Published in final edited form as:
Lancet Infect Dis. 2014 May ; 14(5): 388–396. doi:10.1016/S1473-3099(14)70020-9.

The phase 2b HVTN 503/Phambili study test-of-concept HIV vaccine study, investigating a recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up

Glenda E Gray, MBBCH[1,2], **Zoe Moodie, PhD**[3], **Barbara Metch, MS**[3], **Peter B. Gilbert, PhD**[3], **Linda-Gail Bekker, PhD**[4], **Gavin Churchyard, PhD**[5], **Maphoshane Nchabeleng, MBChB**[6], **Koleka Mlisana, MBChB**[7], **Fatima Laher, MBBCH**[1], **Surita Roux, MBChB**[4], **Kathryn Mngadi, MPhil**[7], **Craig Innes, MBChB**[5], **Matsontso Mathebula, MBChB**[6], **Mary Allen, MS**[8], **M Julie McElrath, MD**[3], **Michael Robertson, MD**[9], **James Kublin, MD**[3], and **Lawrence Corey, MD**[3] **on behalf of the HVTN 503 study team**

[1]Perinatal HIV Research Unit, University of Witwatersrand [2]South African Medical Research Council, South Africa [3]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA [4]Desmond Tutu HIV Foundation, University of Cape Town, South Africa [5]Aurum Institute for Health Research, Johannesburg, South Africa [6]MEDUNSA HIV Clinical Research Unit, University of Limpopo, South Africa [7]Centre for AIDS Programme for Research in South Africa, University of KwaZulu-Natal [8]Vaccine Research Program, Division of AIDS, NIAID, NIH, USA [9]ID/Vaccines Clinical Research, Merck and Company, INC, USA

Abstract

Background—The Phambili study, conducted in South Africa amongst a predominantly heterosexual population, evaluated the efficacy of the MRK Ad5 *gag/pol/nef* subtype B HIV-1 preventive vaccine. Enrollment and vaccinations were stopped, participants unblinded, and follow-up extended when the Step study evaluating the same vaccine in the Americas, Caribbean and Australia was unblinded for non-efficacy with more HIV infections amongst vaccinee than placebo recipients [ZM1]. Extensive analyses over the complete follow-up period, most of which was unblinded, are reported.

Methods—Phambili participants were HIV-1 uninfected, sexually active men and women aged 18–35 years, followed for 3.5 years. HIV testing and risk reduction counseling occurred at weeks 0, 12, 30 and were switched to a 3 monthly schedule after unblinding. Cox proportional hazards

Correspondence: G. Gray, Perinatal HIV Research Unit (PHRU), University of the Witwatersrand, Chris Hani Baragwanath Hospital& South African Medical Research Council, PO Box 114, Diepkloof, Johannesburg 1864, South Africa; gray@pixie.co.za. Mobile: +27834592680.

models were used to estimate HIV-1 infection hazard ratios (HR) comparing vaccine to placebo recipients, overall and within subgroups. Long-term vaccine efficacy was evaluated in participants who were unblinded early in follow-up.

Results—Of the 801 participants enrolled (400 vaccine, 401 placebo), 112 (28%) received 1 vaccination, 259 (65%) 2 vaccinations and 29(7%) 3 vaccinations. More infections occurred in vaccinees (n=63) as compared to placebo (n=37) (adjusted HR (vaccine:placebo) 1.70, 95% CI 1.13–2.55, p = 0.01). We found no increase in infections with the number of vaccinations received and that the HRs did not differ by gender, circumcision, or Ad5 serostatus. Differences in risk behavior at baseline or during the study, or differential drop-out (p=0.40) are unlikely explanations for the increased rate of HIV-1 infections seen in vaccinees.

Conclusion—The increased HR of HIV-1 acquisition, irrespective of number of doses received, warrants further investigation to understand the biological mechanism. Further use of the Ad5 vector for HIV vaccines is not warranted

