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Conflicts of Interest

DVM, RM, DRC, KMG, JAC, MNR all are paid employees of Merck & Co., Inc and own Merck stock and have Merck stock options; DL has Merck stock; SPB, MM and MJM have all served as investigators on Merck-funded research; AD, DWF, PB, JRL, CDR, and LC have no conflicts of interest.

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Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial

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Abstract

Background—Observational data and non-human primate challenge studies suggest that cell-mediated immune (CMI) responses may provide control of HIV replication. The Step Study is the first direct assessment of the efficacy of a CMI vaccine to protect against HIV infection or alter early plasma HIV levels in humans.

Method—HIV-seronegative participants (3000) were randomized (1:1) to receive 3 injections of MRKAd5 HIV-1 gag/pol/nef vaccine or placebo. Randomization was pre-stratified by gender, baseline adenovirus type 5 (Ad5) titer, and study site. Participants were tested ~every 6 months for HIV acquisition; early plasma HIV RNA was measured ~3 months post-HIV diagnosis.

Findings—The vaccine elicited IFN-γ ELISPOT responses in 75% of vaccinees. In a pre-specified interim analysis among participants with baseline Ad5 ≤200, 24 of 741 vaccinees became HIV infected, versus 21 of 762 placebo recipients. All but one infection occurred in men. The early geometric mean plasma HIV RNA was comparable in infected vaccine and placebo recipients. In exploratory multivariate analyses, HIV incidence was higher in vaccinees versus placebo recipients among Ad5 seropositive men (5.1% versus 2.2% per year, respectively) and uncircumcised men (5.2% versus 1.4% per year, respectively). HIV incidence was similar in vaccinees versus placebo recipients among Ad5 seronegative men and circumcised men.

Interpretation—This CMI vaccine did not prevent HIV infection or lower early viral level. Mechanisms for failure of the vaccine to protect and for the increased HIV infection rates in subgroups of vaccinees are being explored. Additional follow-up will determine if elevated HIV incidence in vaccinee subgroups persists.

Keywords

HIV vaccine; efficacy; adenovirus; HIV acquisition; viral load; male circumcision; test of concept

Introduction

The development of an efficacious HIV vaccine is one of the world's greatest public health challenges. The lack of a known correlate of protection and the widespread genetic diversity of the virus pose significant scientific hurdles (1). Traditional methods of vaccine design, such as use of live attenuated virus, whole killed virus, or subunit proteins are either thought to be too dangerous or have been found to be ineffective in generating robust immune responses or protecting against HIV (2). No effective strategies have yet been developed to generate broadly neutralizing antibody against HIV, although considerable work is being done in this field (3–6). A substantial body of data points to the importance of CMI responses in controlling viral replication and disease progression in long-term nonprogressors (7–13) and in non-human primate challenge models (14–17). Substantial effort has been devoted to designing and evaluating CMI based vaccines.

Adenovirus type 5 (Ad5) vector-based vaccines have proven to be among the most immunogenic of CMI vaccines in Phase I clinical trials (18;19), surpassing immune responses generated by DNA plasmids (20;21), and many poxvirus vectors (22–24). Non-human primate challenge studies have also demonstrated that SIV Ad5 prototype vaccines led to control of viremia, in some, but not all, challenge models (14;25–27).

Building on this early promising data from prototype vaccines containing a single gene (gag) (28), a candidate vaccine using a mixture of rAd5 vectors expressing the HIV-1 *gag*, *pol* and *nef* genes (18)was developed. These antigens were selected because they are commonly recognized during natural infection and are relatively conserved across different clades of HIV-1. This vaccine mixture was shown in phase 1 trials to elicit immune responses in both Ad5 seronegative and Ad5 seropositive, immunocompetent participants (18). However, because of considerable uncertainty about what would be required of a CMI vaccine to control HIV viral replication, we designed a test-of-concept trial (29) to evaluate the potential public health impact of this CMI vaccine. Test-of-concept trials provide a preliminary assessment of efficacy and allow exploration of immune correlates of protection while being substantially smaller than phase III licensure trials (30).

Methods

Study population

The Step Study is a multicenter, double-blind, randomized, placebo-controlled phase II test of concept study of the MRKAd5 HIV-1 gag/pol/nef vaccine in HIV-1 negative individuals at high risk of HIV-1 acquisition. This trial is being conducted in regions of the world where clade B is the predominant HIV-1 subtype. The trial was initially designed to enroll 1500 participants with low (≤200) Ad5 antibody titers at enrollment, based on reduced levels of immunogenicity seen in persons with higher baseline Ad5 titers (28). After data from a Phase I trial demonstrated robust immune responses even in subjects with pre-existing immunity to Ad5 (18), the trial was expanded to include a cohort of 1500 participants with Ad5 titers >200, to increase the potential global relevance of this vaccine candidate.

Participants were 18–45 years of age, HIV-1 seronegative, with serum alanine transaminase levels ≤ 3 times the upper limit of normal, and at high risk of HIV-1 acquisition based on reported risk behavior in the 6 months prior to enrollment. Men were eligible if they reported: 1) unprotected anal intercourse with a male partner or 2) anal intercourse with ≥ 2 male partners. Heterosexual men from Caribbean sites were also eligible if they reported: 1) a diagnosis of syphilis or genital ulcer disease; 2) ≥2 sexual partners; 3) exchanging sex for money, drugs, services, or gifts; or 4) using crack cocaine ≥3 times. Women were eligible if they reported: 1) unprotected vaginal or anal intercourse with an HIV positive man or an injection drug user;

2) exchanging sex for money, drugs, services, or gifts, or 3) using crack cocaine ≥3 times. Women from Caribbean sites were also included if they reported a diagnosis of syphilis or pelvic inflammatory disease. Participants were excluded if they had a history of immunodeficiency, malignancy, anaphylaxis or allergy to vaccine components, receipt of an experimental HIV vaccine, or other conditions that would interfere with their study participation. Women who were pregnant at screening were excluded; women who became pregnant during the study did not receive further study injections but followed all other study procedures.

Vaccine Description

The MRKAd5 HIV-1 gag/pol/nef vaccine, consisted of a 1:1:1 mixture of 3 separate replication-defective Ad5 vectors, one each expressing the *gag* gene from the HIV-1 strain CAM-1, the *pol* gene from HIV-1 strain IIIB and the *nef* gene from HIV-1 strain JR-FL, as previously described (18). Vaccine was administered as a 1.0 ml injection of 1.5×10^{10} adenovirus genomes, equivalent to the 3×10^{10} viral particle dose used in previous vaccine trials (18). The placebo was a 1.0 ml injection of the vaccine diluent only, with no Ad5 vector.

Study procedures

Participants underwent a thorough written informed consent process. The protocol was approved by the Ethical Review Committee of each site, and the study was conducted in conformance with applicable local and country requirements.

Study participants were randomized in a 1:1 ratio to receive 3 doses of the MRK Ad5 gag/pol/nef vaccine or placebo on Day 1 (study enrollment), Week 4, and Week 26. Randomization was pre-stratified by study site, gender, and baseline Ad5 titer (<18 (lower limit of detection of assay), 18–200, 201–1000, >1000). Study participants were seen at Day 1 and Weeks 2, 4, 8, 12, 26, 30, 52, and every 26 weeks thereafter through week 208. Clinical evaluation and risk reduction counseling were conducted at each visit. Local and systemic reactogenicity was assessed for the 14 days following study injections. Behavioral risk was assessed by self-report at screening and every 26 weeks thereafter, and included standardized interviewer-administered questionnaires about sexual risk, drug use, and sexually transmitted infections in the previous 6 months.

