MAJOR ARTICLE

# Placebo-Controlled Phase 3 Trial of a Recombinant Glycoprotein 120 Vaccine to Prevent HIV-1 Infection

**The rgp120 HIV Vaccine Study Group**[a]

**(See the article by Gilbert et al., on pages 666–77, and the editorial commentary by Graham and Mascola, on pages 647–9.)**

***Background.*** A vaccine is needed to prevent human immunodeficiency virus type 1 (HIV-1) infection.

***Methods.*** A double-blind, randomized trial of a recombinant HIV-1 envelope glycoprotein subunit (rgp120) vaccine was conducted among men who have sex with men and among women at high risk for heterosexual transmission of HIV-1. Volunteers received 7 injections of either vaccine or placebo (ratio, 2:1) over 30 months. The primary end point was HIV-1 seroconversion over 36 months.

***Results.*** A total of 5403 volunteers (5095 men and 308 women) were evaluated. The vaccine did not prevent HIV-1 acquisition: infection rates were 6.7% in 3598 vaccinees and 7.0% in 1805 placebo recipients; vaccine efficacy (VE) was estimated as 6% (95% confidence interval, −17% to 24%). There were no significant differences in viral loads, rates of antiretroviral-therapy initiation, or the genetic characteristics of the infecting HIV-1 strains between treatment arms. Exploratory subgroup analyses showed nonsignificant trends toward efficacy in preventing infection in the highest risk (VE, 43%; $n = 247$) and nonwhite (VE, 47%; $n = 914$) volunteers ($P = .10$, adjusted for multiple subgroup comparisons).

***Conclusions.*** There was no overall protective effect. The efficacy trends in subgroups may provide clues for the development of effective immunization approaches.

The creation of a vaccine to combat the global HIV-1 pandemic is an international public-health priority [1, 2]. Although infection leads to the development of an HIV-specific immune response, the immune system is generally unable to effectively control replication of the virus or to prevent immunosuppression [3]. Nonetheless, there is evidence of a protective immune response in certain special circumstances [4–9]. There has also been considerable debate with regard to whether antibody-mediated or cell-mediated responses are of primary importance in providing protective immunity [3, 10, 11].

Protection of chimpanzees from intravenous and mucosal challenge with homologous and heterologous HIV-1 strains has been achieved with recombinant HIV-1 envelope glycoprotein subunit (rgp120 and rgp160) vaccines [12–14]. Phase 1 and 2 studies in uninfected humans have demonstrated that rgp120 is safe and able to generate antibody responses similar to those observed in the protected chimpanzees [15–17].

Two versions of an rgp120 vaccine candidate advanced to phase 3 studies in 1998–1999. The first study was to evaluate a bivalent subtype B/B rgp120 vaccine in individuals in North America and The Netherlands who were at risk for infection via sexual exposure, whereas the second study was to evaluate a bivalent subtype B/E rgp120 vaccine in injection drug users in Thailand [17, 18]. Here, we report the results of the first of these studies designed to evaluate whether an rgp120 vaccine can confer protection against HIV-1 infection.

## VOLUNTEERS, MATERIALS, AND METHODS

### Study Design

In this double-blind, randomized trial (known as "VAX004"), the volunteers were healthy, 18–62 years old, did not use intravenous drugs, and were either

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Potential conflicts of interest: listed after the text with the members of the Writing and Analysis Committee.

[a] Study group members and members of the Writing and Analysis Committee are listed after the text.

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men who have sex with men (MSM) or women at high risk for heterosexual transmission of HIV-1. Men were eligible if they had had any anal intercourse during the preceding 12 months but were excluded if they had had a continuously monogamous sexual relationship with an HIV-1–uninfected male partner for ⩾12 months. Women were eligible if they had had sexual intercourse with an HIV-1–infected male during the preceding 30 days or met at least 1 of the following criteria: had smoked crack cocaine during the preceding 12 months, had exchanged sex for drugs or money during the preceding 12 months, or had ⩾5 male sex partners during the preceding 12 months. A computer-generated block randomization list, stratified by the 61 sites that participated in the study, was designed to satisfy a 2:1 vaccinee to placebo recipient ratio. The eligibility criteria for and screening and enrollment of these volunteers have been described in detail elsewhere [19]. Volunteers who met the eligibility criteria, which included a negative test for HIV-1, were to be enrolled within 30 days of screening. The actual screening interval ranged from 1 to 51 days (median, 15 days), and 99% were enrolled within the required 30 days.