Keywords

HIV; rAd5 HIV vaccines; HIV-1 vaccine efficacy studies; South Africa

Introduction

Recombinant adenovirus type 5 (rAd5) vectors have been described as ideal platforms for use in HIV-1 vaccine research because they are highly immunogenic, able to express large amounts of antigen/s, and are easily manufactured[1]. Despite well-documented pre-clinical studies[2,3,4,5,6,7,8,9], clinical safety and immunogenicity in multiple phase1/2 studies[10,11,12,13,14,15,16,17,18], two phase 2b proof-of-concept trials of a rAd5-vectored HIV-1vaccine failed to show vaccine efficacy[19,20,21]. The first of these trials, the Step study[19], raised concerns about vaccine enhanced HIV-1 acquisition. Step tested the MRK rAd5 polyvalent HIV-1 *gag/pol/nef* subtype B vaccine in a cohort consisting primarily of men who had sex with men (MSM) and at risk women in the Americas, Caribbean and Australia, where the circulating HIV-1 subtype is B. In September 2007, the first interim efficacy analysis concluded that the trial met the pre-specified criteria for futility, thus vaccinations were halted, follow-up continued, and participants were informed of their treatment assignments. Unplanned subgroup analyses of Step showed that the increased susceptibility to HIV-1 acquisition appeared restricted to vaccinated Ad5 seropositive and/or uncircumcised men (based on interaction tests).[19] A subsequent analysis of the study follow-up showed a higher risk of HIV-1 infection among male vaccinees as compared to male placebo recipients over all the follow-up time (hazard ratio [HR]1.40; 95% CI 1.03–1.92; p=0.03).[22] The vaccine-induced enhanced risk of HIV-1 acquisition seen in the uncircumcised and/or Ad5 seropositive men was more evident in the first 18 months and appeared to wane over time, whereas for circumcised, Ad5 seronegative men, the HR increased over time from 0.38 (95% CI 0.16–0.90) in the first 18 months to 2.18 (95% CI (0.97–4.92) after 18 months of follow-up.

The second trial of the MRK rAd5 vaccine, HVTN 503 or Phambili,[20] was undertaken in South Africa, where clade C is the predominant HIV-1 subtype, in a principally heterosexual

population. Enrollment and vaccinations were halted in September 2007 when Step met the non-efficacy criteria. Because of the increased susceptibility to HIV-1 acquisition seen in certain sub-groups of Step, participants were rapidly unblinded in Phambili and follow-up was extended. The initial analysis of Phambili based on an average of 22.5 months of follow-up found that although there were more infections amongst vaccinees as compared to placebos, this was not statistically significant [HR 1.25 adjusted for gender (95% CI 0.76–2.05)]. Because of the concern for increased acquisition and the lack of sufficient HIV-1 infections in the Step trial to assess the effect of vaccination in women, long-term follow-up of the Phambili participants occurred. This report describes HIV-1 acquisition over the 3.5 years of participant follow-up and recent recall of the Phambili cohort.

Methods

Study Design and Population

This two-arm, double-blind, placebo controlled randomized clinical trial, initiated in January 2007 was designed to enroll 3000 healthy HIV-1 uninfected, heterosexual adults aged 18–35 at 5 sites within SA (Soweto, Cape Town, Klerksdorp-Orkney-Stilfontein-Hartbeesfontein [KOSH], eThekwini, and MEDUNSA).[20] On 19 September 2007, enrollment and vaccinations were halted and in October 2007, unblinding of participants began, with concomitant HIV-1 testing, risk evaluation and counseling.

The study was registered with the Food and Drug Administration in the USA; and approved by the SA Medicines Control Council; the Genetically Modified Organism Review Committee of the SA Department of Agriculture; and the ethical review committees and institutional biosafety committees of the University of the Witwatersrand, University of Cape Town, University of Limpopo and the University of KwazuluNatal. Participants provided written informed consent in English or their local language.

Products and Procedures

MRKAd5 HIV-1 *gag/pol/nef* vaccine (Merck and Co., Inc) was given as a dose of 1.5×10^{10} Ad viral genomes/1mL. Placebo was a 1ml solution of the vaccine diluent with no Ad5 vector. Study products were administered by intramuscular injection on a 0, 1, 6 month schedule.

Initially, HIV-1 evaluation was done on blood drawn on the day of first vaccination, weeks 12, 30, 52, and every six months. Risk was assessed six months prior to screening and at HIV-1 evaluation. After unblinding and receipt of appropriate approvals, follow-up visits were changed to every three monthly for HIV-1 evaluation, risk assessment, and risk reduction counseling. In June 2013, a follow-up study of the 695 Phambili participants who were alive and HIV-1 uninfected at their last study visit was implemented.

Statistical Analysis

Randomization was 1:1 between vaccine and placebo, stratified by site and gender based on computer-generated random numbers and provided to site pharmacists by a central statistical and data monitoring center (SDMC). Analyses were of the modified-intent-to-treat cohort

(MITT), consisting of all participants HIV-1 uninfected at enrollment. Unless otherwise specified, analyses included the total follow-up period of 3.5 years per HIV-1 uninfected participant, including blinded and unblinded follow-up periods. A formal comparison of rates of infection pre- and post-unblinding was not possible due to the small number of infections pre-unblinding.