Serum alanine transferase and a complete blood count were measured immediately prior to and two weeks following the first vaccination to assess any hepatic or hematological toxicity from the vaccine. HIV-1 testing was conducted at Day 1, Weeks 12 and 30, 52, and every 26 weeks thereafter through Week 208. If HIV was diagnosed at any visit, stored plasma specimens from earlier time points were tested to accurately time the onset of HIV-1 infection. All HIV-1 tests were performed at a central laboratory. Specimens were screened with an immunoassay (Uni-Gold™ Recombigen® HIV test from Trinity BioTech or the Multispot HIV-1/HIV-2 Rapid Test from Bio-Rad) that only contained HIV envelope antigens, which are not included in the vaccine. Reactive tests were confirmed with an HIV-1 Western blot and HIV-1 plasma viral RNA assay (Amplicor Monitor Version 1.5 from Roche) conducted on the original specimen and a confirmatory specimen. A blinded Endpoint Adjudication Committee consisting of 3 independent experts in HIV-1 diagnostics made the final determination of HIV-1 infection status. All cases were unanimously confirmed by this committee. Participants who became HIV-infected during the study were provided counseling and linkage to local HIV medical and psychosocial care. HIV-1 infected participants underwent clinical and laboratory assessment 1, 2, 8, 12, and 26 weeks after their initial HIV-1 diagnosis, and every 26 weeks thereafter through week 78 post-diagnosis.

Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-anticoagulated blood obtained at weeks 8, 30, 52, and 104, and were cryopreserved within 12 hours of venipuncture, using previously described methods (31). Validated IFN-γ ELISPOT assays (32) were performed on cryopreserved PBMC at the weeks 8 and 30 timepoints on a random sample of 25% of study participants, stratified by treatment assignment and study site.

Study Objectives and Endpoints

The primary objectives were to demonstrate the safety, tolerability, and efficacy of the MRK Ad5 gag/pol/nef HIV-1 vaccine in the study population with baseline Ad5 titers ≤200. The primary objectives focused on the subpopulation initially targeted for this trial and the one likely to have the most robust immune response, based on data from phase I trials. Efficacy was defined as demonstrating a reduction in HIV-1 acquisition rates (infection endpoint) and/or a decrease in HIV-1 viral load set-point (average of 2 $\log_{10}$ HIV-1 RNA values at ~ 3 months after HIV-1 diagnosis) (viral load endpoint), among vaccine versus placebo recipients.

Secondary objectives were to evaluate the safety, tolerability, and efficacy of the vaccine in the entire study population, regardless of baseline Ad5 titer, and to identify immune responses that correlated with efficacy endpoints. Exploratory objectives included evaluation of associations between the co-primary efficacy endpoints (infection and viral load) and prognostic factors such as gender, baseline Ad5 titer, age, race, HLA type, and circumcision status (for males).

Statistical analysis

Pre-specified analyses—All serious vaccine-related adverse experiences, injection-site reactions (within 5 days of each study injection), body temperatures and systemic adverse events (within 15 days of each study injection), and laboratory measures (at pre-specified time points) were summarized. The safety analyses included all randomized subjects that received at least one dose of vaccine or placebo.

To assess vaccine efficacy for the infection endpoint, the number of acquired HIV-1 infections ("events") in the vaccine arm was compared to the corresponding number in the placebo arm using a test for stratified Poisson data (33). To assess vaccine efficacy for the viral load endpoint, viral load set-points for subjects who became HIV-1 infected were compared between treatment groups using a stratified Wilcoxon rank sum test; a pre-specified multiple imputation approach was used to resolve the problem of altered or missing viral load data associated with antiretroviral therapy (ART) initiation or premature study discontinuation, respectively (34).

Two analysis populations were pre-defined. The per-protocol (PP) analysis population included all randomized subjects who received the first two doses of either vaccine or placebo, except those who were either diagnosed with HIV-1 infection before or at week 12 (i.e., 8 weeks post-dose 2) and/or were identified as protocol violators based on predefined criteria. The modified intention-to-treat (MITT) analysis population included all randomized subjects who received at least one dose of vaccine or placebo, except those who had a positive HIV-1 screening test prior to randomization.

The Step Study was an event-driven trial, designed to accrue at least 50 PP events in the Ad5 ≤ 200 stratum and 50 PP events in the Ad5 > 200 stratum (100+ events overall). An alpha-spending interim analysis for the primary efficacy hypotheses was to be conducted when 30 PP events had accrued in the Ad5 ≤ 200 stratum and the corresponding viral load set-point data for the HIV-1 infected subjects were available. Similarly, an alpha-spending interim analysis for the secondary efficacy hypotheses was to be conducted when 30 PP events had accrued in

the Ad5 > 200 stratum and at least 30 PP events had accrued in the Ad5 ≤ 200 stratum (60+ total PP events), and the corresponding viral load data for the HIV-1 infected subjects were available. At the interim analysis for the primary efficacy hypothesis, statistical success for the infection and viral load endpoints was defined as the 1-tailed p-value (in the direction of a vaccine benefit) being less than alpha allocated levels of 0.00025 and 0.025, respectively. The corresponding p-value thresholds for success at the interim analysis for the secondary efficacy hypothesis were 0.000125 and 0.0125, respectively. Futility criteria associated with strong evidence of a lack of vaccine efficacy were also specified upfront: at either planned interim analysis, the vaccine was to be declared ineffective if the 1-tailed p-value was greater than 0.50 for both of the co-primary efficacy endpoints.

For the primary efficacy hypothesis (Ad5 ≤ 200 stratum), 30 events provided 80% power to detect a 1 $\log_{10}$ copies/ml difference (placebo – vaccine) in mean viral load set-point, and 50 events provided 80% power to detect a 60% reduction in the HIV-1 infection rate for vaccine versus placebo. The power calculations were based on a total alpha allocation of 0.05 for the two primary efficacy endpoints, and they accounted for the alpha spending at the interim analysis. Similarly, for the secondary efficacy hypothesis (Ad5 ≤ 200 and Ad5 > 200 strata combined), 60 events provided 80% power to detect a 0.75 $\log_{10}$ copies/ml difference in mean viral load set-point, and 100 events provided 80% power to detect a 50% reduction in the HIV-1 infection rate, based on a total alpha allocation of 0.025 for the two co-primary efficacy endpoints and accounting for the planned interim analysis.

Exploratory analyses—Because the study unexpectedly met the pre-specified futility boundaries at the first interim analysis (see Results), additional analyses were initiated to explore reasons for the vaccine's lack of efficacy and potential for increased HIV-1 acquisition. Data accrued through October 17, 2007, prior to public announcement of study results and participant unblinding, were included in these analyses.

Univariate Cox proportional hazards models were used to quantify treatment effects for various subgroups defined by demographic and/or baseline behavioral risk factors. The time-to-event variable for the Cox model analyses was defined as the time from initial vaccination to the midpoint between the date of the last HIV seronegative visit and the date of the first evidence of HIV infection, as determined by the blinded Endpoint Adjudication Committee. Participants who never showed any evidence of HIV infection were right-censored on the date of their last study visit prior to October 17, 2007. Kaplan-Meier plots were generated to graphically illustrate the treatment effect across the four design-based Ad5 strata (baseline Ad5 titer ≤18, 19–200, 201–1000, >1000). Treatment effects were quantified using estimated hazard ratios (vaccine/placebo) with associated Wald-based 95% confidence intervals (CIs) and two-tailed p-values. Interaction tests were conducted to evaluate whether the treatment effect differed between two given subgroups.

