

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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Summary

Background Observational data and non-human primate challenge studies suggest that cell-mediated immune responses might provide control of HIV replication. The Step Study directly assessed the efficacy of a cell-mediated immunity vaccine to protect against HIV-1 infection or change in early plasma HIV-1 levels.

Methods We undertook a double-blind, phase II, test-of-concept study at 34 sites in North America, the Caribbean, South America, and Australia. We randomly assigned 3000 HIV-1-seronegative participants by computer-generated assignments to receive three injections of MRKAd5 HIV-1 gag/pol/nef vaccine (n=1494) or placebo (n=1506). Randomisation was prestratified by sex, adenovirus type 5 (Ad5) antibody titre at baseline, and study site. Primary objective was a reduction in HIV-1 acquisition rates (tested every 6 months) or a decrease in HIV-1 viral-load setpoint (early plasma HIV-1 RNA measured 3 months after HIV-1 diagnosis). Analyses were per protocol and modified intention to treat. The study was stopped early because it unexpectedly met the prespecified futility boundaries at the first interim analysis. This study is registered with ClinicalTrials.gov, number NCT00095576.

Findings In a prespecified interim analysis in participants with baseline Ad5 antibody titre 200 or less, 24 (3%) of 741 vaccine recipients became HIV-1 infected versus 21 (3%) of 762 placebo recipients (hazard ratio [HR] 1.2 [95% CI 0.6–2.2]). All but one infection occurred in men. The corresponding geometric mean plasma HIV-1 RNA was comparable in infected male vaccine and placebo recipients (4.61 vs 4.41 log₁₀ copies per mL, one tailed p value for potential benefit 0.66). The vaccine elicited interferon-γ ELISPOT responses in 75% (267) of the 25% random sample of all vaccine recipients (including both low and high Ad5 antibody titres) on whose specimens this testing was done (n=354). In exploratory analyses of all study volunteers, irrespective of baseline Ad5 antibody titre, the HR of HIV-1 infection between vaccine and placebo recipients was higher in Ad5 seropositive men (HR 2.3 [95% CI 1.2–4.3]) and uncircumcised men (3.8 [1.5–9.3]), but was not increased in Ad5 seronegative (1.0 [0.5–1.9]) or circumcised (1.0 [0.6–1.7]) men.

Interpretation This cell-mediated immunity vaccine did not prevent HIV-1 infection or reduce early viral level. Mechanisms for insufficient efficacy of the vaccine and the increased HIV-1 infection rates in subgroups of vaccine recipients are being explored.

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Introduction

The development of an efficacious HIV vaccine is one of the world's greatest public-health challenges. The absence of a known correlate of protection and the widespread genetic diversity of the virus pose substantial 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 are ineffective in generating robust immune responses or protecting against HIV.² No effective strategies have yet been developed to generate broadly neutralising antibody against HIV, although much work is being done in this area.^{3–6} A substantial amount of data draws attention to the importance of cell-mediated immune responses in

control of viral replication and disease progression in long-term non-progressors^{7–13} and in non-human primate challenge models.^{14–17} Substantial effort has been devoted to the design and assessment of vaccines based on cell-mediated immunity.

Adenovirus type 5 (Ad5) vector-based vaccines are among the most immunogenic of cell-mediated immunity vaccines in phase I clinical trials,^{18,19} surpassing immune responses generated by DNA plasmids^{20,21} and many poxvirus vectors.^{22–24} Challenge studies in non-human primates have also showed that SIV Ad5 prototype vaccines led to control of viraemia in some, but not all, challenge models.^{14,25–27}

On the basis of this early promising data from prototype vaccines containing a single gene (*gag*),²⁸ a candidate

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vaccine using a mixture of rAd5 vectors expressing the HIV-1 *gag*, *pol*, and *nef* genes was developed.¹⁸ These antigens were selected because they are commonly recognised during natural infection and are fairly conserved across different clades of HIV-1. This vaccine mixture was shown in phase I trials to elicit immune responses in immunocompetent participants who were both Ad5 seronegative and Ad5 seropositive.¹⁸ About two-thirds of adults in North America and western Europe are Ad5 seropositive, and the seroprevalence and average neutralising titre to Ad5 is even higher in South America, sub-Saharan Africa, and south Asia.^{29–32} However, because of substantial uncertainty about what would be needed of a cell-mediated immunity vaccine to control HIV-1 viral replication, we designed a test-of-concept trial³³ to assess the potential public-health effect of this 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.³⁴

Methods

Study design and population

The Step Study is a multicentre, double-blind, randomised, placebo-controlled, phase II test-of-concept study of the MRKAd5 HIV-1 *gag/pol/nef* vaccine in HIV-1 negative individuals who are at high risk of HIV-1 acquisition. This trial opened in December, 2004, and was undertaken in regions where clade B is the predominant HIV-1 subtype. The trial was initially designed to enrol 1500 participants with low (≤ 200) Ad5 antibody titres at enrolment, on the basis of reduced levels of immunogenicity detected in people with high Ad5 titres at baseline.²⁸ After data from a phase I trial showed robust immune responses even in people with pre-existing immunity to Ad5,¹⁸ we expanded the trial in July, 2005, to include a cohort of 1500 participants with Ad5 antibody titres greater than 200, to increase the potential worldwide relevance of this vaccine candidate. Participants were enrolled through March, 2007, at 34 sites in North America, the Caribbean, South America, and Australia.

Participants were aged 18–45 years, were HIV-1 seronegative, had serum alanine aminotransferase concentrations that were three times or less the upper limit of normal, and were at high risk of HIV-1 acquisition on the basis of reported risk behaviour in the 6 months before enrolment. Men were eligible if they reported unprotected anal intercourse with a male partner or anal intercourse with two or more male partners. Heterosexual men from Caribbean sites were also eligible if they reported a diagnosis of syphilis or genital ulcer disease; two or more sexual partners; exchanging sex for money, drugs, services, or gifts; or using crack cocaine three or more times. Women were eligible if they reported unprotected vaginal or anal intercourse with an HIV-positive man or an injecting-drug user; exchanging sex for money, drugs, services, or gifts; or using crack

cocaine three or more times. Women from Caribbean sites were also included if they reported a diagnosis of syphilis or pelvic inflammatory disease. We excluded participants if they had a history of immunodeficiency, malignancy, anaphylaxis or allergy to vaccine components, receipt of an experimental HIV vaccine, or other disorders that would interfere with their study participation. We excluded women who were pregnant at screening; women who became pregnant during the study did not receive further study injections but followed all other study procedures.