Kaplan-Meier cumulative incidence plots of time to HIV-1 infection by treatment, overall and by subgroups were provided, with log-rank p-values. Cox proportional hazards models were used to estimate the HR for HIV-1 infection due to vaccination (vaccine:placebo), overall and within subgroups. Time to HIV-1 infection for infected participants was defined as the time from first study injection to the midpoint between the last plasma HIV-1 RNA negative and first RNA positive test; for uninfected participants the censoring time was defined as the time from first study injection to the last study HIV test. Differences in HRs between subgroups were assessed with Wald tests for the vaccine by subgroup interaction. To assess whether HRs varied over time, Grambsch and Therneau[23] tests were used. Differences between vaccinees and placebos in time to dropout and for HIV-1 infected participants' time to CD4 decay were assessed by log-rank tests. Predictors of drop-out were assessed in univariate and multivariate Cox models. Differences in viral load set-point between HIV-1 infected vaccine and placebo recipients were assessed with Wilcoxon rank sum tests. We defined set-point as the geometric mean of HIV-1 plasma viral-load measurements obtained between 2–3 months after diagnosis. All p-values were 2-sided.

Results

801 (26.7%) of the planned 3000 participants were enrolled before vaccinations were suspended and participants informed of their treatment allocation. One woman who received placebo was diagnosed as HIV-1 infected at enrollment and was therefore excluded from the MITT cohort. Men comprised 55% (n=441) of the MITT cohort, with 129 previously circumcised at the time of enrollment and an additional 139 circumcised while on study (supplemental tables 1 and 2). Among those randomized to receive the vaccine product, 112 (28%) received one vaccination, 259 (65%) received two and 29 (7%) received three vaccinations (figure 1). Since participants were unblinded early in the study, only 289 (13%) of the observed 2268 person years of follow-up occurred while the study was blinded (figure 1).

The annualized dropout rate was 7.7% for vaccinees (95% CI 6.2%–9.5%) and 8.8% for placebo (95% CI 7.1%–10.7%)(p=0.40, supplemental figure 1). During the follow-up period, there were 100 HIV-1 infections (table 1). Significantly more HIV-1 infections occurred amongst vaccinees (n=63) as compared to placebo (n=37); the annualized HIV-1 incidence rate was 5.61% (95% CI 4.31%–7.18%) for vaccinees and 3.23% (95% CI 2.28%–4.45%) for placebo recipients (p=0.007, figure 2A). Fourteen of the infections (8 vaccine; 6 placebo) were observed during the 289 person-years of blinded follow-up.

The HR adjusted for baseline HSV-2 status was 1.70 (95% CI: 1.13–2.55, p=0.01, table 2). The overall annualized incidence rate was much higher in women (6.10%) than in men (3.07%). The vaccine:placebo HR was > 1 for both men and women (table 2), with a more

pronounced effect in men (interaction p=0.19, table 2, figure 2D, 2E). There was no evidence that the HR varied by baseline Ad5 status, age, or in men, by time-dependent circumcision status (interaction p-values ≥0 .38, table 2, supplemental table 2). The HR did not significantly differ among the five study sites (p=0.51, Table 2), and the estimated HR was highest in Soweto (p=0.02), the site with the highest enrollment.

While the overall HR did not significantly vary over time (p=0.11), the differences in HIV-1 acquisition rates became evident as infections accumulated during the long follow-up period (figure 2). Among participants who became HIV-1 infected, vaccination had no significant effect on viral load set-point or time to CD4 decay to less than 350. Descriptively, viral load set-point appeared to be slightly lower in the vaccine than placebo group overall and within gender subgroups (supplemental figure 2).

To determine possible explanations for the observed increased rate of HIV-1 infection among vaccinees, we considered the effect of number of vaccinations received and extensively analyzed the dropout and risk behavior data as well as the relationship between on study circumcision and HIV-1 acquisition. We found no evidence that the increase in infection rate amongst vaccinees depended on the number of vaccinations received (supplemental figure 3). The annualized dropout rate for men was higher than for women (9.7% vs. 6.4%), although neither subgroup showed a significant difference in dropout between vaccine and placebo (supplemental figure 1). Of the 100 documented HIV-1 infections, 37 occurred in Soweto and 27 in Cape Town. The annualized dropout rate in the Soweto site was only 4.6%, with no difference between the vaccine and placebo groups (p=0.49). The Cape Town site experienced higher rates of drop-out with more men in the placebo group dropping out compared to the vaccine group although this was not significant (supplemental table3, supplemental figure 1G).

To further assess if differential dropout could account for the increased HR among men, we looked for risk behaviors that were associated with HIV-1 infection and if more of these behaviors were significant predictors of dropout among the placebo men than among vaccine men. Among all men, we found significant associations between HIV-1 infection and certain self-reported risk factors during the trial (multiple partners; casual/anonymous partner; unprotected vaginal/anal sex; apart regularly from main partner), but these behaviors were not associated with dropout among the male vaccine or placebo recipients. Adjustment of the HR estimate for men for behavioral risk in addition to baseline HSV-2 status had little effect on the HR estimate: HR=2.30 after adjustment for behavioral risk (95% CI: 1.14–4.62, p=0.02) versus HR=2.46 (table 2).