Multivariate Cox models were used to estimate the treatment effect after adjusting for potential confounding variables. Candidate confounders were pre-selected on the basis of their plausibility to impact HIV infection risk. The candidate confounders were all dichotomous for simplicity, stabilizing the model fitting, and reducing the modeling assumptions. The backwards elimination procedure for building the multivariate models used a Wald p-value threshold of 0.15 for removing variables; similar results were observed using a threshold of 0.10.

Role of the Data Safety Monitoring Board

The trial was monitored by an independent Data Safety Monitoring Board (DSMB) consisting of seven experts in clinical trials, vaccinology, statistics, and bioethics. The DSMB met three times per year to review safety data; serious adverse events were reviewed by the DSMB chair

in real time. In September 2007, the DSMB met to review the unblinded data on HIV acquisition and viral load endpoints at the pre-specified interim analysis.

Role of the funding sources

This study was funded by Merck Research Laboratories; the Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), in the US National Institutes of Health (NIH); and the NIH-sponsored HIV Vaccine Trials Network (HVTN). Each of the partners was involved in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

The Step Study opened in December 2004 to study participants with Ad5 titers ≤200 at screening, and was amended to include participants with Ad5 titers >200 at screening in July 2005. Three thousand participants were enrolled through March 2007 at 34 sites in North America, the Caribbean, South America, and Australia. Protocol adherence was excellent, with 94% of the vaccine and placebo groups receiving all 3 study injections (Figure 1). Overall, 6.5% and 5.8% of vaccine and placebo recipients, respectively, had discontinued follow-up in the study. Baseline demographic and risk characteristics are shown in Table 1, stratified by gender and baseline Ad5 titer. Overall, the study cohort was diverse and reported substantial levels of HIV risk. More than 75% of each stratum reported multiple male sex partners of unknown HIV serostatus, while a substantial proportion of men also reported having known HIV- positive male partners; only 68 men were exclusively heterosexual. Pregnancy rates in female vaccine and placebo participants were 12.8% and 9.9%, respectively, at the time of the interim analysis, indicating substantial levels of unprotected vaginal sex. Within pre-specified Ad5 strata, vaccine and placebo recipients were well-matched on demographic and risk characteristics at baseline. However, there were substantial differences in several important demographic and HIV risk factors between individuals in the low vs. high Ad5 strata. For example, men with baseline Ad5 titers >200 were significantly more likely to have been enrolled outside of North America, to be non-white, and to be uncircumcised. Men with high Ad5 titers were also less likely to have known HIV-positive partners or to use recreational drugs.

Side effects from the vaccine were similar to those reported earlier (18). Injection site pain (70% of vaccinees and 34% of placebo recipients) and headache (32% of vaccinees and 27% of placebo recipients) were most common. There were no clinically significant differences in safety laboratory results between vaccine and placebo recipients. Of 40 serious adverse events reported by blinded study investigators, only 2 (fever, rigors) were reported in the vaccine group that were deemed related to study vaccine.

Among the 25% pre-specified random sample of study volunteers evaluated for IFN-y ELISPOT responses at the week 8 time-point, 75% of vaccinees responded to one or more HIV antigens, with geometric mean titers of several hundred (Table 2). Response rates were higher among those with baseline Ad5 titers ≤200 than those with Ad5 >200; overall responses did not differ between men and women.

Interim efficacy results

As pre-specified in the protocol, an interim analysis of HIV incidence and early HIV-1 viral load was conducted when there were 30 per-protocol events in the Ad5 ≤200 stratum. Results of this interim analysis are presented in Table 3. Overall HIV-1 seroincidence at the time of the interim analysis in the modified intention-to-treat (MITT) population was 3.6% per year in men (95% CI 2.6 – 4.8) and 0.2% per year in women (95% CI 0.0 - 1.3). HIV infection rates

and mean viral load setpoint were no different or slightly higher in vaccine than placebo recipients in both the PP and MITT analysis subsets. The p-values for a beneficial effect exceeded 0.5 for both primary endpoints, thereby meeting the pre-specified futility criteria. Based on these results, the Step Study Protocol Team immediately halted all additional immunizations in the trial, and began notifying the study investigators, study participants, and the general public of the trial results within 72 hours of the DSMB meeting. After extensive discussions with study investigators, staff, and community representatives about the benefits of continuing blinded versus unblinded follow-up, the Step Study Protocol team decided to unblind study participants in November 2007.

Exploratory efficacy analyses in male participants

Because the study unexpectedly met the pre-specified futility boundaries at the first interim analysis, additional analyses were initiated to explore reasons for the vaccine's lack of efficacy. The interim data were expanded to include an additional 8 HIV-1 infections in participants with Ad5 titers ≤200 and 30 in participants with Ad5 titers >200 accrued through October 17, 2007. Because only 1 HIV-1 infection had occurred in a female participant, all subsequent analyses are limited to male participants in the MITT population.

In this expanded analysis of data through October 17, 2007, 49 of the 914 male vaccine recipients became HIV infected (annual HIV incidence 4.6%, 95% CI 3.4 to 6.1) and 33 of the 922 male placebo recipients became HIV infected (annual incidence 3.1%, 95% CI 2.1 to 4.3). The overall treatment effect hazard ratio from the univariate Cox model was 1.5 (95% CI 0.97 to 2.3, p=0.07). As randomization occurred within each of 4 pre-specified Ad5 strata, data are presented for each stratum (Figure 2). Although HIV acquisition rates were similar in vaccine and placebo recipients with baseline Ad5 titers ≤18 (Ad5 seronegative participants), surprisingly, rates appeared to be more than twice as high in vaccinees compared with placebo recipients in Ad5 strata >18, (overall HIV acquisition rate 5.1% vs. 2.3%/year, unadjusted two-tailed p-value 0.013). There was also evidence that the hazard ratio increased with increasing $\log_{10}$(Ad5) (univariate Cox model trend test p-value = .06).

Viral load setpoints were not materially different between vaccine and placebo recipients in either the Ad5 seronegative or Ad5 seropositive stratum (Figure 3, p>.25 for all comparisons).

Factors associated with HIV infection risk

Univariate Cox proportional hazard analyses were conducted to evaluate if vaccine effects on HIV acquisition rates were different for different subgroups of participants (Table 4). The hazard ratio (HR) of HIV acquisition in vaccine versus placebo recipients was consistently close to 1.5 among all subgroups defined by age, race, unprotected receptive anal sex, unprotected insertive anal sex, drug use, and number of male sex partners. The elevated risk of HIV acquisition seen in Ad5 seropositive men appeared absent in Ad5 seronegative men (HR 2.3 versus 1.0 respectively, interaction test p = .08). Similarly, the HR was elevated in uncircumcised men, but not in circumcised men (HR = 3.8 vs. 1.0, interaction test p = .01). The univariate results did not materially change after adjusting for other baseline and demographic covariates in multivariate models (data not shown).

To evaluate whether Ad5 serostatus or circumcision status were independent risk factors for HIV infection in the placebo group alone, we applied the Cox model adjusting for baseline demographic and risk variables. The adjusted hazard ratio was 1.6 (95% CI 0.7 to 3.6, p=0.23) for Ad5 >18 versus Ad5 ≤18 placebo recipients, and 2.5 (95% CI 0.7 to 8.7, p=0.14) for circumcised versus uncircumcised placebo recipients. These results do not support either variable as a significant independent predictor of HIV infection; however, they must be

interpreted with caution because the study did not randomize participants to Ad5 or circumcision groups, only to vaccine or placebo.