Participants underwent a thorough written informed consent process. The protocol was approved by the ethics review committee of every site, and the study was undertaken in conformance with applicable local and country requirements.

Procedures

The MRKAd5 HIV-1 *gag/pol/nef* vaccine consisted of a 1:1:1 mixture of three 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 given as a 1.0 mL injection of 1.5×10^{10} adenovirus genomes, which was 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. The vaccine and placebo vials and their contents looked identical. Vials were only labelled by number, without indication about whether it contained vaccine or placebo.

We randomly assigned study participants in a 1:1 ratio to receive three doses of the MRK Ad5 *gag/pol/nef* vaccine or placebo on day 1 (study enrolment), week 4, and week 26. Participant allocation schedules were generated by computer, and randomised assignments to vaccine or placebo were assigned with an interactive voice response system. Randomisation was prestratified by study site, sex, and baseline Ad5 antibody titre (≤ 18 [lower limit of detection of assay], 19–200, 201–1000, and >1000). Study participants were seen at day 1 and weeks 2, 4, 8, 12, 26, 30, 52, and every 26 weeks thereafter through to week 208. We undertook clinical assessment and counselling for risk reduction at every visit. We assessed local and systemic reactogenicity for the 14 days after study injections. Behavioural risk was assessed by self-report at screening and every 26 weeks thereafter, and included standardised interviewer-administered questionnaires about sexual risk, drug use, and sexually transmitted infections in the previous 6 months.

We measured serum alanine aminotransferase and a complete blood count immediately before and 2 weeks after the first vaccination to assess any hepatic or haematological toxic effects from the vaccine. HIV-1 testing was done at day 1; weeks 12, 30, 52; and every 26 weeks thereafter through to week 208. If HIV-1 was diagnosed at any visit, we tested stored plasma

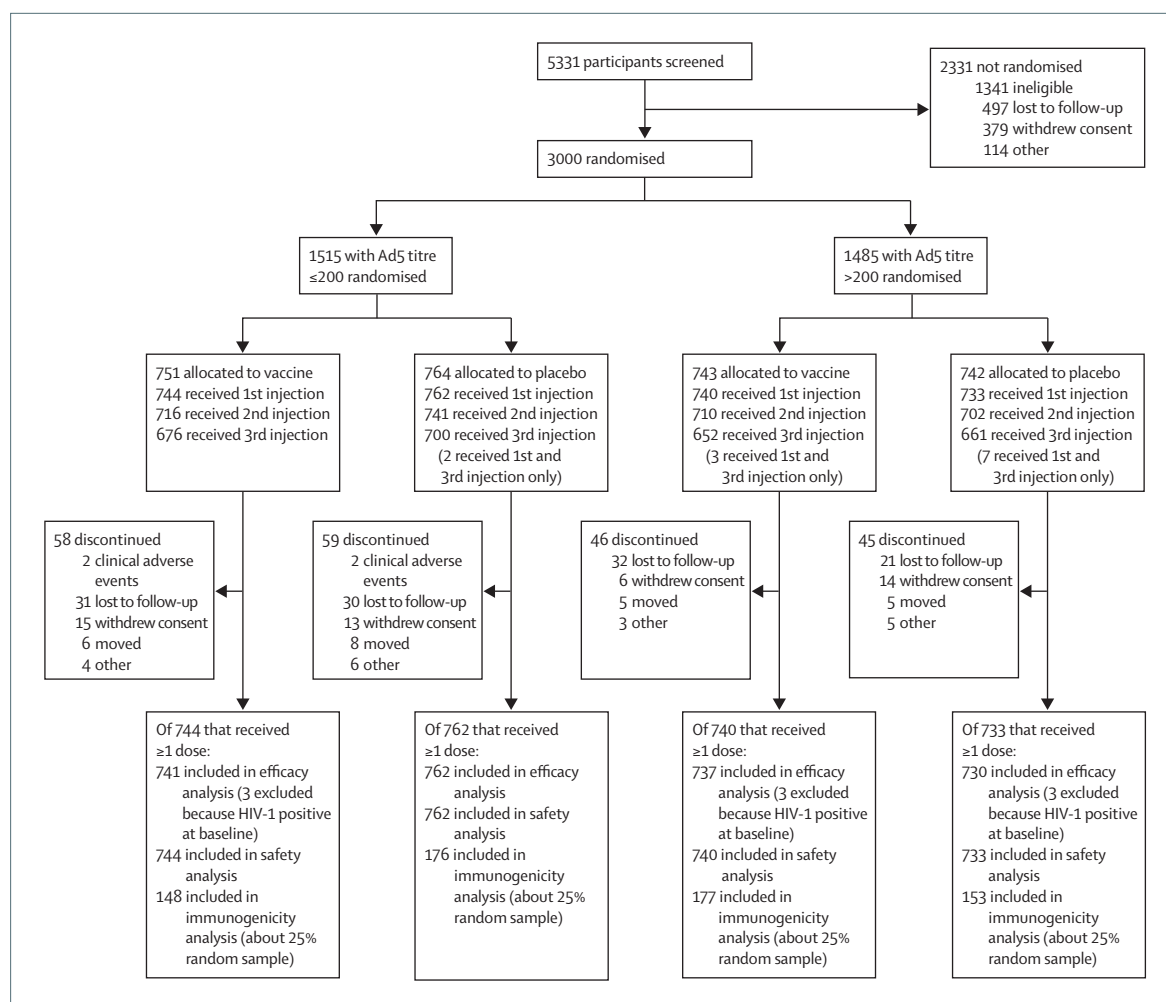


Figure 1: Trial profile

Study participants who discontinued included those who were unwilling or unable to continue follow-up in the trial at the time that 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-intention-to-treat subgroup who were also HIV-1 negative at baseline. We undertook the immunogenicity analysis on a 25% random sample of the entire cohort.

specimens from earlier time points to accurately time the onset of HIV-1 infection. All HIV-1 tests were done at a central laboratory. Specimens were screened with an immunoassay (Uni-Gold Recombigen HIV test, Trinity BioTech, Jamestown, NY, USA; or the Multispot HIV-1/HIV-2 Rapid Test, Bio-Rad, Hercules, CA, USA) that only contained HIV envelope antigens, which are not included in the vaccine. We confirmed reactive tests with an HIV-1 Western blot and HIV-1 plasma viral RNA assay (Amplicor Monitor version 1.5, Roche, Basel, Switzerland), which we undertook on the original specimen and a confirmatory specimen. A blinded endpoint adjudication committee consisting of three independent experts in HIV diagnostics made the final identification of HIV-1 infection status. All cases were unanimously confirmed by this committee. Participants who became infected with HIV-1 during the study were given counselling and linkage to local HIV medical

and psychosocial care. Participants infected with HIV-1 underwent clinical and laboratory assessment at 1, 2, 8, 12, and 26 weeks after their initial HIV-1 diagnosis, and every 26 weeks thereafter through to week 78 after the diagnosis.

