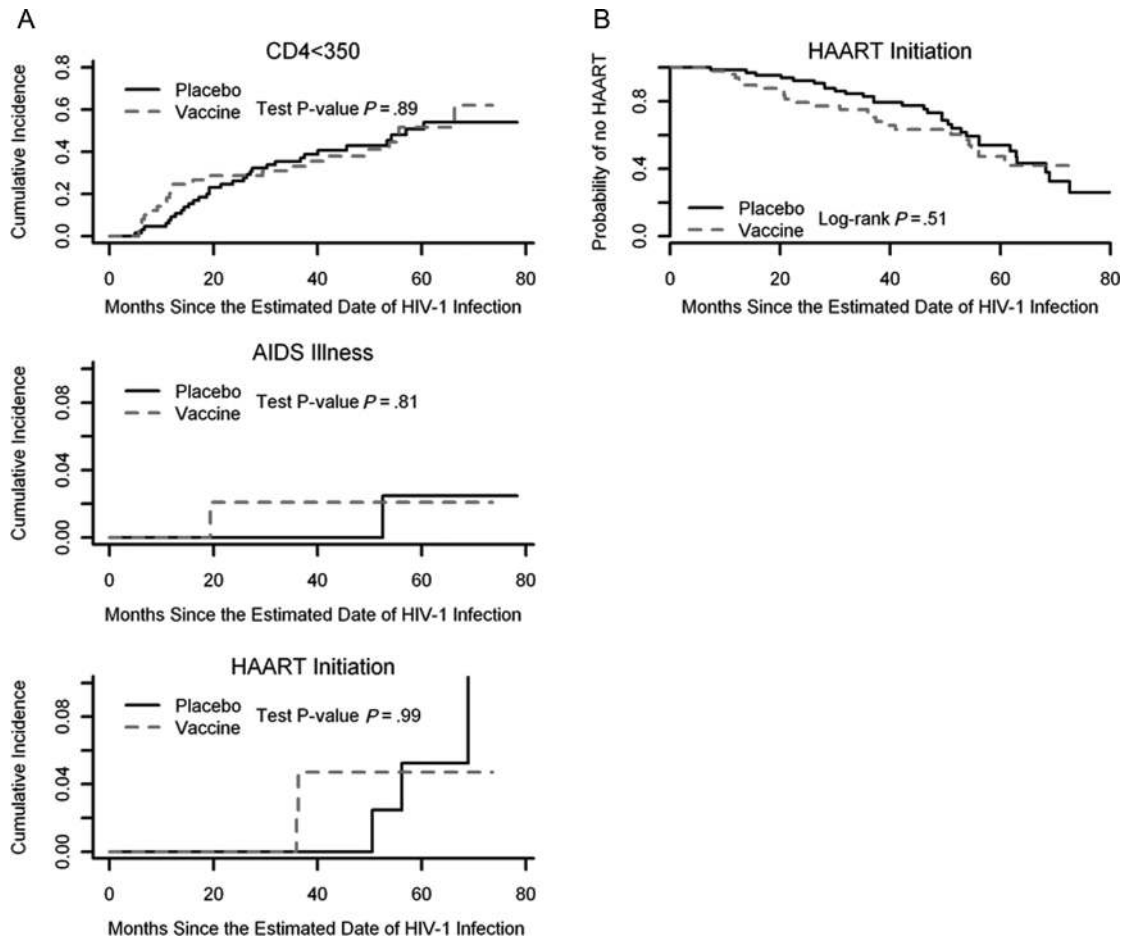
**Figure 2.** *A*, Cause-specific cumulative incidences of the 3 component endpoints of the primary composite endpoint. Each graph represents data for those individuals who met criteria for CD4 count, AIDS-defining illness, or highly active antiretroviral therapy (HAART) initiation as a primary endpoint. *B*, Kaplan-Meier curves of the time from estimated date of infection with human immunodeficiency virus type 1 (HIV-1) until HAART initiation (modified intent-to-treat [mITT] cohort) for all participants.

overall test of whether the vaccine had an effect on pre-HAART log10 viral load was not statistically significant ($P = .21$ from MIGEE and $P = .43$ from restricted maximum likelihood models).