To evaluate if differential loss to follow-up was a major factor influencing the higher rates of infection among vaccinees, we implemented a follow-up study in June 2013 to bring back all 695 HIV-1 uninfected participants. To date, of the 422 participants seen, 28 additional HIV-1 infections have been detected (table 3). Of the 189 participants who dropped out, 58 have been located and enrolled into the sub-study. The HIV-1 incidence amongst drop-outs was 5.29% (95% CI: 1.94%–11.51%; n=5 infected men/1 infected woman) in vaccinees vs. 0.96% (95% CI: 0.02%–5.34%; n=1 infected woman) among placebos. Including the

additional follow-up time, the adjusted HR is 1.57(95% CI: 1.10–2.23) compared to1.70 (95% CI: 1.13–2.55) for the Phambili study alone.

Discussion

While not significant in our earlier analysis at an average of 22.5 months of follow-up, our long-term follow-up analysis shows a significantly higher rate of HIV-1 infection among vaccinees than placebos. This finding was not related to the number of vaccinations received nor explained by any covariate imbalances between the vaccinated and placebo recipients. The increased vaccine:placebo HR is consistent across all sub-groups (p-values 0.01–0.27), albeit weaker in women than men.

Our data support the Step findings that the MRK Ad5 gag/pol/nef HIV-1 vaccine induced enhancement of HIV-1acquisition among male vaccinees with no significant change in the vaccine: placebo HR over time.[22] Step men were primarily MSM, received three vaccinations and were exposed to sub-type B HIV-1. Step demonstrated significant changes in the HR for two sub-groups over time. Among uncircumcised, Ad5 seropositive men, the initially high HR in the first 18 months decreased (interaction test p=0·04) and the initial low HR among circumcised, Ad5 seronegative men increased after 18 months (p=0·04). Phambili participants were predominantly heterosexual, exposed to sub-type C HIV-1 and received one or two vaccinations. The increased risk of HIV-1 acquisition due to vaccine was significant in the Ad5 seropositive subgroup (p=0.02) and increased with time in both the Ad5 seronegative subgroup (p=0.01) and in circumcised men (p=0.02).

Given that most of the follow up occurred post-unblinding, we have looked extensively for confounders such as treatment differences in risk behavior to explain our results. Although we did not find any differences in behavior at baseline or during study follow-up between the treatment groups, our staff-administered risk assessments may have led to inaccurate or under-reporting of risk or recall bias. In the future, use of novel platforms to capture sexual practices contemporaneously, or ACASI use at the clinic may improve the reliability of reported sexual practices. After unblinding, under-reporting may have occurred more frequently among vaccinees as they received from staff a stronger message regarding risk based on the Step results. We have previously reported that between 8–11 months after unblinding, more participants in the vaccine group felt they were more likely to get HIV/AIDS than most people, and attributed this to receiving the vaccine.[24]

To address concerns of ascertainment bias, we initiated a follow-up study to perform HIV testing for all HIV-1 uninfected participants to assess whether higher risk among placebo participants who dropped out could provide an explanation for our results. Based on data accrued to date, we still find a disproportionate amount of HIV-1 infections amongst male vaccinees, particularly those who dropped out of the primary study, consistent with an interpretation of vaccine induced enhancement (table 3). Interestingly, the evidence for enhancement in women lessened based on the additional data.

Our findings differ from that seen in HVTN 505, a study that used DNA priming before a single Ad5 boost.[25] The vaccine regimen used in HVTN 505 contained HIV-1 *env* antigen

inserts that induced binding antibodies to HIV-1 envelope. The HVTN 505 study, performed in circumcised Ad5 seronegative MSM, did not demonstrate efficacy nor was there evidence of vaccine-induced enhancement. This suggests that any potential enhancement could have been overcome by anti HIV-1 envelope specific responses, implying that our findings may not be applicable to all Ad5 HIV-1 vaccines.