### Vaccine and Placebo Preparations

The study vaccine contained 2 rgp120 HIV-1 envelope antigens (300 $\mu$g each of MN and GNE8 rgp120/HIV-1) (AIDSVAX B/B; VaxGen) that had been derived from 2 different subtype B strains and that were adsorbed onto 600 $\mu$g of alum. GNE8 gp120 was cloned directly from peripheral-blood mononuclear cells and had the CCR5 phenotype; the GNE8 gp120 DNA sequence was deposited in GenBank (accession no. AY771703). Placebo consisted of alum only.

### Ethics Considerations

The present study was conducted in accordance with the Declaration of Helsinki and local institutional review board requirements and with approval from appropriate regulatory authorities. Written, informed consent was obtained from all volunteers. Before enrollment in the study, a thorough discussion of possible issues and risks associated with participation was conducted with each potential volunteer [20]. At each visit that included screening, trained counselors provided comprehensive education and pre- or post-HIV test and risk-reduction counseling, according to a comprehensive manual. Safety was monitored every 6 months by an independent data and safety monitoring board, which performed 1 interim efficacy analysis 40 months after initiation of the study.

### Vaccination and Study Assessments

Vaccine or placebo was administered by intramuscular injection at months 0, 1, 6, 12, 18, 24, and 30, with a final follow-up visit at month 36. At each visit, adverse events and possible social harms were assessed; blood was obtained for assessment of HIV-1 status and immunogenicity. HIV-1 status was determined by detection of HIV-1 antibodies, using a standard HIV-1 ELISA and confirmatory immunoblot. The date of HIV-1 infection was estimated as follows: if HIV-1 RNA was undetectable in serum by a highly sensitive and specific nucleic acid–based amplification test (NAT; Procleix HIV-1 Discriminatory Assay) at the date of the last seronegative test, then the date of HIV-1 infection was estimated as the midpoint of the dates of the last negative and first positive ELISA/immunoblot test results. Otherwise, the infection date was estimated as the date of the earliest sample with detectable HIV-1 RNA. For volunteers who became infected during the study, plasma HIV-1 RNA load and $CD4^+$ lymphocyte counts were assessed at <1 month and at months 1, 2, 4, 8, 12, 16, 20, and 24 after diagnosis of infection. Self-reported risk behaviors, including sexual activity and alcohol and drug use, and occurrence of sexually transmitted diseases were assessed by use of standard interviewer-administered questionnaires at baseline and every 6 months thereafter.

### Sequencing of Viral gp120

HIV-1 RNA was isolated from the earliest postinfection plasma sample; full-length gp120 genes were amplified and cloned. Three full-length gp120 sequences were recovered from each of 336 of 368 infected volunteers. With the exception of 1 subtype C virus, all isolates were subtype B.

### Immune Responses to the rgp120 Vaccine

A cytopathicity bioassay was used to determine 50% neutralizing titers for HIV-$1_{MN}$ infection of MT-4 cells. Binding antibodies were measured in 5 indirect ELISAs with an MN/GNE8 gp120 mixture and GNE8 V2, MN V2, GNE8 V3, and MN V3 peptides as the antigens. Two competitive ELISAs were used to measure antibody blocking of the binding of MN or GNE8 gp120 to recombinant soluble CD4 [21, 22]. The 8 assays were performed on samples obtained 2 weeks after the last immunization before HIV-1 infection for infected vaccinees and on samples obtained 2 weeks after each immunization for a 5% random sample of uninfected vaccinees.

### Statistical Analysis

***Primary end-point analysis.*** Vaccine efficacy (VE) was defined as $(1 - \text{the relative risk of infection}) \times 100$ and was estimated by use of a Cox proportional hazards model, with time to HIV infection grouped over six 6-month intervals and with the Efron method used for correction for ties [23]. The sample size of the trial was selected as that which would provide, by a 2-sided log-rank test, 90% power to reject the null hypothesis—VE ⩽30% if the true VE ⩾60%. The Lan-DeMets implementation of the O'Brien-Fleming stopping boundary was used for 1 interim efficacy analysis.

***Secondary end-point analyses.*** A generalized-estimating-equations model, which was based on all viral loads from samples obtained before initiation of antiretroviral therapy (ART), was used to estimate the mean difference between the vaccine and placebo arms in pre-ART viral load at each of the 9 post-infection visits. The time between detection of HIV infection and initiation of ART was compared between the 2 study arms by use of a log-rank test.