### Impact of Vaccination on Pre-HAART CD4⁺ T-Cell Count

At each of the timepoints after infection diagnosis (between months 1 and 54), the mean pre-HAART square-root CD4⁺ T-cell count was comparable between the vaccine and placebo groups, although the vaccine group had a slightly lower mean CD4⁺ T-cell count between visit months 1 and 42 (Figure 4*B*). At the last 2 visit time points (months 60 and 66), the mean pre-HAART square-root CD4⁺ T-cell count was significantly higher in the vaccine group compared to the placebo group. Again the instability of the statistical inference at these late time points suggests the evidence for a vaccine effect is weak. Accounting for all time points, an overall test of whether the vaccine had an effect on pre-HAART CD4⁺ T-cell count was

not statistically significant ($P = .20$ from MIGEE and $P = .30$ from REML).

### Genital Fluid Viral Load

Viral load was measured in semen and cervical vaginal lavage at the first RV152 study visit. As the cervical vaginal lavage yields are highly variable despite a constant amount of lavage fluid (5 mL), seminal viral load is an intrinsically more accurate measure of virus in mucosal secretions. Vaccination was associated with lower genital fluid viral load in the mITT analysis (Figure 5. The association of vaccination and lower viral load was borderline significant [Wilcoxon rank sum test $P = .04$ adjusting for specimen type and mucosal