Because circumcision rates were substantially higher in the Ad5 seronegative than Ad5 seropositive participants (77.6% vs. 40.4%, p<0.001), hazard ratios were calculated for 4 different subpopulations of men in the trial, based on Ad5 and circumcision status. The unadjusted hazard ratio for risk of HIV acquisition among vaccinees compared with placebo recipients was highest among uncircumcised, Ad5 seropositive men (n= 620, HR 3.9, 95% CI 1.3 – 11.9). Risk was intermediate among uncircumcised, Ad5 seronegative men (n=168, HR 3.3, 95% CI 0.7 – 15.8) and circumcised, Ad5 seropositive men (n=421, HR 1.6, 95% CI 0.7 – 3.8). Risk did not appear to be elevated in men who were both circumcised and Ad5 seronegative (n=578, HR 0.7, 95% CI 0.3 – 1.4). These results did not change significantly when using adjusted hazard ratios from any of several multivariate Cox models.

To evaluate whether the increased hazard of HIV acquisition seen within these subgroups occurred only in peri-vaccination periods or persisted over time, the relative HIV incidence (vaccine:placebo) was evaluated during 3 semi-annual periods from the time of enrollment (Figure 4). Overall and within subgroups, HIV incidence was approximately constant over time for both vaccinees and placebo recipients through 78 weeks of follow-up.

Risk behavior

If the vaccine increased the risk of HIV acquisition in uncircumcised male participants, a likely mechanism would be through insertive anal sex exposures and therefore, relative hazards should be particularly high in men reporting this risk. Therefore, the relative hazard of HIV infection was compared between men who had and had not reported unprotected insertive anal sex with HIV positive or unknown serostatus partners at baseline. Among uncircumcised men, the hazard ratios appeared to be even higher in men who reported unprotected insertive anal sex at baseline than in men who did not report this risk (HR 6.1 vs. 2.5 respectively). No such relationship was seen with unprotected receptive anal sex with HIV positive or unknown partners; the hazard ratio for uncircumcised men who reported this risk at baseline was lower than in uncircumcised men who did not report this risk (HR 3.7 vs. 5.7 respectively). Among circumcised men, hazard ratios were consistently near 1.0, regardless of reported baseline risk.

The difference in infection rates between vaccine and placebo recipients could be attributed to differences in risk practices between vaccine and placebo groups, particularly if any substantial levels of unblinding had occurred. Risk data were compared between vaccine and placebo recipients through 18 months of follow-up. For both vaccine and placebo recipients, the proportion of study participants reporting risk declined substantially during the first 6 months of the study, and then remained relatively level throughout follow-up (data not shown). The frequency of all measured risk behavior variables was similar for Ad5 seropositive vaccine and placebo recipients over time, and for uncircumcised vaccine and placebo recipients over time (all p-values > 0.20 data not shown).

Discussion

This is the first completed efficacy evaluation of a CMI-based HIV vaccine. Thirty-three months after the first participant was enrolled in the Step Study, this trial determined that the MRKAd5 gag/pol/nef HIV-1 vaccine neither prevented HIV-1 infection nor lowered viral load setpoint in participants with baseline Ad5 titers ≤200, despite generating IFN-γ ELISPOT responses in the majority of vaccinees. High levels of protocol adherence provide further confidence in this study's conclusions about the vaccine's lack of protective efficacy. Unfortunately, vaccine efficacy could not be conclusively evaluated in women in this trial because of low HIV acquisition rates, possibly driven by low HIV prevalence in male partners.

The challenge of identifying high-seroincidence cohorts of women has been demonstrated in a number of other studies (35;36).

Because CMI vaccines act by killing HIV infected cells, they would likely have their biggest impact in controlling viral replication, rather than preventing infection (37). However, there was no indication that early plasma viral levels were reduced in vaccinees compared with placebo recipients in this study. A companion manuscript explores the immunologic response among vaccinees in greater detail, and begins to explore potential explanations for the failure of this vaccine to provide protection. What is not yet clear is whether the magnitude, quality, specificity, or homing of the immune response generated by this specific vaccine was insufficient to control viral replication, or if this represents a more fundamental challenge of CMI vaccines to alter the clinical course of HIV disease. By providing the first direct test of a CMI vaccine to alter clinical outcome, the STEP study has provided important data for the field, and a repository of specimens with which to explore reasons for failure (e.g., mismatch between vaccine-induced immune response and viral sequences, inadequate magnitude or quality of the vaccine-induced immune response) and potential immune correlates of protection (e.g., presence or magnitude of a functional assay associated with lower viral load in subgroups of vaccinees).

Surprisingly, there was an increase in the number of HIV-1 infections in male vaccine recipients. These effects appeared to be limited to men who were Ad5 seropositive and/or uncircumcised on multivariate analyses, and not to be confounded by other measured baseline demographic and risk variables. However, this does not rule out confounding by as-yet unmeasured variables such as baseline herpes simplex type 2 (HSV-2) serostatus or host genetic factors, which are currently being measured in cryopreserved specimens. Other potential confounders, such as sexual network clustering, are being explored through viral genotyping.

There is little in the published literature that point to a mechanism for increased acquisition risk associated with this candidate or other adenovirus-based HIV vaccines. Antibody-dependent enhancement has been described for a number of viral infections (38;39). To date, such enhancement has been directed at surface envelope proteins and the MRKAd5 trivalent vaccine did not contain envelope inserts. There is one published report of a candidate HIV vaccine using a recombinant varicella-zoster virus (VZV) vector that led to enhanced SIV replication and disease progression in rhesus macaques, although the effects on SIV acquisition were not assessed (40). The VZV vaccine elicited a robust SIV-specific CD4+ T cell response without a measurable CD8+ T cell response, quite different from the immunologic profile of the Merck trivalent vaccine.

The mechanism for enhanced HIV acquisition risk in vaccinated Ad5 seropositive men is likely to be complex. All vaccinees are likely to have developed both Ad5 antibodies as well as T cell responses to the vector; thus, none of the vaccinees were likely Ad5 seronegative after the first immunization. However, natural Ad5 infection occurs via the nasopharynx or gut, may persist at mucosal surfaces over several years, and preferentially infect lymphocytes that home to mucosa (41). Vaccinees with pre-existing Ad5 immunity may generate an Ad5-specific immune response that homes to mucosal surfaces, while those with vaccine-induced Ad5 immunity may not. Studies are underway to further explore differences in the mucosal immune response between participants with and without pre-existing Ad5 immunity. Conversely, the repeated administration of the Ad5 vector may cause an as yet undefined effect on the immune response that led to increased HIV acquisition. It is not yet clear whether the effects of this vaccine apply to other adenovirus-based HIV vaccines, including those using alternate serotypes. Until the mechanism for these effects can be clarified, clinical trials of novel adenovirus-based HIV vaccine candidates should include safeguards to minimize potential risk to study volunteers (e.g., limiting study enrollment to subgroups without evidence of vaccine-

associated elevated risk, close study monitoring, and extensive discussion of risk during informed consent).

Circumcision has been shown to be associated with a halving of the risk of HIV acquisition in MSM in a longitudinal study (42), although data from cross-sectional studies and smaller longitudinal studies have been mixed (43–45). The protective effect of circumcision may be more difficult to demonstrate for men who engage in both insertive and receptive anal sex, and may therefore be most concentrated among men reporting unprotected insertive anal sex with HIV positive or unknown serostatus partners. In this study, uncircumcised vaccinees were at increased risk of HIV acquisition compared with uncircumcised placebo recipients, especially among men reporting high-risk insertive anal sex. Conversely, the risk of HIV acquisition did not appear to be more concentrated in uncircumcised men reporting high-risk receptive anal sex at baseline, nor did circumcised men appear to be at elevated risk, regardless of their sexual practices. These results call for further inquiry into evaluating the mucosal response to this and other vaccines, and the potential interaction of mucosal immune responses to pre-existing vector immunity.