***Exploratory analyses.*** Tests for interaction in Cox proportional hazards models were used to evaluate whether VE differed by age ($\leqslant$30 or >30 years), sex, education (less than a college degree or a college or graduate degree), race (white, black, Hispanic, Asian, and other and white vs. nonwhite), and baseline behavioral risk (low, medium, and high) [24]. The binary categories for age and education were determined before the unblinded analysis was conducted by collapsing the 5 age categories and the 4 education categories into 2 binary categories with approximately equal sample size. Because there was limited power to evaluate the VE for particular nonwhite subgroups, race was also dichotomized as white and nonwhite, with the latter category including volunteers who designated their race as Hispanic. Volunteers were classified as having low, medium, or high baseline behavioral risk on the basis of self-reported behaviors during the 6 months before enrollment that were predictive of HIV infection in men pooled over the treatment arms. Behaviors that were statistically significant ($P < .05$) in univariate Cox proportional hazards models were further assessed in multivariate models. Nine behaviors were identified as independent predictors of HIV infection. A behavioral risk score for each volunteer was computed as the total number of these behaviors the person reported at baseline. The score was highly predictive of HIV infection, with an estimated hazard ratio of 1.66 (95% confidence interval [CI], 1.56 to 1.77) per 1 risk-factor increase ($P < .0001$). Behavioral risk scores ranged from 0 to 7; 0 was categorized as low, 1–3 was categorized as medium, and 4–7 was categorized as high. The baseline behavioral risk score was based on data for men only, because only 6 HIV-1 infections were observed among the 308 female volunteers and because the important risk factor of insertive anal sex does not apply to women. The results reported below on VE by behavioral risk category did not change appreciably when the risk model was based on data for both men and women.

To account for multiple comparisons in subgroup analyses, a rerandomization procedure (with 10,000 permutations) was used to test the omnibus null hypothesis that $VE = 0$ for all subgroups versus the alternative hypothesis that $VE \neq 0$ for at least 1 subgroup. A bootstrap resampling procedure was used to compute adjusted $P$ values [25]. The estimate and 95% CI of the VE value within each subgroup was also computed by use of a Cox proportional hazards model. A Cox proportional hazards model was used to estimate VE values for particular HIV-1 genotypes and to test whether VE differed by viral genotype [26].

## RESULTS

### Demographics, Risk Behavior, and Conduct of Study

Between June 1998 and October 1999, 7185 volunteers were screened for study eligibility criteria (figure 1). Of these, 5417 eligible volunteers (5108 men and 309 women) were enrolled and were randomized to receive either vaccine or placebo. Of the 1768 volunteers not enrolled, 966 did not return after the initial screening visit, 328 met the study eligibility criteria but chose not to enroll, and 474 were excluded; the major reasons for exclusion were HIV-1 infection (161), serious underlying disease (148), and not meeting risk-behavior criteria (141). Despite being HIV-1 antibody negative at screening, 14 (11 vaccinees and 3 placebo recipients) volunteers had HIV-1 infection detected at baseline (month 0) and were excluded from all efficacy, but not safety, analyses. Of these, 12 were positive by NAT at month 0, although they were antibody negative; 1 was positive by NAT and intermediate by immunoblot; and 1 was positive by NAT and antibody positive. The vaccine and placebo arms were similar in terms of demographic characteristics (table 1). The study population was predominantly male (94%), white (83%), young (median age, 36 years), and well educated (61% had a college or graduate degree).

Self-reported risk behaviors, including sexual activity and alcohol and drug use, and rates of sexually transmitted diseases were similar in the vaccine and placebo arms at baseline and during follow-up (table 1 and figure 2); they were also similar when stratified by race (figure 3) and by behavioral risk group (figure 4). For the 9 behaviors reported at baseline that were predictive of HIV-1 infection, borderline statistically significant differences between the vaccine and placebo arms were observed for unprotected receptive anal sex with an HIV-1–uninfected partner reported at month 6 (i.e., occurring during the interval between baseline and the month 6 visit) and unprotected receptive anal sex with an HIV-1–infected partner reported at month 18. Most behaviors, except amphetamine use and unprotected receptive anal sex with an HIV-1–uninfected partner, decreased over time, with the major decrease occurring between baseline and month 6.

The rate of compliance with study vaccinations and the rate of loss to follow-up were well balanced between the vaccine and placebo arms (figure 1 and table 2), although, in the high behavioral risk subgroup, the dropout rate was higher in the placebo arm (24%) than in the vaccine arm (13%) ($P = .052$, Fisher's exact test). There were no statistically significant differences in the 9 baseline risk behaviors between the vaccinees and placebo recipients who dropped out of the study.