specimen collection time (≤6 months, 6–12 months, and >12 months postinfection) and $P = .06$ unadjusted. The effect was more pronounced in men ($P = .04$) than women ($P = .68$). On the basis of the mixed effects model, the estimated mean seminal viral load was 1.75 versus 2.55 for vaccine versus placebo in the unadjusted model
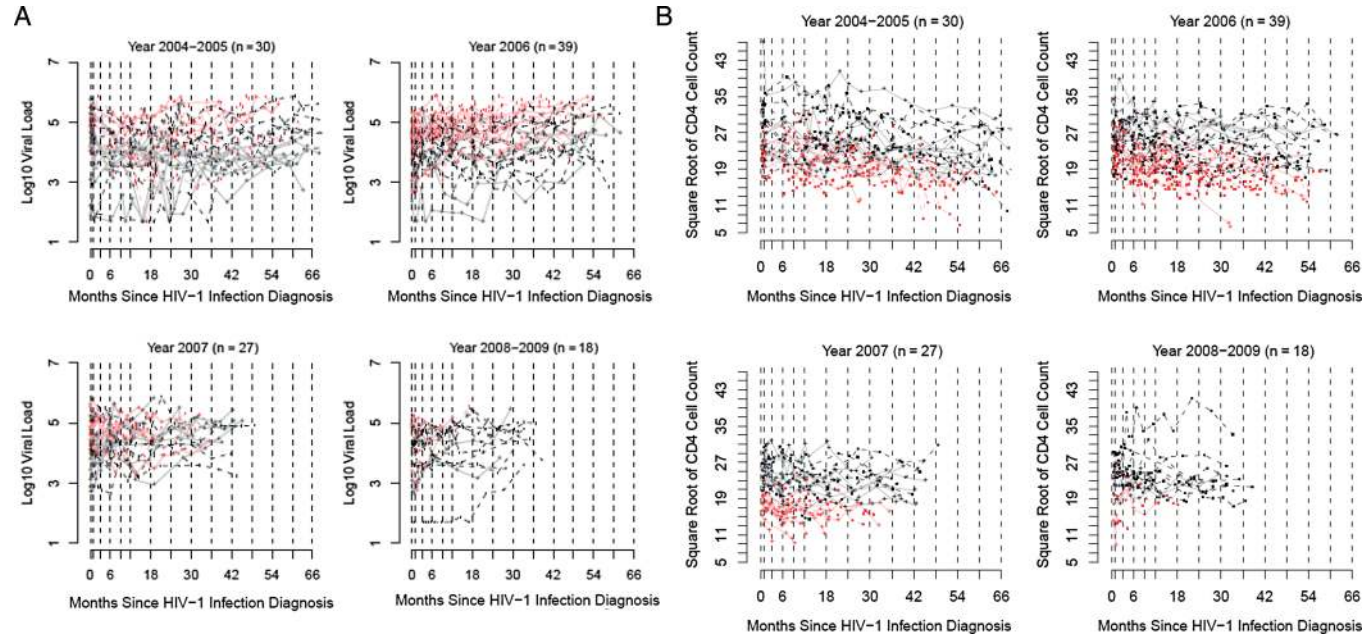
**Figure 3.** Individual longitudinal pre-HAART (highly active antiretroviral therapy) biomarker trajectories for (*A*) log10 viral load and (*B*) square root CD4$^+$ T-cell count by calendar year of infection diagnosis (modified intent-to-treat [mITT] cohort). *Red lines*: Pre-HAART trajectories for subjects who later started HAART; *black lines*: Pre-HAART trajectories for subjects who never started HAART; *plus sign*, vaccine recipient; *solid circle*, placebo recipient.
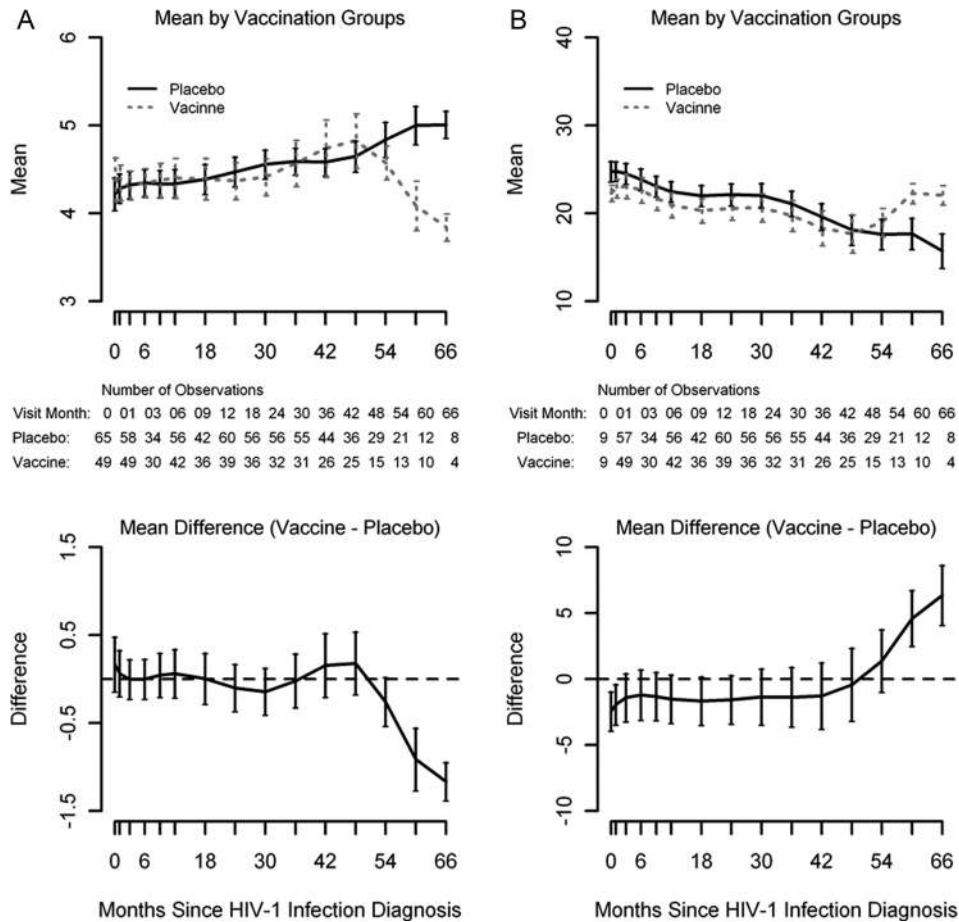
**Figure 4.** *A*, Estimated pre-HAART (highly active antiretroviral therapy) mean log10 viral loads and difference between vaccine and placebo arms. *B*, Estimated pre-HAART mean square root of CD4+ T-cell counts and difference between vaccine and placebo arms at scheduled visits from infection with human immunodeficiency virus type 1 (HIV-1) diagnosis through 66 months using generalized estimating equations (GEE) with multiple imputations to fill in missing values due to HAART initiation, drop-out, or missed visits (mITT cohort).