The Step Study has also been a landmark trial in deepening our understanding of the potential, and potential pitfalls, of current non-human primate challenge models. A prototype replication incompetent Ad5 vaccine based on an earlier Merck Ad5 gag-only vaccine provided substantial and durable control of viral replication against SHIV 89.6P challenge (14), particularly in animals with genetic markers associated with virologic control (25;25;27;27). The Step trial results suggest that this model is not a useful predictor of the clinical utility of T cell based vaccines (46). Other non-human primate challenge studies of this candidate vaccine have demonstrated more transient protection against $SIV_{mac}239$ that may depend upon a DNA prime (25) or inclusion of additional gene inserts (26); however, the utility of these challenge models is also as yet unproven (47). If vector-based immunity plays an important role in the quality of the immune response generated to vaccines, animal models may not predict clinical experience, particularly when the vector's host range is limited.

The Step Study successfully addressed the pre-specified primary study outcomes. Furthermore, it has challenged the field to more fully understand the role of vector-based immunity, the potential for vaccine-induced increased acquisition, and to mine the wealth of data and specimens in this human trial of a CMI vaccine, to understand the vaccine's failure. It will take the additional, coordinated efforts of laboratory, non-human primate, and clinical scientists to provide definitive answers to these questions, and ultimately to develop a safe and effective HIV vaccine.

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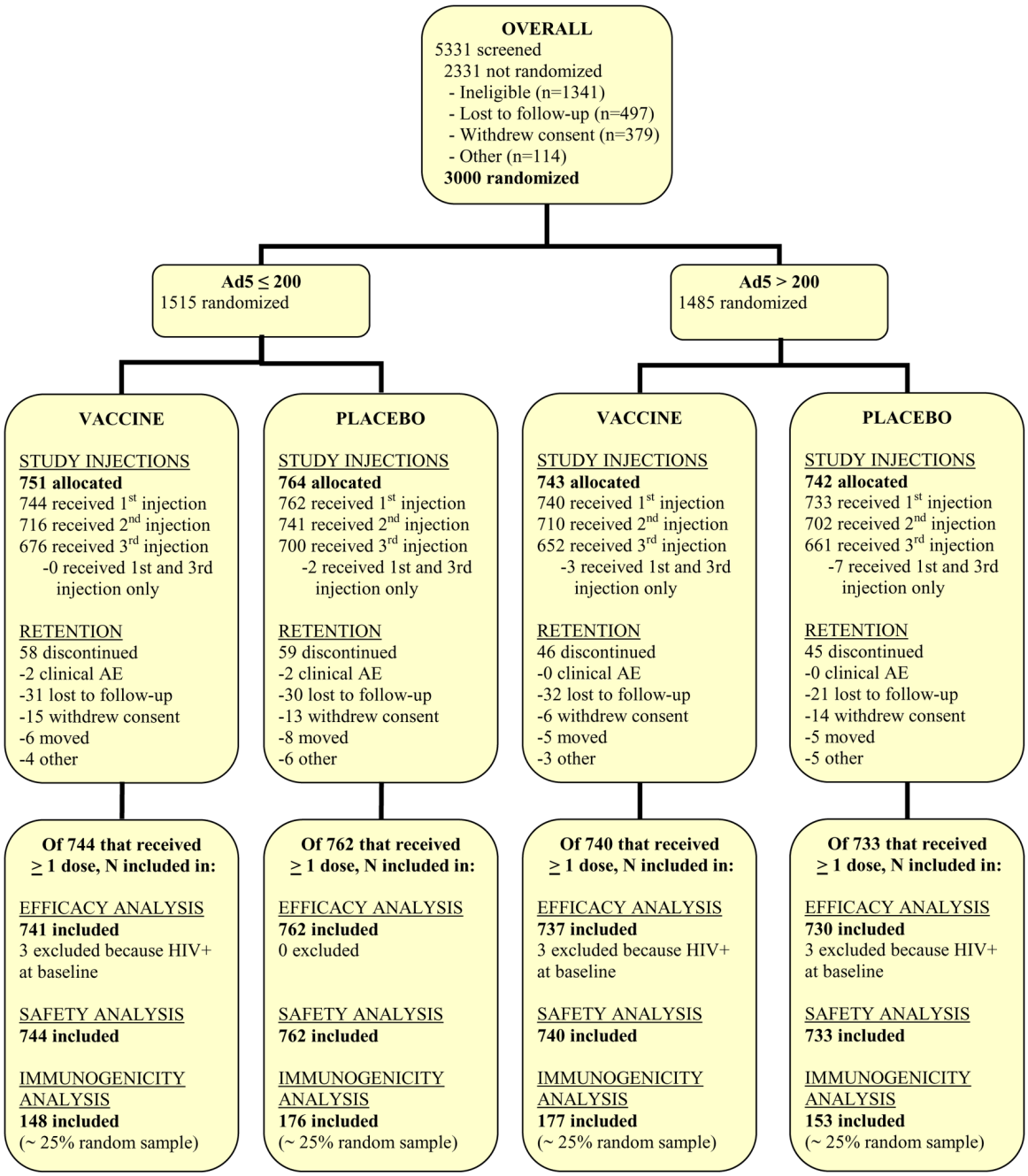

Figure 1.

Trial profile. Discontinued includes study participants who were unwilling or unable to continue follow-up in the trial at the time the dataset was frozen. All participants who received at least one dose of vaccine or placebo were included in the safety analysis; efficacy analysis was limited to the modified intent-to-treat subgroup who were also HIV negative at baseline. The immunogenicity analysis was performed on a 25% random sample of the entire cohort.