While unidentified, confounding behavioral factors are potential explanations for our data; the differences between men and women and the consistency between the Step and Phambili studies point to a potential biological explanation for our observation. A recent meta-analysis combining the Step and Phambili data has shown an estimated HR of 1.41 (95% CI: 1.11–1.78, p = 0.005)[26] between vaccinees and placebos, that is constant over time. Sub-clinical genital inflammation is a well-recognized risk factor for HIV-1 acquisition,[27, 28] as has been demonstrated with HSV-2 infection. We do not have a plausible biological mechanism for this increased susceptibility seen, and postulate that vaccination increased the number of target cells in the genital mucosa. This has been postulated as a mechanism in HSV-2 infection, which has been shown to lead to increased rates of HIV-1 acquisition.[29] As with HSV-2, it is possible that similar interactions could exist for potent vectors such as Ad5. Ad5 vector immunization, as well as naturally occurring adenovirus infection have been shown to lead to an expansion of activated Ad5-specific T-cells thereby increasing the number of HIV-1 target cells[30, 31, 32]. Persistence of adenovirus antigen in the gut from natural infection may further facilitate migration and expansion of Ad5-specific T-cells in the intestinal mucosa with trafficking of target cells to the penile surface. As we did not use an empty viral vector as a control, we cannot determine whether the increased risk of acquisition could be attributed either to an Ad5-vector specific response or to the HIV-1 antigens (gag/pol/nef) used in the vaccine. A study conducted in NHPs utilizing an Ad5-based SIV gag/pol/nef vaccine demonstrated enhancement after a penile challenge, whereas, NHPs immunized with the empty Ad5 vector were not susceptible to low-dose SIV challenge, implying that the SIV-specific immune responses were responsible for the enhanced susceptibility[33].

The persistence of our effect may be attributed to antigen-experienced CD4 T-cells resident in the long-lasting resting subset of CD4 T-cells that have traditionally been described as “naive”[34,35] The increased rate of infection seen in the Phambili vaccine group was weakest amongst women. Given the high background prevalence of genital inflammation seen in women in SA and its association with HIV-1 acquisition, any additional vaccine-related subclinical inflammation may be masked by these already high rates of genital tract inflammation.[36] Given the gender differences seen in this study, future vaccine trials should be stratified by sex as was recommended when we published our initial findings[37]

Given the lack of efficacy of all tested Ad5-vectored HIV-1 vaccines[19,20,26], and our observations of vaccine induced enhancement, we do recommend caution with the use of this vaccine platform in high HIV-1 prevalence areas. Further investigations with this vaccine delivery platform in HIV-1 vaccine trials should inform participants of these findings and include judicious monitoring for potential increased risk of HIV acquisition.. In assessing Ad5-vectored vaccines for other diseases, we recommend monitoring participants

for HIV-1 acquisition, particularly when trials are conducted in high HIV-1 prevalence areas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Our study was funded by grants from the National Institute of Allergy and Infectious Diseases to the HIV Vaccine Trials Network (5U01 AI068614, 5U01 AI068618, 5U01 AI068635, 5U01 AI069453, 5U01 AI069519, 5U01 AI069469) as well as Merck and Co Inc. The South African AIDS Vaccine initiative (SAAVI) of the Medical Research Council provided support to the clinical trial sites. We thank the Phambili Study volunteers and the staff and community members at each of the Phambili Study sites, the staff at the HVTN Administrative Core, SCHARP Statistical Center, and Central Laboratory; the staff at Merck, and Elizabeth Adams at the Division of AIDS in the National Institute of Allergy and Infectious Diseases for their facilitation of the study design, and conduct. The opinions expressed in this Article are those of the authors and do not represent the official views of the US National Institute of Allergy and Infectious Diseases.