($P = .04$) and was 1.91 versus 2.47 for vaccine versus placebo adjusting for the same variables as the Wilcoxon test, together with age and behavioral risk ($P = .14$). There was a greater frequency of undetectable viral load (VL <50 copies/mL) in genital fluids of vaccinated volunteers in both the mITT ($P = .03$) and per-protocol analyses ($P = .09$).In a logistic regression model that included treatment group, sex, age, baseline behavior risk, specimen collection time, and concurrent plasma viral load as covariates, vaccination (odds ratio [OR], 1.72, $P = .04$), female sex (OR, 1.76, $P = .03$), medium behavior risk (OR, 0.52, $P = .03$), and concurrent plasma viral load (OR, 0.17, $P < .001$) were associated with having undetectable HIV-1 viral RNA in the mucosal compartment. There was moderate correlation between plasma and seminal fluid viral load (Spearman $r = 0.58$, $P < .001$). A weak correlation also existed for cervical vaginal lavage viral load (Spearman $r = 0.38$, $P = .22$).

In an effort to assess confounding that could arise with the primary and secondary analyses of this nonrandomized HIV-infected cohort, the postinfection endpoint data were also analyzed from the time of randomization in the entire original HIV-1–negative randomized cohort (16 395 mITT subjects) [29]. Because this analysis can only be performed over the follow-up period for capturing HIV-1 infections (42 months), the number of composite endpoints is too low to appropriately power this post hoc analysis. Although the survival curves (primary endpoint) appear to show a benefit when counted from the time of initial vaccination, ultimately, a statistically significant result is not seen (Supplementary Figure 1).

## DISCUSSION

The impact of vaccination on the course of postvaccination (breakthrough) HIV infection has clinical, scientific, and regulatory implications. In HIV-infected persons who have not received HIV vaccination (reviewed in Gurunathan et al [30]),
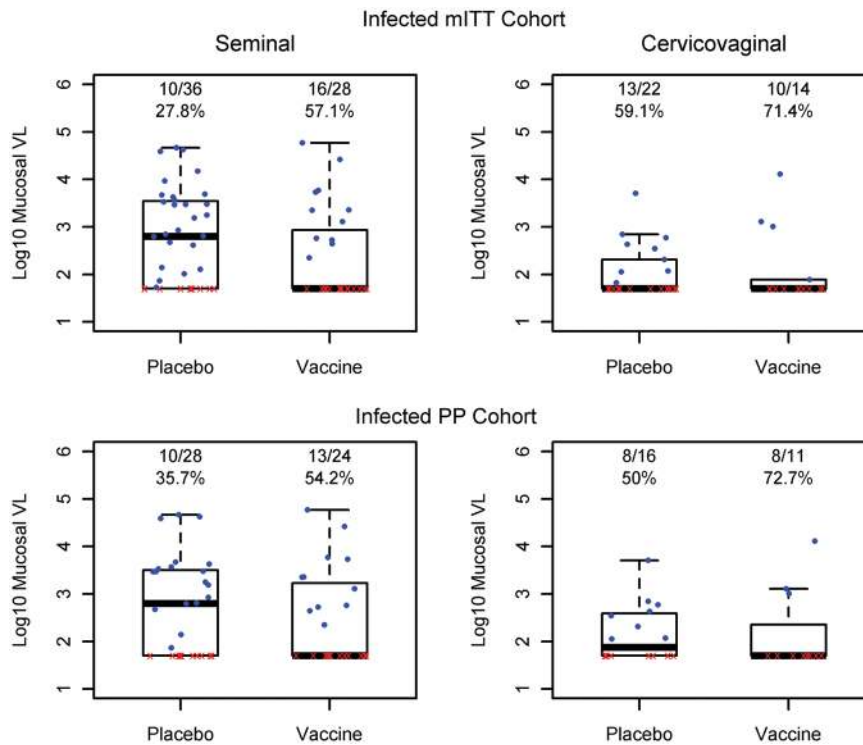
**Figure 5.** Box plots of seminal and cervicovaginal pre-HAART viral RNA ($\log_{10}$ copies/mL); percentages undetectable (copies/mL <50 shown in x's) are listed above the boxplots for both mITT and per-protocol cohorts. Both means and percent undetectable viral loads were statistically significantly different for semen ($P = .04$ and .03, respectively).

postinfection prognosis is associated with set-point viral load [31], CD4^{+} T-cell count, and cellular immune activation [32, 33], HIV DNA[25, 34], and combinations of these factors. The Thai Phase III trial did not demonstrate an immediate (early postinfection) effect on viral load or CD4^{+} T-cell count [1]. Extended follow-up of RV144 breakthrough infections was undertaken to see if the vaccination altered the longer term course of postinfection CD4^{+} T-cell decline or viral load and ultimately affect clinical outcome. In one study of nonhuman primates immunized with recombinant DNA and an adenovirus serotype 5 vector prime-boost combination, vaccination was associated with longer survival and a lower viral load as measured by area under the curve (AUC), but only prior to viral set point [24]—an analysis unfeasible in human clinical trials given the need for multiple viral load measurements in very early acute infection.