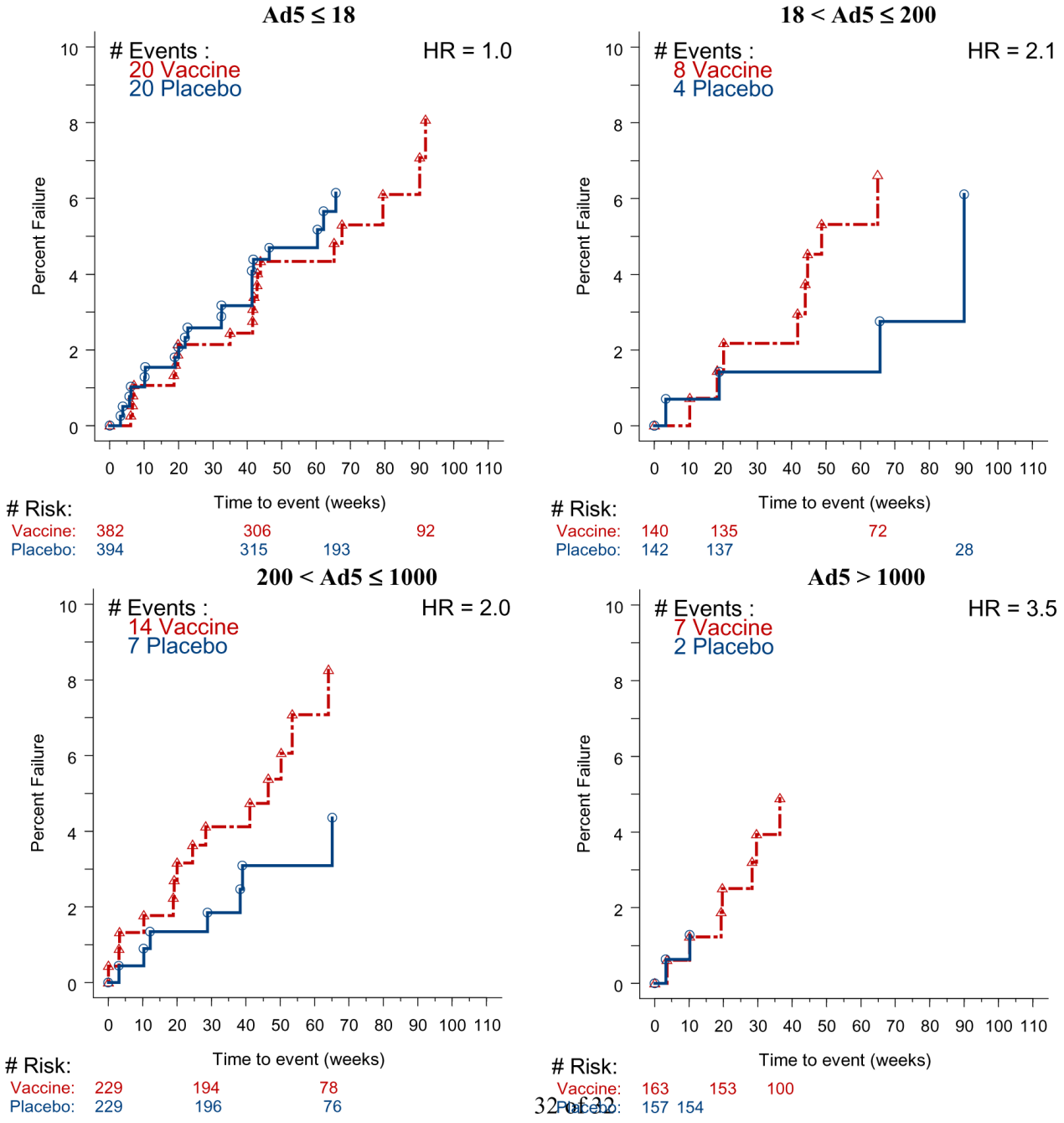

Figure 2.

Kaplan Meier plots of HIV infection for male vaccine and placebo groups by A) baseline Ad5 ≤18; B) baseline Ad5 >18 and ≤200; C)baseline Ad5 >200 and ≤1000; and D) baseline Ad5 >1000. Each hazard ratio (HR) is from a univariate Cox regression model.

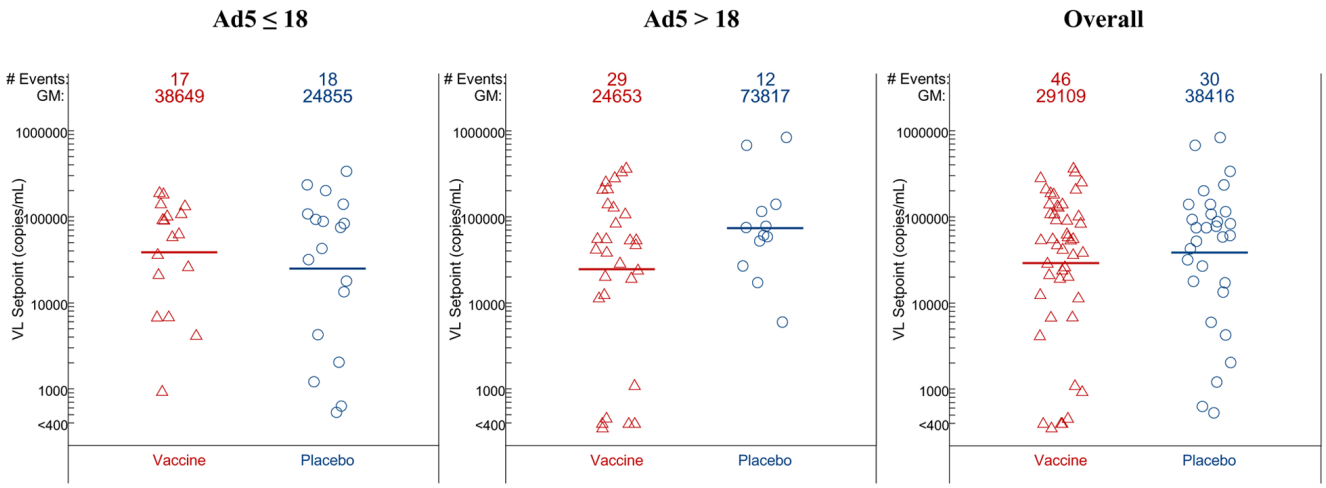

Figure 3.

Early plasma viral load (VL) at ~ 3 months after detection of infection in male study participants by A) baseline Ad5 ≤ 18; B) baseline Ad5 >18; and C) all participants. The bar in each panel denotes the geometric mean titers of the plasma VL.