Role of the funding source

The study was reviewed by the Division of Acquired Immunodeficiency Syndrome of the US National Institute of Allergy and Infectious Diseases, and the report was reviewed by the sponsors. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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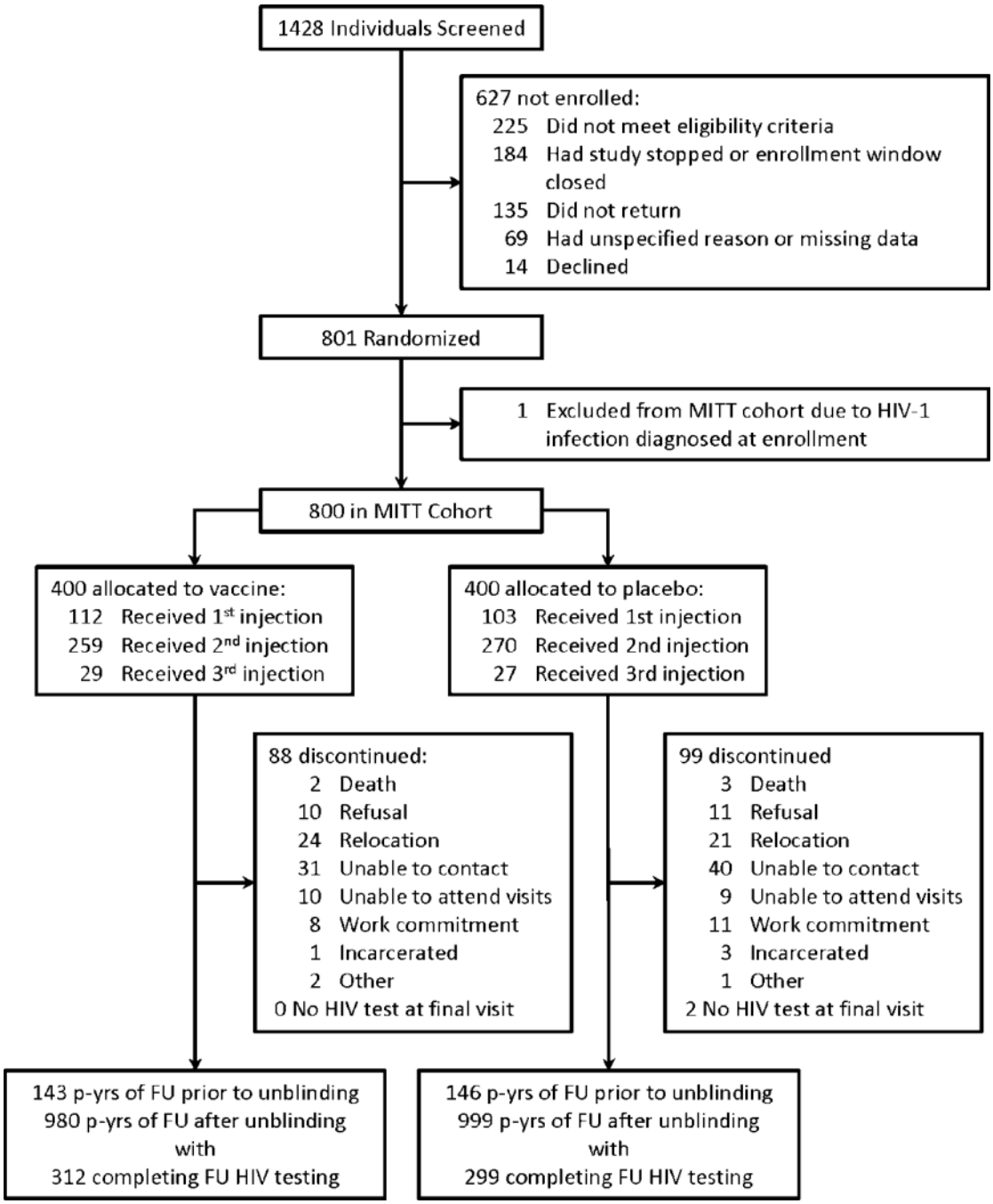
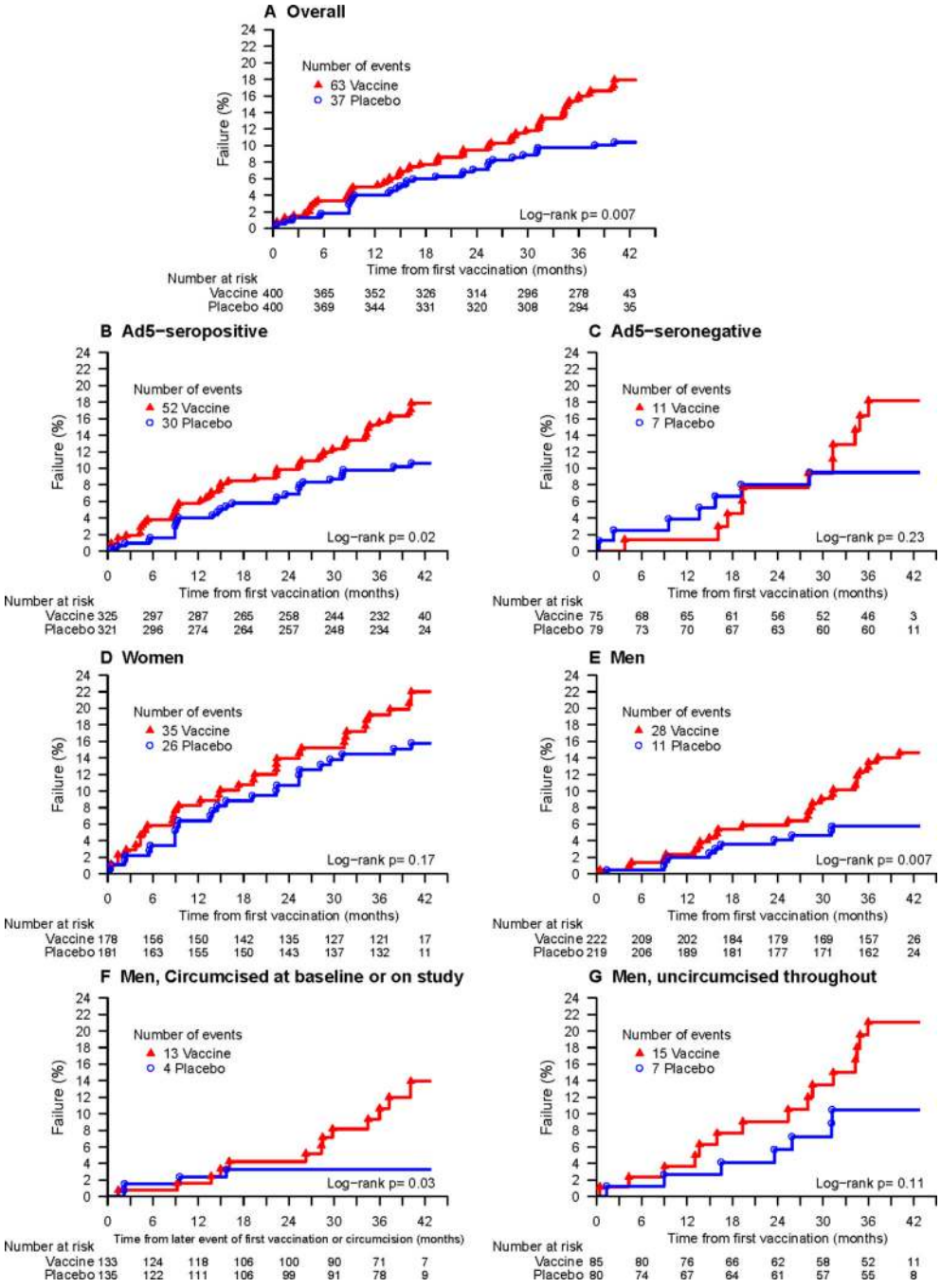