In this study, prime-boost vaccination with ALVAC-HIV and AIDSVAX B/E did not affect the occurrence of the composite endpoint, with the majority of endpoints represented by the component endpoint of time to a CD4^{+} T-cell count <350 cells/μL. Longitudinal assessment of pre-HAART viral loads showed no difference between vaccine and placebo groups through 48 months after infection diagnosis. Beginning at week 48, however, there is weak evidence for a lower viral

load in the vaccine group, and this corresponds to a higher CD4^{+} T-cell count in vaccinees, suggesting a potential benefit. Possible explanations include a late effect of vaccination resulting in delayed secondary vaccine-induced immune responses or early elimination of rapid HIV progressors from the vaccinated pool of breakthrough infection volunteers. Several lines of evidence suggest that postinfection cellular immune responses in vaccine recipients are different from those seen in placebo recipients (de Souza et al, submitted). Whether or not the lower viral load and higher CD4^{+} T-cell counts are a transient effect or presage a longer term effect on survival related to unspecified host genetic or vaccine-induced response is speculative. Interestingly, the finding of reduced genital fluid viral load in vaccinated recipients compared to placebo recipients suggests that there may be vaccine-induced effects, potentially caused by mucosal immune responses that are not reflected by the peripheral blood immune assessments performed in RV 144.

Little is known about the control of viral replication in genital mucosa, so the finding of lower viral RNA in vaccine recipients is unexpected and, if confirmed, would suggest a potential public health benefit. This hypothesis-generating finding may have methodological problems in that female genital fluid viral load was obtained from measurements of

cervical vaginal lavage, which are notably variable [35–37]. Previous data demonstrate the seminal viral load is usually lower than the plasma viral load, and we found that both were moderately correlated in this study [35, 38]. It is sometimes argued that seminal virus represents a sanctuary (reviewed in [39]), but the absence of apparent immune effects in the periphery with the lower viral load in genital fluid of vaccinees is difficult to explain as mucosal immune responses were not evaluated in these volunteers. Future clinical trials of the RV144 regimen are being designed to include more rigorous collection of mucosal specimens to describe innate and adaptive mucosal immune responses.

Recently, Barouch et al [40], using a heterologous SIV challenge of adenovirus type 26/modified vaccinia Ankara-based SIV vaccines showed differential effects in the prevention of acquisition and control of viremia postinfection in macaques. Analyses demonstrated that $CD8^+$ CTLs correlated with viremic control but that total binding antibody and Tier 1 neutralization correlated with protection from acquisition. Data from Letvin et al [41], in a trial of DNA/rAd5 vaccination with heterologous challenge of SIVsmE660, suggested that protection from acquisition was also associated with neutralization when a PBMC assay was used. In those nonhuman primates showing viremic control, a survival effect was seen. In addition, studies evaluating replicating CMV-SIV vectors show strong T-cell effect or memory responses elicited by the vaccine are associated with control of viremia [11, 42]. Collectively, these data suggest that improving cellular immune responses could potentially yield both survival and acquisition benefit, and HIV vaccines that effectively bring both humoral and cellular mechanisms into play may provide greater efficacy.

Findings from RV152 highlight some of the following considerations when evaluating breakthrough HIV infections from a vaccine study: The lack of randomization inherent to these studies entails additional evaluation of the results, usually with sensitivity analyses to assess the level of this potential selection bias. Consequently, there would be reduced power for detecting causal vaccine effects on postinfection endpoints. The use of HAART by infected trial participants requires that posttherapy data be imputed to approximate pretherapy differences in viral load and $CD4^+$ T-cell trajectories. These challenges may confound and complicate future HIV vaccine trials, which may need to accommodate other prevention interventions in the face of data from preexposure prophylaxis and vaginal microbicide studies [43, 44]. Earlier initiation of antiretroviral therapy advocated in many guidelines may also limit long-term evaluation of breakthrough infections [45, 46]. Finally, the impact of viral and host genetics on both acquisition and postinfection sequelae should also be considered in HIV vaccine trials and is part of ongoing analyses of data from this study, which we hope will provide further insight for HIV vaccine development.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

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