Peripheral blood mononuclear cells were isolated from EDTA (edetic acid)-anticoagulated blood obtained at weeks 8, 30, 52, and 104, and were cryopreserved within 12 h of venipuncture, by previously described methods.³⁵ We undertook validated interferon- γ ELISPOT assays³⁶ on cryopreserved peripheral blood mononuclear cells at weeks 8 and 30 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 show the safety, tolerability, and efficacy of the MRK Ad5 gag/pol/nef

	Men				Women			
	Ad5 antibody titre ≤200		Ad5 antibody titre >200		Ad5 antibody titre ≤200		Ad5 antibody titre >200	
	Vaccine (N=525)	Placebo (N=536)	Vaccine (N=394)	Placebo (N=389)	Vaccine (N=219)	Placebo (N=226)	Vaccine (N=346)	Placebo (N=344)
Demographics								
Age (years)	31 (18-45)	31 (18-45)	28 (18-46)	28 (18-45)	27 (18-45)	30 (18-45)	27 (18-45)	28 (18-45)
Race/ethnic origin								
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%)
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 enrolment								
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 sexual partners								
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 (IQR)	6 (3-12)	6 (3-15)	5 (3-15)	5 (3-15)	25 (3-318)	30 (3-360)	20 (4-250)	15 (3-261)
Serostatus of male sexual 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%)
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	2 (0.5%)	0	11 (5.0%)	10 (4.4%)	17 (4.9%)	16 (4.7%)
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%)

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HIV-1 vaccine in the study population with baseline Ad5 antibody titres of 200 or less. The primary objectives focused on the subpopulation that was initially targeted for this trial and the one that was likely to have the most

robust immune response, on the basis of data from phase I trials. We defined efficacy as showing a reduction in HIV-1 acquisition rates (infection endpoint) or a decrease in HIV-1 viral-load setpoint (average of two log₁₀

	Men				Women			
	Ad5 antibody titre ≤ 200		Ad5 antibody titre > 200		Ad5 antibody titre ≤ 200		Ad5 antibody titre > 200	
	Vaccine (N=525)	Placebo (N=536)	Vaccine (N=394)	Placebo (N=389)	Vaccine (N=219)	Placebo (N=226)	Vaccine (N=346)	Placebo (N=344)
(Continued from previous page)								
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%)
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%)

Data are median (range) or number (%), unless otherwise indicated. *Self-reported gonorrhoea or chlamydia.

Table 1: Baseline characteristics, stratified by sex and baseline Ad5 antibody titre

HIV-1 RNA values at roughly 3 months after HIV-1 diagnosis (viral-load endpoint), or both, in vaccine versus placebo recipients.

Secondary objectives were to assess the safety, tolerability, and efficacy of the vaccine in the entire study population, irrespective of Ad5 antibody titre at baseline, and to identify immune responses that correlated with efficacy endpoints. Exploratory objectives included assessment of associations between the coprimary efficacy endpoints (infection and viral load) and prognostic factors such as sex, Ad5 antibody titre at baseline, age, ethnic origin, HLA type, and circumcision status (for men).

We summarised all serious vaccine-related adverse events, 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 prespecified timepoints). The safety analyses included all randomly assigned participants who received at least one dose of vaccine or placebo.

Statistical analysis

To assess vaccine efficacy for the infection endpoint, we compared the number of acquired HIV-1 infections (so-called events) in the vaccine group to that in the placebo group using a test for stratified Poisson data.³⁷ To assess vaccine efficacy for the viral-load endpoint, we compared viral-load setpoints for participants who became HIV-1 infected between treatment groups with use of a stratified Wilcoxon rank sum test; we used a prespecified multiple imputation approach to resolve the problem of altered or missing viral-load data that is associated with start of antiretroviral therapy or premature study discontinuation, respectively.³⁸

We predefined two analysis populations. The population in the per-protocol analysis included all randomly assigned participants who received the first two doses of either vaccine or placebo, apart from those who were either diagnosed with HIV-1 infection before or at week 12 (ie, 8 weeks after the second dose) or who violated the protocol on the basis of predefined criteria. The population in the modified-intention-to-treat analysis

	Ad5 antibody titre ≤ 200 (n=166)	Ad5 antibody titre > 200 (n=188)	Overall (N=354)
Gag	125 (75%); GMT=277	102 (54%); GMT=170	227 (64%); GMT=213
Pol	118 (71%); GMT=489	88 (47%); GMT=245	206 (58%); GMT=339
Nef	116 (70%); GMT=251	97 (52%); GMT=164	213 (60%); GMT=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%)

Data are number (%) of responders. GMT=geometric mean titre. Responders were defined as those with ELISPOT ≥ 55 spot-forming cells per 10^6 peripheral blood mononuclear cells and four-fold or more over negative control. Week 8 is 4 weeks after the second vaccination. ELISPOT assay was done for a random sample of roughly 25% of the study cohort; we excluded volunteers with evidence of HIV-1 infection by week 8 from the summaries. Geometric mean is based on data for responders and non-responders combined.

Table 2: Interferon γ ELISPOT summaries at week 8 for the vaccine group

included all randomly assigned participants who received at least one dose of vaccine or placebo, apart from those who had a positive HIV-1 screening test before randomisation.