Figure 1. Trial profile

Abbreviations: MITT – modified intent-to-treat; p-yrs – person years; FU – follow-up



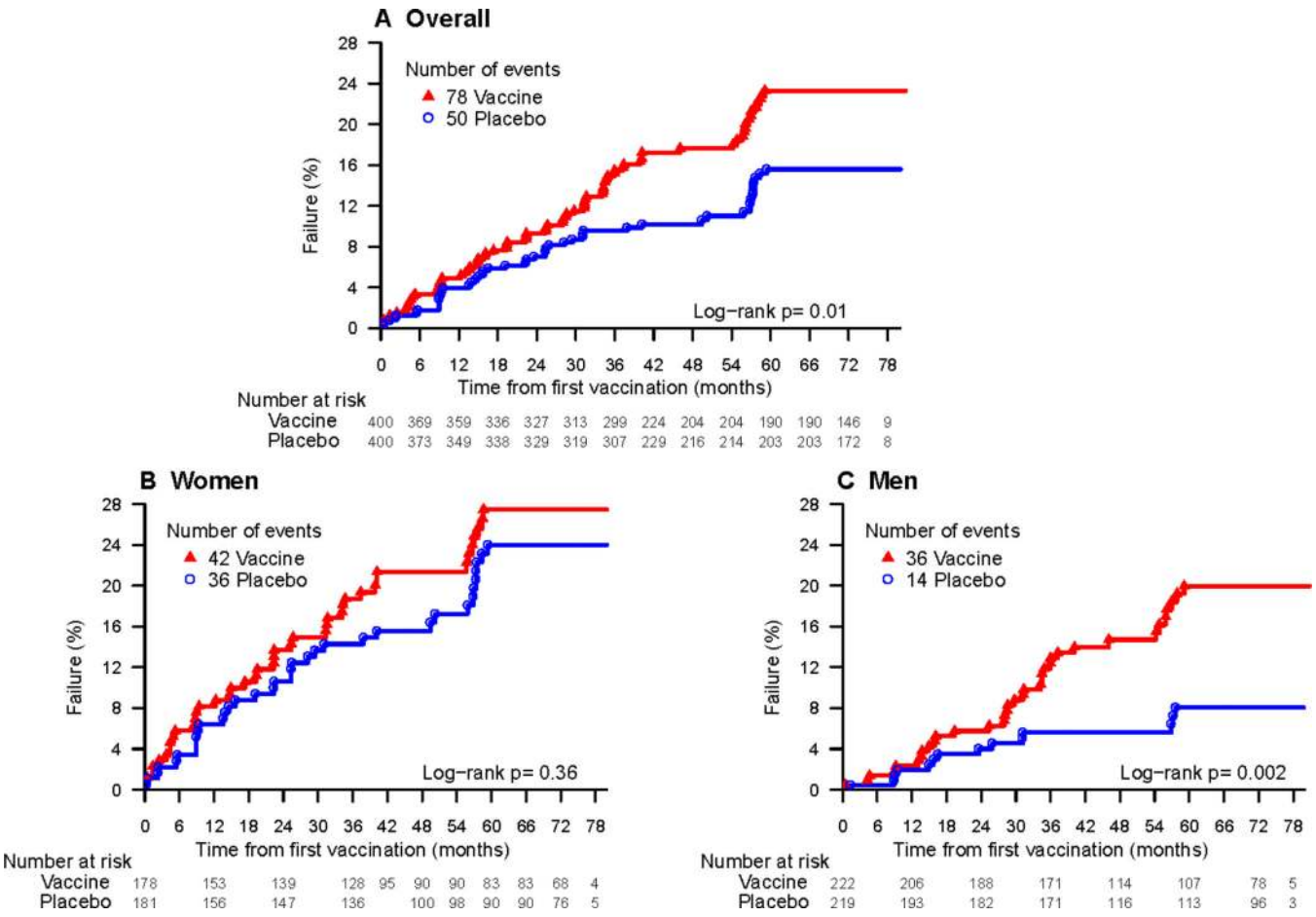


Figure 2.

a. Cumulative HIV-1 incidence curves for vaccine and placebo groups for main Phambili study (excluding sub-study)

b. Cumulative HIV-1 incidence curves for vaccine and placebo groups including additional follow-up from follow-up study

Table 1

Total number of incident HIV-1 infections by baseline adenovirus serotype 5 (Ad5) serostatus, gender, and circumcision status

	Vaccine				Placebo			
Category	**No. at risk**	**No. HIV-1 infections**	**Annualized HIV-1 incidence rate**	**(95% CI)**	**No. at risk**	**No. HIV-1 infections**	**Annualized HIV-1 incidence rate**	**(95% CI)**
Overall	400	63	5.61%	(4.31%, 7.18%)	400	37	3.23%	(2.28%, 4.45%)
Ad5-seronegative	75	11	5.43%	(2.71%, 9.72%)	79	7	3.05%	(1.23%, 6.28%)
Ad5-seropositive	325	52	5.65%	(4.22%, 7.41%)	321	30	3.28%	(2.21%, 4.68%)
Women	178	35	7.21%	(5.02%, 10.03%)	181	26	5.06%	(3.31%, 7.41%)
Men	222	28	4.39%	(2.92%, 6.35%)	219	11	1.74%	(0.87%, 3.12%)
Circumcised men[a]	133	13	3.76%	(2.00%, 6.43%)	135	4	1.14%	(0.31%, 2.93%)
Uncircumcised[a] men	157	15	5.14%	(2.87%, 8.47%)	147	7	2.50%	(1.01%, 5.15%)

Abbreviations: CI, confidence interval; HIV-1, human immunodeficiency virus 1; Ad5, adenovirus serotype 5.

[a]For men who were circumcised on study, their person-years of follow-up from enrollment to end of study is split between the two circumcision subgroups. Their person-years from enrollment to circumcision were counted as uncircumcised time and person-years from circumcision to end of follow-up or HIV infection was counted as circumcised time. Eight men who were uncircumcised at enrollment and did not have a follow-up circumcision assessment were excluded.

Table 2

HIV-1 infection hazard ratios (vaccine:placebo), adjusted for baseline HSV-2

Cohort	Est. (95% CI); P-value	Interaction P-value	Time-varying HR P-value
Overall	1.70 (1.13, 2.55); 0.01	-	0.11
Ad5 seropositive	1.70 (1.08, 2.66); 0.02	0.98	0.51
Ad5 seronegative	1.70 (0.66, 4.40); 0.27		0.01
Women	1.42 (0.85, 2.36); 0.18	0.19	0.65
Men	2.46 (1.22, 4.93); 0.01		0.08
Men circumcised at baseline or on study	3.58 (1.16, 11.01); 0.03	0.38	0.02
Men uncircumcised throughout study	1.83 (0.74, 4.54); 0.19		0.29
Age ≤25 years	1.72 (1.04, 2.84); 0.03	0.96	0.41
Age > 25 years	1.66 (0.83, 3.32); 0.15		0.09
Soweto	2.40 (1.19, 4.86); 0.02	0.51	0.15
Cape Town	1.86 (0.84, 4.14); 0.13		0.51
KOSH	0.87 (0.33, 2.25); 0.77		0.93
eThekwini	1.91 (0.62, 5.94); 0.26		0.44
Medunsa	1.05 (0.21, 5.24); 0.95		0.50

Table 3

New HIV-1 infections detected among participants enrolled in the follow-up study with available HIV test results[a]

	Total	Vaccine	Placebo
All	28/421 (6.7%)	15/205 (7.3%)	13/216 (6.0%)
Women	17/190 (8.9%)	7/90 (7.8%)	10/100 (10.0%)
Men	11/231 (4.8%)	8/115 (7.0%)	3/116 (2.6%)

[a]Among the 422 participants enrolled in the follow-up study, one woman who received vaccine is pending HIV testing results.