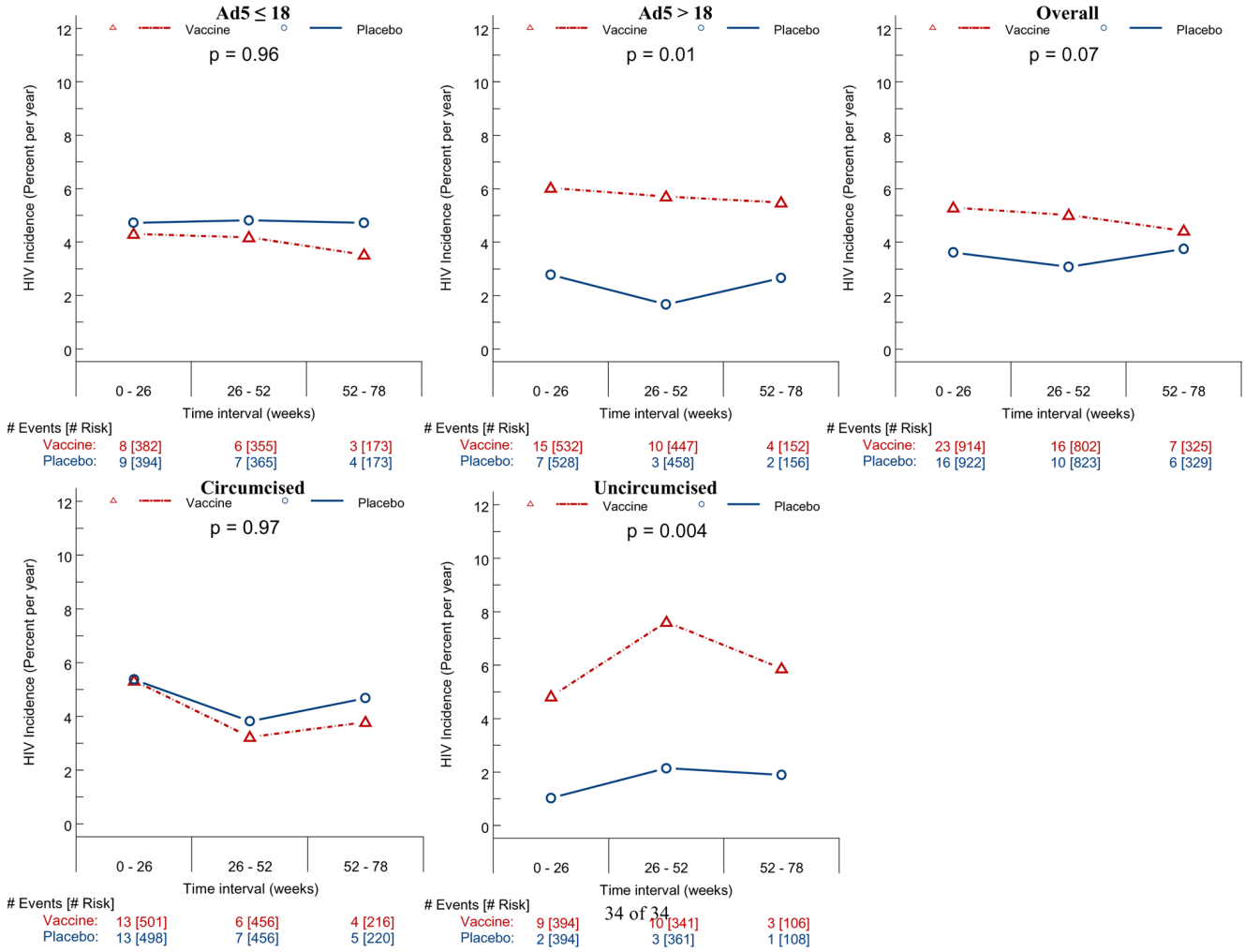

Figure 4.

HIV incidence during 6-month time intervals for male vaccine and placebo groups by A) baseline Ad5 titer ≤18; B) baseline Ad5 >18; C) overall; D) circumcised; and E) uncircumcised. Each 2-tailed p-value (p) is from a univariate Cox regression model.

Table 1

Baseline characteristics, stratified by gender and baseline Ad5 antibody titer

	Men				Women			
	Ad5 ≤ 200		Ad5 > 200		Ad5 ≤ 200		Ad5 > 200	
	Vaccine	Placebo	Vaccine	Placebo	V	P	V	P
	N=525	N=536	N=394	N=389	N=219	N=226	N=346	N=344
Baseline Characteristics	n(%)	n (%)	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)
Demographics								
Age								
Median	31	31	28	28	27	30	27	28
Range	18–45	18–45	18–46	18–45	18–45	18–45	18–45	18–45
Race/ethnicity								
Black	53 (10.1)	51 (9.5)	41 (10.4)	40 (10.3)	151 (68.9)	149 (65.9)	199 (57.5)	205 (59.6)
Hispanic	39 (7.4)	42 (7. 8)	44 (11.2)	50 (12.9)	37 (16.9)	42 (18.6)	95 (27.5)	91 (26.5)
Multiracial	104 (19.8)	100 (18.7)	161 (40.9)	149 (38.3)	18 (8.2)	15 (6.6)	32 (9.2)	28 (8.1)
White	312 (59.4)	332 (61.9)	136 (34.5)	133 (34.2)	11 (5.0)	18 (8.0)	12 (3.5)	13 (3.8)
Other	17 (3.2)	11 (2.1)	12 (3.0)	17 (4.4)	2 (0.9)	2 (0.9)	8 (2.3)	7 (2.0)
Circumcision status								
Circumcised	345 (65.7)	349 (65.1)	159 (40.4)	150 (38.6)	NA	NA	NA	NA
Uncircumcised	165 (31.4)	167 (31.2)	231 (58.6)	228 (58.6)				
Unknown	15 (2.9)	20 (3.7)	4 (1.0)	11 (2.8)				
Site of enrollment								
Caribbean	12 (2.3)	12 (2.2)	22 (5.6)	23 (5.9)	66 (30.1)	63 (27.9)	171 (49.4)	167 (48.5)
North America & Australia	404 (77.0)	417 (77.8)	189 (48.0)	186 (47.8)	134 (61.2)	145 (64.2)	142 (41.0)	145 (42.2)
South America	109 (20.8)	107 (20.0)	183 (46.4)	180 (46.3)	19 (8.7)	18 (8.0)	33 (9.5)	32 (9.3)
Sexual risk (previous 6 months)								
Number of male sex partners								

	Men				Women			
	Ad5 ≤ 200		Ad5 > 200		Ad5 ≤ 200		Ad5 > 200	
	Vaccine	Placebo	Vaccine	Placebo	V	P	V	P
	N=525	N=536	N=394	N=389	N=219	N=226	N=346	N=344
Baseline Characteristics	n(%)	n (%)	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)
0	12 (2.3)	12 (2.2)	22 (5.6)	22 (5.7)	2 (0.9)	1 (0.4)	1 (0.3)	1 (0.3)
1	23 (4.4)	24 (4.5)	13 (3.3)	15 (3.9)	14 (6.4)	24 (10.6)	26 (7.5)	24 (7.0)
2–4	173 (33.0)	160 (29.9)	139 (35.3)	123 (31.6)	54 (24.7)	42 (18.6)	65 (18.8)	94 (27.3)
5–9	130 (24.8)	130 (24.3)	85 (21.6)	88 (22.6)	23 (10.5)	18 (8.0)	38 (11.0)	38 (11.0)
10–19	88 (16.8)	94 (17.5)	46 (11.7)	60 (15.4)	12 (5.5)	16 (7.1)	34 (9.8)	19 (5.5)
≥20	99 (18.9)	116 (21.6)	89 (22.6)	81 (20.8)	114 (52.1)	125 (55.3)	182 (52.6)	168 (48.8)
Median	6	6	5	5	25	30	20	15
Serostatus of male sex partners								
Any HIV positive	162 (30.9)	161 (30.0)	70 (17.8)	74 (19.0)	16 (7.3)	17 (7.5)	23 (6.7)	23 (6.7)
Any HIV unknown	424 (80.8)	424 (79.1)	311 (78.9)	307 (78.9)	194 (88.6)	201 (88.9)	316 (91.6)	316 (91.9)
All HIV negative	317 (60.4)	322 (60.1)	210 (53.3)	208 (53.5)	94 (42.9)	92 (40.7)	102 (29.5)	101 (29.4)
Unprotected receptive anal sex								
With HIV positive partner	36 (6.9)	37 (6.9)	14 (3.6)	22 (5.7)	4 (1.8)	3 (1.3)	3 (0.9)	2 (0.6)
With HIV unknown partner	155 (29.5)	164 (30.6)	135 (34.3)	132 (33.9)	37 (16.9)	34 (15.0)	42 (12.1)	46 (13.4)
With HIV negative partner	151 (28.8)	151 (28.2)	98 (24.9)	94 (24.2)	13 (5.9)	19 (8.4)	16 (4.6)	17 (4.9)
None	257 (49.0)	266 (49.6)	200 (50.8)	200 (51.4)	171 (78.1)	181 (80.1)	293 (84.7)	286 (83.1)
Unprotected insertive anal sex								
With HIV positive partner	73 (13.9)	67 (12.5)	30 (7.6)	33 (8.5)	NA	NA	NA	NA
With HIV unknown partner	202 (38.5)	196 (36.6)	169 (42.9)	155 (39.8)				
With HIV negative partner	157 (29.9)	176 (32.8)	111 (28.2)	101 (26.0)				
None	203 (38.7)	211 (39.4)	160 (40.6)	167 (42.9)				
Unprotected vaginal sex								
With HIV positive partner	2 (0.4)	0 (0)	2 (0.5)	0 (0)	11 (5.0)	10 (4.4)	17 (4.9)	16 (4.7)