The Step Study was an event-driven trial, which was designed to accrue at least 50 per-protocol events in the subgroup with Ad5 antibody titres 200 or less and 50 per-protocol events in the subgroup with Ad5 antibody titres more than 200 (>100 events overall). An α -spending interim analysis for the primary efficacy hypotheses was to be undertaken when 30 per-protocol events had accrued in the subgroup with Ad5 antibody titres 200 or less and the corresponding viral-load setpoint data for the participants infected with HIV-1 were available. Similarly, an α -spending interim analysis for the secondary efficacy hypotheses was to be undertaken when 30 per-protocol events had accrued in the subgroup with Ad5 antibody titres greater than 200 and at least 30 per-protocol events had accrued in the subgroup with Ad5 antibody titres 200 or less (>60 total per-protocol events), and the corresponding viral-load data for participants infected with HIV-1 were available. At the interim analysis for the primary efficacy hypothesis, we defined statistical success for the infection and viral-load endpoints as the one-tailed p value (in the direction of a vaccine benefit) being less than α allocated levels

	Number in analysis population	Number of events	Person-years of follow-up*	HIV infection rate (% per year)	Viral-load setpoint† (log ₁₀ copies/mL)
Per-protocol population ‡					
Men					
Vaccine	489	19	475	4.00%	4.60
Placebo	495	10	471	2.12%	4.57
Women					
Vaccine	183	0	145	0.00%	NA
Placebo	196	1	152	0.66%	4.31
Modified-intention-to-treat population§					
Men					
Vaccine	522	24	607	3.95%	4.61
Placebo	536	20	618	3.24%	4.41
Women					
Vaccine	219	0	215	0.00%	NA
Placebo	226	1	218	0.46%	4.31

Per-protocol analysis includes all vaccinated participants who received at least two vaccinations, apart from those diagnosed as HIV positive on or before week 12 visit or identified as protocol violators as per the statistical analysis plan, or both. Modified-intention-to-treat analysis includes all vaccinated participants, apart from those diagnosed as HIV positive on or before day 1 visit. NA=not applicable. *For the per-protocol population, follow-up was calculated as the time from the day of the week 12 (or day 1 for the modified-intention-to-treat analysis) visit to the last day of study follow-up for uninfected participants and to the day of HIV diagnosis for infected participants. †Viral-load setpoint was the average of log₁₀ HIV-1 RNA values at 2 and 3 months after HIV diagnosis. ‡One-tailed p values (to assess a potential vaccine benefit): 0.949 for infection endpoint and 0.528 for viral-load endpoint. §One-tailed p values (to assess a potential vaccine benefit): 0.743 for infection endpoint and 0.656 for viral-load endpoint.

Table 3: Results of prespecified interim analysis for the subgroup with Ad5 antibody titre 200 or less (infection and viral-load endpoints)

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 an absence of vaccine efficacy were also specified upfront: at either planned interim analysis, the vaccine was to be declared ineffective if the one-tailed p value was greater than 0.50 for both the coprimary endpoints for efficacy.

For the primary efficacy hypothesis (Ad5 antibody titres ≤200), 30 events provided 80% power to detect a difference of 1 log₁₀ copies per mL (placebo minus vaccine) in mean viral-load setpoint, 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 α allocation of 0.05 for the two primary efficacy endpoints, and they accounted for the α spending at the interim analysis. Similarly, for the secondary efficacy hypothesis (Ad5 antibody titres ≤200 and Ad5 antibody titres >200 combined), 60 events provided 80% power to detect a difference of 0.75 log₁₀ copies per mL in mean viral-load setpoint, and 100 events provided 80% power to detect a 50% reduction in the HIV-1 infection rate, on the basis of a total α allocation of 0.025 for the two coprimary efficacy endpoints and accounting for the planned interim analysis.

Because the study unexpectedly met the prespecified futility boundaries at the first interim analysis, we

started additional analyses to explore reasons for the vaccine's insufficient efficacy and potential for increased HIV-1 acquisition. In these analyses, we included data accrued through Oct 17, 2007, before participant unblinding. Because only one HIV-1 infection had arisen in a female participant, all subsequent analyses are limited to male participants in the modified-intention-to-treat population.

We used univariate Cox proportional hazards models to quantify treatment effects for various subgroups that were defined by demographic or baseline behavioural risk factors, or both. 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-1 seronegative visit and the date of the first evidence of HIV-1 infection, as established by the blinded endpoint adjudication committee. Participants who never showed any evidence of HIV-1 infection were right censored on the date of their last study visit before Oct 17, 2007. We generated Kaplan-Meier plots to graphically show the treatment effect across the four design-based Ad5 titre subgroups (baseline Ad5 antibody titres ≤18, 19–200, 201–1000, and >1000). Treatment effects were quantified with estimated hazard ratios (HRs) (vaccine/placebo) with associated Wald-based 95% CIs and two-tailed p values. We undertook interaction tests to assess whether the treatment effect differed between two given subgroups.

We used multivariate Cox models to estimate the treatment effect after adjustment for potential confounding variables. Candidate confounders were preselected on the basis of their plausibility to affect risk of HIV-1 infection. The candidate confounders were all dichotomous for simplicity, stabilising the model fitting, and reducing the modelling assumptions. The backwards elimination procedure for building the multivariate models used a Wald p value threshold of 0.15 for removing variables; we recorded similar results with a threshold of 0.10.

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 for HIV-1 acquisition and viral-load endpoints at the prespecified interim analysis.

Because the study unexpectedly met the prespecified futility boundaries at the first interim analysis, the Step Study protocol team immediately stopped all additional immunisations in the trial, and began notifying study investigators, study participants, and the general public of the trial results within 72 h 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.

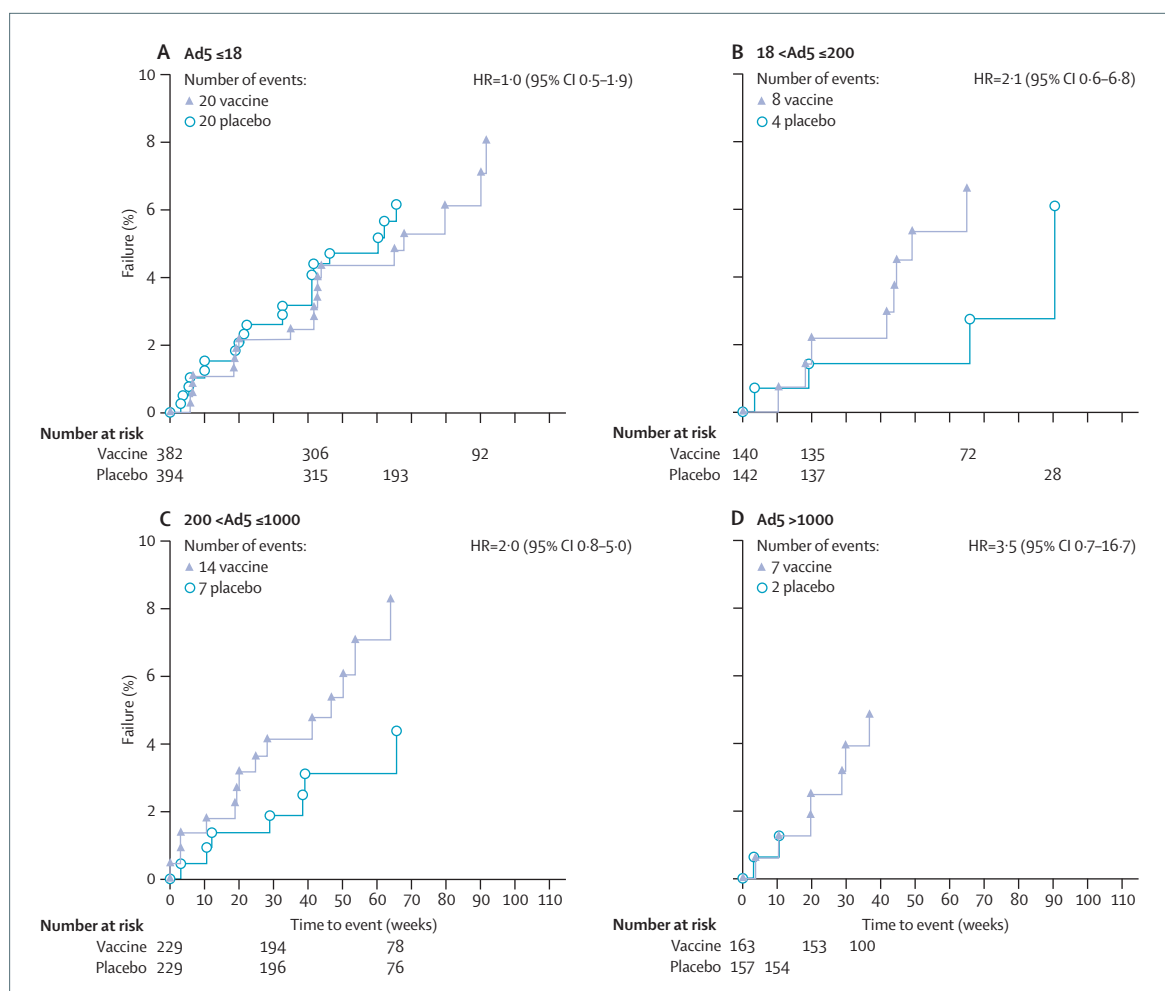


Figure 2: Kaplan-Meier plots of HIV infection for male vaccine and placebo groups by baseline Ad5 antibody titre ≤18 (A); baseline Ad5 antibody titre between >18 and ≤200 (B); baseline Ad5 antibody titre between >200 and ≤1000 (C); and baseline Ad5 antibody titre >1000 (D). Each hazard ratio (HR) is from a univariate Cox regression model.

This study is registered with ClinicalTrials.gov, number NCT00095576.

Role of the funding sources

The sponsors of the study were involved in the study design, data collection, data analysis, data interpretation, writing of the report, and in the decision to submit for publication. The sponsors had full access to all the data in the study and shared final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Protocol adherence was excellent, with 2677 (94%) participants in the vaccine and placebo groups receiving all three study injections. Overall, 104 (7%) of people receiving vaccine and 104 (7%) receiving placebo had discontinued follow-up in the study. Table 1 shows baseline demographic and risk characteristics, stratified by sex and Ad5 antibody titre at baseline. Overall, the study cohort was diverse and

reported substantial levels of HIV risk. More than three-quarters of each prespecified group of Ad5 antibody titre reported multiple male sex partners of unknown HIV-1 serostatus, whereas a substantial proportion of men also reported having known HIV-positive male partners; only 68 men were exclusively heterosexual (table 1). 12.8% and 9.9% of female participants in the vaccine and placebo groups, respectively, reported pregnancies up to the time of the interim analysis, indicating substantial levels of unprotected vaginal sex. Within prespecified groups of Ad5 antibody titre, vaccine and placebo recipients were well matched on demographic and risk characteristics at baseline (table 1). However, we recorded substantial differences in several important demographic and HIV-1 risk factors between individuals in the low versus high Ad5 antibody titre groups. For example, men with baseline Ad5 antibody titres greater than 200 were notably more likely to have been enrolled outside of North America, to be non-white, and to be uncircumcised. Men with high Ad5 antibody titres were

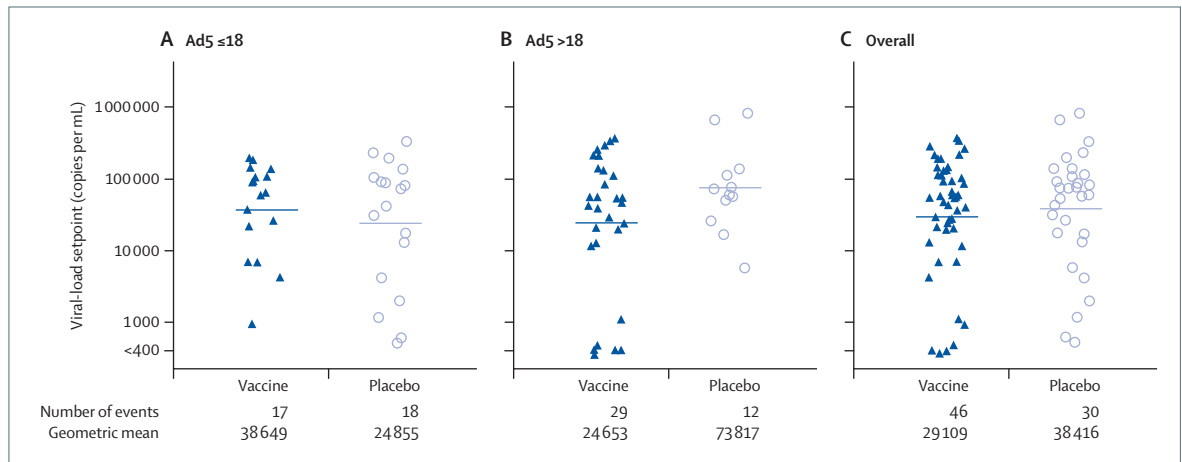


Figure 3: Early plasma viral load at around 3 months after detection of infection in male participants by baseline Ad5 antibody titre ≤ 18 (A); by baseline Ad5 antibody titre > 18 (B); and overall (C)

The bar denotes the geometric mean titres of the plasma viral load.

also less likely to have known HIV-positive partners or to use recreational drugs (table 1).

Side-effects from the vaccine were much the same to those reported previously.¹⁸ Pain at injection site (712 [49%] participants receiving vaccine and 305 [21%] receiving placebo) and headache (331 [22%] vs 269 [18%]) were most common. Safety laboratory results were not clinically significantly different between vaccine and placebo recipients. Of 40 serious adverse events reported by blinded study investigators, only two (fever, rigors) in the vaccine group were deemed related to study vaccine.