	Men				Women			
	Ad5 ≤ 200		Ad5 > 200		Ad5 ≤ 200		Ad5 > 200	
	Vaccine	Placebo	Vaccine	Placebo	V	P	V	P
	N=525	N=536	N=394	N=389	N=219	N=226	N=346	N=344
Baseline Characteristics	n(%)	n (%)	n (%)	n (%)	n(%)	n(%)	n(%)	n(%)
With HIV unknown partner	31 (5.9)	34 (6.3)	71 (18.0)	68 (17.5)	165 (75.3)	159 (70.4)	223 (64.5)	228 (66.3)
With HIV negative partner	35 (6.7)	29 (5.4)	54 (13.7)	48 (12.3)	69 (31.5)	69 (30.5)	80 (23.1)	80 (23.3)
None	469 (89.3)	484 (90.3)	292 (74.1)	294 (75.6)	28 (12.8)	38 (16.8)	84 (24.3)	77 (22.4)
Sexually transmitted disease*	81 (15.4)	73 (13.6)	65 (16.5)	44 (11.3)	34 (15.5)	33 (14.6)	39 (11.3)	40 (11.6)
Drug use (previous 6 months)								
Any use	251 (47.8)	242 (45.1)	150 (38.1)	152 (39.1)	134 (61.2)	141 (62.4)	157 (45.4)	155 (45.1)
Methamphetamines	44 (8.4)	36 (6.7)	17 (4.3)	24 (6.2)	4 (1.8)	4 (1.8)	6 (1.7)	8 (2.3)
Amyl nitrites	109 (20.8)	95 (17.7)	50 (12.7)	54 (13.9)	NA	NA	NA	NA
Cocaine/crack	30 (5.7)	28 (5.2)	20 (5.1)	18 (4.6)	80 (36.5)	102 (45.1)	92 (26.6)	91 (26.5)

* self-reported gonorrhea or chlamydia

Table 2

IFN-gamma ELISPOT summaries at week 8 for the vaccine group

	Frequency (%) of Responders ***Geometric Mean (SFC/10^6 PBMC)***		
	Ad5 ≤ 200	**Ad5 > 200**	**Overall**
	n = 166	**n = 188**	**n = 354**
Gag	125 (75%) *277*	102 (54%) *170*	227 (64%) *213*
Pol	118 (71%) *489*	88 (47%) *245*	206 (58%) *339*
Nef	116 (70%) *251*	97 (52%) *164*	213 (60%) *200*
≥ 1 antigen	140 (84%)	127 (68%)	267 (75%)
≥ 2 antigens	122 (73%)	96 (51%)	218 (62%)
All 3 antigens	97 (58%)	64 (34%)	161 (45%)

"Responder": ELISPOT ≥ 55 SFC/10^6 PBMC and ≥ 4-fold over negative control. Week 8 is 4 weeks after the 2nd vaccination. ELISPOT assay was done for a random sample of approximately 25% of the study cohort; volunteers with evidence of HIV infection by week 8 were excluded from the summaries. Geometric mean is based on data for responders and non-responders combined.

Table 3

Results of Pre-specified Interim Analysis for the Ad5 ≤ 200 Subgroup (Infection and Viral Load (VL)[#] Endpoints)

Analysis Population	Gender	Treatment Group	N	n	Person-years of Follow-up[*]	HIV infection rate(% per year)	Viral Load Setpoint(log10 c/mL)
Per Protocol (PP)	Male	Vaccine	489	19	475	4.00	4.60
		Placebo	495	10	471	2.12	4.57
	Female	Vaccine	183	0	145	0.00	NA
		Placebo	196	1	152	0.66	4.31
1-tailed p-values (to assess a potential vaccine benefit): 0.949 for infection endpoint and 0.528 for VL endpoint.							
Modified Intent-to-Treat (MITT)	Male	Vaccine	522	24	607	3.95	4.61
		Placebo	536	20	618	3.24	4.41
	Female	Vaccine	219	0	215	0.00	NA
		Placebo	226	1	218	0.46	4.31
1-tailed p-values (to assess a potential vaccine benefit): 0.743 for infection endpoint and 0.656 for VL endpoint.							

N=Number in respective analysis population; n = number of events; NA = Not applicable.

PP analysis includes all vaccinated subjects who received at least two vaccinations except those diagnosed as HIV+ on or before Week 12 visit and/or identified as protocol violators per the statistical analysis plan. MITT analysis includes all vaccinated subjects except those diagnosed as HIV+ on or before Day 1 visit.

[*] For the PP (MITT) population, follow-up was calculated as the time from the day of the Week 12 (Day 1) visit to the last day of study follow-up for uninfected subjects and to the day of HIV diagnosis for infected subjects.

[#] Viral load (VL) setpoint was the average of $\log_{10}$ HIV-1 RNA values at 2 and 3 months after HIV diagnosis.

Table 4

Hazard Ratios of HIV Infection for Male Subgroups Defined by Demographic and Baseline Behavioral Risk Factors (Univariate Cox Model Analyses)

MITT Population	N	Number of HIV infections		HIV infection rate (% per year)		Hazard Ratio(Vaccine/ Placebo) (95% CI)	Interaction p-value[a]
		Vaccine	Placebo	Vaccine	Placebo		
Demographic factors							
Ad5– (titer ≤ 18)	776	20	20	4.1	4.0	1.0 (0.5 to 1.9)	0.08
Ad5+ (titer > 18)	1060	29	13	5.1	2.2	2.3 (1.2 to 4.3)	
Circumcised	999[b]	26	26	4.1	4.2	1.0 (0.6 to 1.7)	0.01
Uncircumcised	788[b]	22	6	5.2	1.4	3.8(1.5 to 9.3)	
Whites	907	24	18	4.4	3.2	1.4 (0.8 to 2.6)	0.71
Non-Whites	929	25	15	4.8	2.9	1.6 (0.9 to 3.1)	
Age≤ 30 yrs	970	28	19	5.0	3.5	1.4 (0.8 to 2.6)	0.81
Age > 30 yrs	866	21	14	4.1	2.6	1.6 (0.8 to 3.1)	
North America	1171	37	29	5.2	4.0	1.3 (0.8 to 2.1)	0.18
Others	665	12	4	3.4	1.1	3.0 (1.0 to 9.4)	
Behavioral risk factors							
UIAS: yes	1097	36	25	5.6	3.9	1.4 (0.9 to 2.4)	0.75
UIAS: no	739	13	8	3.1	1.8	1.7 (0.7 to 4.1)	
URAS: yes	916	37	25	7.2	4.7	1.5 (0.9 to 2.5)	0.99
URAS: no	920	12	8	2.2	1.5	1.5 (0.6 to 3.7)	
Any drug use: yes	792	29	19	6.2	4.3	1.5 (0.8 to 2.6)	0.96
Any drug use: no	1044	20	14	3.3	2.2	1.5 (0.8 to 3.0)	

MITT Population	N	Number of HIV infections		HIV infection rate (% per year)		Hazard Ratio(Vaccine/ Placebo) (95% CI)	Interaction p-value[a]
		Vaccine	Placebo	Vaccine	Placebo		
> 4 male sex partners	1101	32	23	5.1	3.5	1.5 (0.9 to 2.5)	0.88
≤ 4 male sex partners	735	17	10	3.9	2.4	1.6 (0.7 to 3.5)	

UIAS = unprotected insertive anal sex, URAS = unprotected receptive anal sex; behavioral risk data are based on self-reported behavior within 6 months prior to randomization. N = number of men in the univariate Cox model analysis;

[a] 2-tailed p-value for a test of difference between the hazard ratios for the two subgroups, not corrected for multiplicity;

[b] circumcision data unknown for 49/1836 males, including one infected male from each of the vaccine and placebo groups.