In the 25% prespecified random sample of study volunteers who we assessed for interferon- γ ELISPOT responses at week 8, 267 (75%) participants in the vaccine group responded to one or more HIV-1 antigens, with geometric mean titres of several hundred (table 2). Response rates were higher in participants with baseline Ad5 antibody titres 200 or less than in those with Ad5 antibody greater than 200 (table 2); overall responses did not differ between men and women (data not shown).

As prespecified in the protocol, we undertook an interim analysis of HIV-1 incidence and early HIV-1 viral load when 30 per-protocol events had arisen in the group with Ad5 antibody titre 200 or less. Table 3 shows the results of this interim analysis. Overall HIV-1 seroincidence at the time of the interim analysis in the modified-intention-to-treat population was 3.6% (95% CI 2.6–4.8) per year in men and 0.2% (0.0–1.3) per year in women. Rates of HIV-1 infection and mean viral-load setpoint did not differ or were slightly higher in vaccine than in placebo recipients in both the per-protocol and modified-intention-to-treat analysis subsets (table 3). The p values for a beneficial effect exceeded 0.5 for both primary endpoints, thereby meeting the prespecified futility criteria.

In the expanded analysis of data through Oct 17, 2007, 49 of the 914 male recipients receiving vaccine became

infected with HIV-1 (yearly HIV-1 incidence 4.6% [95% CI 3.4–6.1]) and 33 of the 922 male recipients receiving placebo (yearly incidence 3.1% [2.1–4.3]). The overall HR for the treatment effect from the univariate Cox model was 1.5 (95% CI 0.97–2.3; $p=0.07$). Since randomisation occurred within each of four prespecified Ad5 antibody titre groups, data are presented for each group (figure 2). Although HIV-1 acquisition rates were much the same in vaccine and placebo recipients with baseline Ad5 antibody titres 18 or less (ie, Ad5-seronegative participants), surprisingly, rates seemed to be more than twice as high in participants receiving vaccine than in those receiving placebo in the group with Ad5 antibody titre greater than 18, (overall HIV-1 acquisition rate 5.1% [95% CI 3.4–7.3] vs 2.3% [1.2–3.8] per year, unadjusted two-tailed p value 0.013). We also noted that the HR increased with increasing \log_{10} (Ad5) (univariate Cox model trend test p value 0.06). Viral-load setpoints did not differ materially between vaccine and placebo recipients in either the Ad5-seronegative or Ad5-seropositive groups (figure 3; $p>0.25$ for all comparisons).

We undertook univariate Cox proportional hazard analyses to assess whether vaccine effects on HIV-1 acquisition rates differed for different subgroups of participants (table 4). The HR of HIV-1 acquisition in vaccine versus placebo recipients was consistently close to 1.5 in all subgroups that were defined by age, race, unprotected receptive anal sex, unprotected insertive anal sex, drug use, and number of male sex partners (table 4). The increased risk of HIV-1 acquisition that we recorded in Ad5-seropositive men seemed to be absent in Ad5-seronegative men (table 4). Similarly, the HR was increased in uncircumcised men, but not in circumcised men (table 4). The univariate results did not materially change after adjustment for other baseline and demographic covariates in multivariate models (data not shown).

	N	Number of HIV infections		HIV infection rate (% per year)		Hazard ratio (vaccine/placebo) (95% CI)	Interaction p value*
		Vaccine	Placebo	Vaccine	Placebo		
Demographic factors							
Ad5 negative (titre ≤18)	776	20	20	4.1%	4.0%	1.0 (0.5-1.9)	0.08
Ad5 positive (titre >18)	1060	29	13	5.1%	2.2%	2.3 (1.2-4.3)	
Circumcised	999†	26	26	4.1%	4.2%	1.0 (0.6-1.7)	0.01
Uncircumcised	788†	22	6	5.2%	1.4%	3.8 (1.5-9.3)	
White race	907	24	18	4.4%	3.2%	1.4 (0.8-2.6)	0.71
Non-white race	929	25	15	4.8%	2.9%	1.6 (0.9-3.1)	
Age ≤30 years	970	28	19	5.0%	3.5%	1.4 (0.8-2.6)	0.81
Age >30 years	866	21	14	4.1%	2.6%	1.6 (0.8-3.1)	
North America	1171	37	29	5.2%	4.0%	1.3 (0.8-2.1)	0.18
Other sites	665	12	4	3.4%	1.1%	3.0 (1.0-9.4)	
Behavioural risk factors							
UIAS: yes	1097	36	25	5.6%	3.9%	1.4 (0.9-2.4)	0.75
UIAS: no	739	13	8	3.1%	1.8%	1.7 (0.7-4.1)	
URAS: yes	916	37	25	7.2%	4.7%	1.5 (0.9-2.5)	0.99
URAS: no	920	12	8	2.2%	1.5%	1.5 (0.6-3.7)	
Any drug use: yes	792	29	19	6.2%	4.3%	1.5 (0.8-2.6)	0.96
Any drug use: no	1044	20	14	3.3%	2.2%	1.5 (0.8-3.0)	
>4 male sex partners	1101	32	23	5.1%	3.5%	1.5 (0.9-2.5)	0.88
≤4 male sex partners	735	17	10	3.9%	2.4%	1.6 (0.7-3.5)	

Data are for the modified-intention-to-treat population. Behavioural risk data are based on self-reported behaviour within 6 months before randomisation. N=number of men in the univariate Cox model analysis. UIAS=unprotected insertive anal sex. URAS=unprotected receptive anal sex. *Two-tailed p value for a test of difference between the hazard ratios for the two subgroups, not corrected for multiplicity. †Circumcision data unknown for 49 of 1836 men, including one infected man from each of the vaccine and placebo groups.

Table 4: Hazard ratios of HIV infection for male subgroups, defined by demographic and baseline behavioural risk factors (univariate Cox model analyses)

To assess whether Ad5 serostatus or circumcision status were independent risk factors for HIV-1 infection in the placebo group alone, we applied the Cox model with adjustment for baseline demographic and risk variables. The adjusted HR was 1.6 (95% CI 0.7-3.6; $p=0.23$) for placebo recipients with Ad5 antibody titre greater than 18 versus Ad5 antibody titre 18 or less, and 2.5 (0.7-8.7; $p=0.14$) for circumcised versus uncircumcised placebo recipients. These results do not lend support to either variable as a significant independent predictor of HIV-1 infection; however, they should be interpreted with caution because the study did not randomly assign participants to Ad5 or circumcision groups, only to vaccine or placebo.

Because circumcision rates were substantially higher for Ad5-seronegative than for Ad5-seropositive participants (77.6% vs 40.4%, $p<0.0001$), we calculated HRs for four different subpopulations of men in the trial, on the basis of Ad5 and circumcision status. The unadjusted HR for risk of HIV-1 acquisition in participants receiving vaccine compared with those receiving placebo was highest in men who were uncircumcised and Ad5 seropositive ($n=620$, HR 3.9 [95% CI 1.3-11.9]). Risk was intermediate in men who were uncircumcised and Ad5 seronegative ($n=168$, HR 3.3 [0.7-15.8]) and in men who were circumcised and Ad5 seropositive ($n=421$, HR 1.6 [0.7-3.8]). Risk did not seem to be increased in men who were both

circumcised and Ad5 seronegative ($n=578$, HR 0.7 [0.3-1.4]). These results did not change significantly when we used adjusted HRs from any of several multivariate Cox models (data not shown).

To assess whether the increased hazard of HIV-1 acquisition recorded within these subgroups occurred only in peri-vaccination periods or persisted over time, we assessed the relative HIV-1 incidence (vaccine:placebo) during three 26-week periods over 78 weeks from the time of enrolment (figure 4). Overall and within subgroups, HIV-1 incidence was roughly constant over time for participants receiving both vaccine and placebo through 78 weeks of follow-up.

If the vaccine increased the risk of HIV-1 acquisition in uncircumcised male participants, a probable mechanism would be through exposure to insertive anal sex; therefore, relative hazards should be especially high in men reporting this risk. Therefore, we compared the relative hazard of HIV-1 infection between men who had and had not reported unprotected insertive anal sex with HIV-positive or unknown serostatus partners at baseline. In uncircumcised men, the HRs were even higher in men who reported unprotected insertive anal sex at baseline than in those who did not report this risk (HR 6.1 [95% CI 1.4-27.0] vs 2.5 [0.8-8.0]). We recorded no such relation with unprotected receptive anal sex with HIV-positive or unknown partners; the HR for

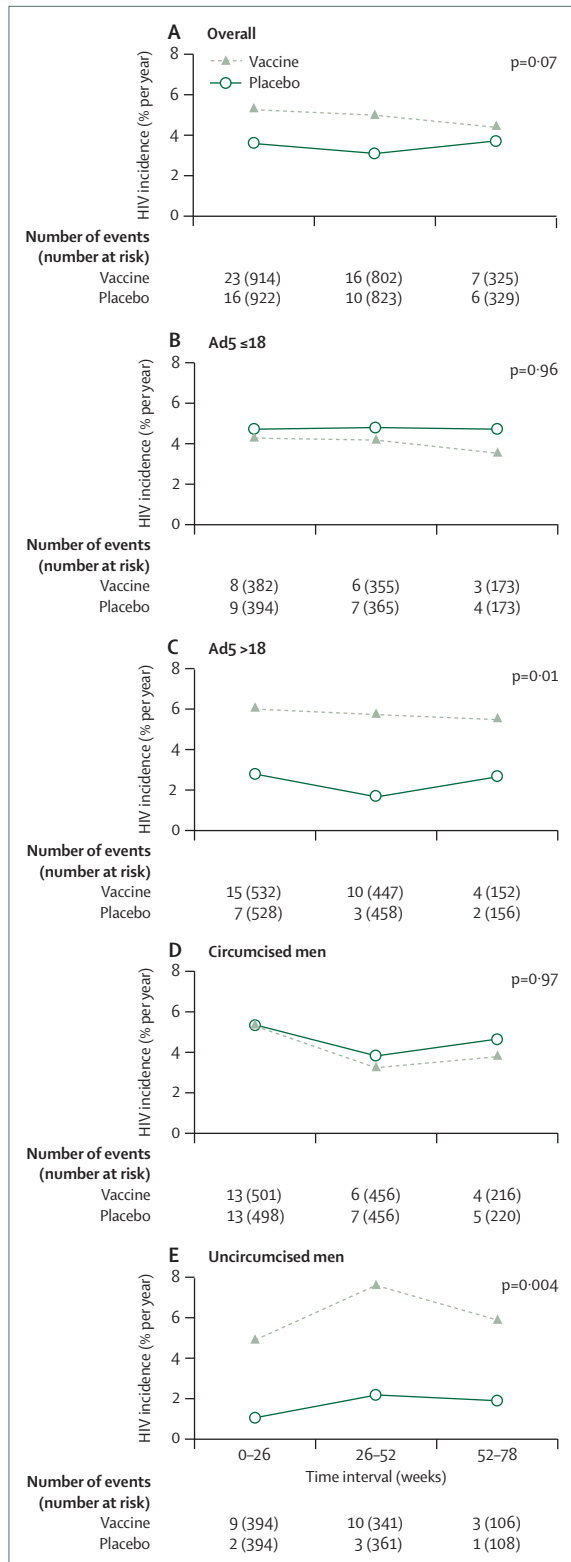


Figure 4: HIV incidence during 6-month intervals for male vaccine and placebo groups overall (A); by baseline Ad5 antibody titre ≤18 (B); baseline Ad5 antibody titre >18 (C); circumcised men (D); and uncircumcised men (E). Each two-tailed p value is from a univariate Cox regression model.

uncircumcised men who reported this risk at baseline was lower than that for uncircumcised men who did not report this risk (HR 3·7 [1·3–10·0] vs 5·7 [0·7–47·5]). In circumcised men, HRs were consistently near 1·0, irrespective of reported baseline risk.

Since the infection rates between vaccine and placebo recipients could be attributed to differences in risk practices between vaccine and placebo groups, especially if any substantial levels of unblinding had occurred, we compared risk data between vaccine and placebo recipients through 18 months of follow-up. For both groups, the proportion of study participants reporting risk decreased substantially during the first 6 months of the study, and then remained fairly level throughout follow-up (data not shown). The frequency of all measured variables for risk behaviour was similar for Ad5-seropositive participants receiving vaccine and placebo over time, and for uncircumcised vaccine and placebo recipients over time (all p values >0·20; data not shown).

Discussion

To our knowledge, no study has yet completed its efficacy assessment of a cell-mediated immunity HIV-1 vaccine. 33 months after the first participant was enrolled in the Step Study, this trial established that the MRKAd5 gag/pol/nef HIV-1 vaccine neither prevented HIV-1 infection nor lowered viral-load setpoint in participants with baseline Ad5 antibody titres 200 or less, despite generating interferon-γ ELISPOT responses in most participants receiving vaccine. High levels of protocol adherence provide further confidence in this study's conclusions about the vaccine's absence of protective efficacy. Unfortunately, we were unable to conclusively assess vaccine efficacy in women in this trial because of low rates of HIV-1 acquisition, which were possibly driven by low HIV-1 prevalence in male partners. The challenge of identifying high-seroincidence cohorts of women in the Americas has been shown in several other studies.^{39,40}

Because cell-mediated immunity vaccines act by killing HIV-infected cells, they would probably have their biggest effect in control of viral replication, rather than prevention of infection.⁴¹ However, we noted no indication that early plasma viral concentrations were reduced in vaccine recipients compared with placebo recipients in this study. A companion Article⁴² explores the immunological response in people receiving the vaccine 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 whether this insufficiency represents a more fundamental challenge of cell-mediated immunity vaccines to change the clinical course of HIV-1 disease. The Step Study has provided important data for this field, and a repository of specimens with which to explore reasons for failure (eg, 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 (eg, presence or magnitude of a functional assay that is associated with lower viral load in subgroups of participants receiving the vaccine).

Surprisingly, we noted an increase in the number of HIV-1 infections in male recipients of the vaccine. These effects seemed to be restricted to men who were Ad5 seropositive or uncircumcised, or both, on multivariate analyses, and were not confounded by other demographic and risk variables measured at baseline. However, this finding does not rule out confounding by variables that have not yet been measured, such as baseline serostatus of herpes simplex virus type 2 (HSV-2) or host genetic factors, which are presently being measured in cryopreserved specimens. Other potential confounders, such as sexual network clustering, are being explored through viral genotyping.

Evidence for a mechanism for increased acquisition risk associated with this candidate or other adenovirus-based HIV vaccines is scarce in published work. Antibody-dependent enhancement has been described for many viral infections.^{43,44} So far, such enhancement has been directed at surface envelope proteins, and the MRKAd5 HIV-1 gag/pol/nef vaccine did not contain envelope inserts. A candidate HIV vaccine using a recombinant varicella-zoster virus (VZV) vector that led to enhanced SIV replication and disease progression in rhesus macaques has been reported, although the investigators did not assess the effects on SIV acquisition.⁴⁵ The VZV vaccine elicited a robust SIV-specific CD4+ T-cell response without a measurable CD8+ T-cell response, which is quite different from the immunological profile of the trivalent vaccine.

The mechanism for enhanced HIV-1 acquisition risk in vaccinated Ad5-seropositive men is probably complex. All people receiving the vaccine are likely to have developed both Ad5 antibodies and T-cell responses to the vector; thus, none of the recipients were probably Ad5 seronegative after the first immunisation. However, natural Ad5 infection occurs via the nasopharynx or gut, can persist at mucosal surfaces over several years, and preferentially infect lymphocytes that home to mucosa.⁴⁶ People receiving vaccine with pre-existing Ad5 immunity might generate an Ad5-specific immune response that homes to mucosal surfaces, whereas those with vaccine-induced Ad5 immunity might 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 might cause an as yet undefined effect on the immune response that led to increased HIV-1 acquisition. Whether the effects of this vaccine apply to other adenovirus-based HIV vaccines, including those using alternative serotypes, is not yet clear. Until the mechanism for these effects can

be clarified, clinical trials of novel adenovirus-based HIV vaccine candidates should include safeguards to keep potential risk to study volunteers to a minimum (eg, restricting study enrolment to subgroups without evidence of vaccine-associated increased risk, close study monitoring, and extensive discussion of risk during informed consent process).

Circumcision is associated with halving the risk of HIV-1 acquisition in men who have sex with men in a longitudinal study,⁴⁷ although data from cross-sectional studies and smaller longitudinal studies have been mixed.^{48–50} The protective effect of circumcision can be more difficult to show for men who engage in both insertive and receptive anal sex, and might therefore be most concentrated in men reporting unprotected insertive anal sex with HIV-positive partners or those whose serostatus is unknown. In this study, uncircumcised men receiving vaccine were at increased risk of HIV-1 acquisition compared with uncircumcised participants receiving placebo, especially in men reporting high-risk insertive anal sex. Conversely, the risk of HIV-1 acquisition did not seem to be more concentrated in uncircumcised men reporting high-risk receptive anal sex at baseline, nor did circumcised men seem to be at increased risk, irrespective of their sexual practices. These results call for further inquiry into assessment of 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 deepened our understanding of the potential, and potential pitfalls, of present non-human primate challenge models. A prototype replication incompetent Ad5 vaccine based on an earlier Ad5 gag-only vaccine (Merck) provided substantial and durable control of viral replication against SHIV 89.6P challenge,¹⁴ particularly in animals with genetic markers associated with virological control.^{25,27} Results from the Step trial suggest that this model is not a useful predictor of the use of T-cell-based vaccines in human beings.⁵¹ Other non-human primate challenge studies of this candidate vaccine have shown a more transient protection against SIV_{mac}239 that might depend on a DNA prime²⁵ or inclusion of additional gene inserts;²⁶ however, the use of these challenge models has also not yet been proven.⁵² If vector-based immunity has an important role in the quality of the immune response generated to vaccines, animal models might not predict clinical experience, especially when the vector does not naturally infect animals used in these models.

The Step Study has challenged the field to improve understanding of the role of vector-based immunity, the potential for vaccine-induced increased acquisition, and to use the data and specimens in this human trial of a cell-mediated immunity vaccine, to understand the vaccine's failure. Additional coordinated efforts will be needed to provide definitive answers to these questions, and ultimately to develop a safe and effective HIV vaccine.

Contributors

SPB, DVM, AD, DWF, PBG, MJM, DRC, JAC, LC, and MNR participated in the design of the study. SPB, MNR, and DWF co-chaired the study and together with AD, oversaw study implementation. KMG and LC served on the oversight committee. JRL, MM, and CDR participated in the conduct of the study. MJM and DRC led the laboratory components of this study and undertook the immunological assays. DVM, RM, DL, and PBG analysed the study data. SPB, DVM, PBG, and MNR drafted the report, and all co-authors participated in revising the report.

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Conflict of interest statement

DVM, RM, DRC, KMG, JAC, and MNR are all paid employees of Merck 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, PBG, JRL, CDR, and LC declare that they have no conflict of interest.

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