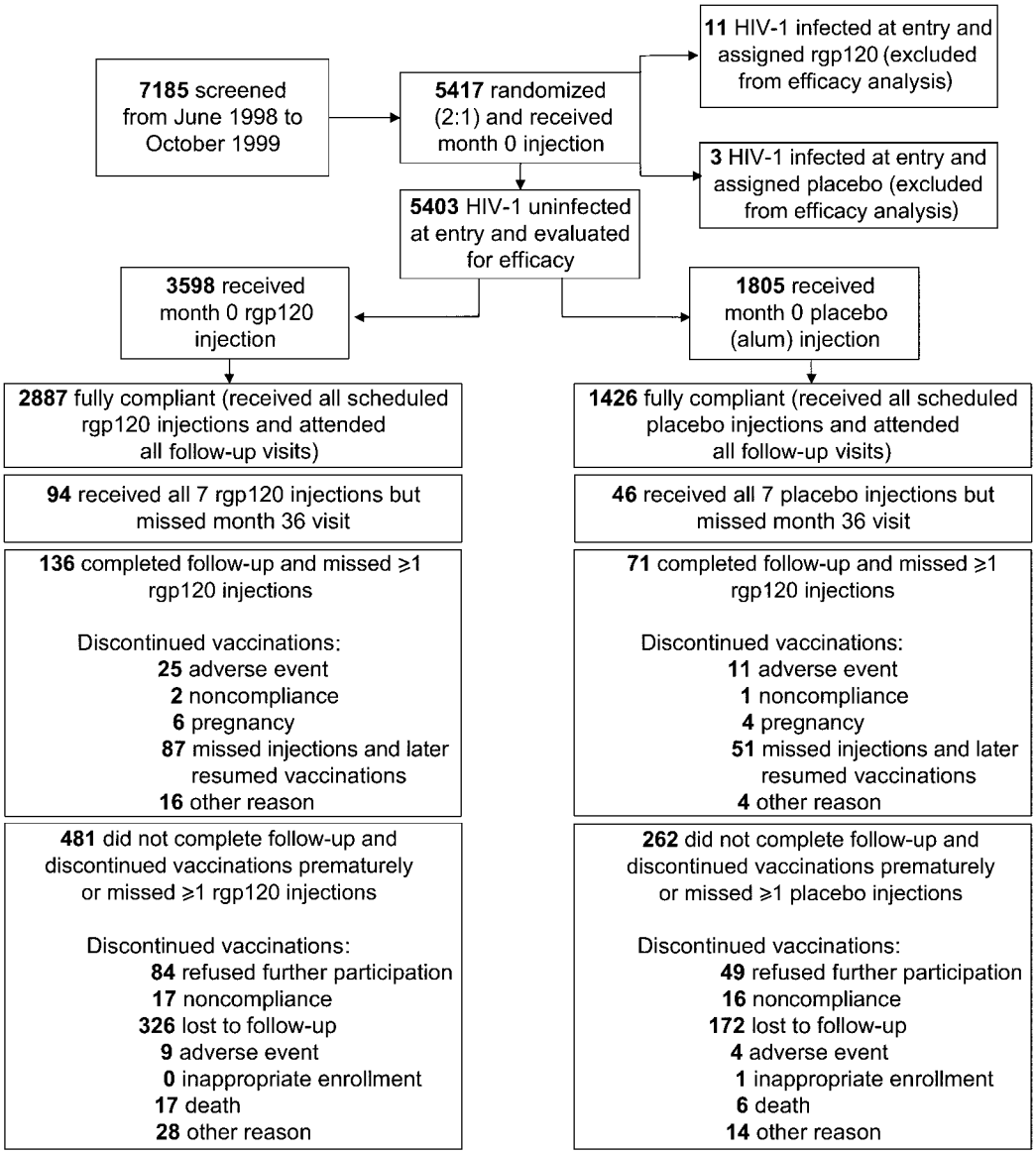


**Figure 1.** Flow of study participants in the present trial (VAX004). rgp, recombinant glycoprotein.

### Adverse Events

The vaccine was generally well tolerated. The most common adverse events were mild or moderate reactogenicity symptoms that occurred during the first 3 days after a vaccination. Rates of local symptoms at the injection site were higher in the vaccinees; local edema, induration, or a subcutaneous nodule reported on at least 1 of the 14 days after any of the vaccinations was reported by 36%, 29%, and 21% of the vaccinees and by 17%, 15%, and 12% of the placebo recipients, respectively. There were no other major differences in the frequency and type of reported adverse events.

### Rates of Infection and VE

Overall, 368 (6.8%) volunteers became HIV-1 infected during the study, giving an annual incidence rate of 2.6% (2.7% in men and 0.8% in women). No reduction of infection in vaccine recipients was observed (VE, 6% [95% CI, −17 to 24]; $P = .59$) (table 3). Kaplan-Meier curves of the time-to-infection showed approximately constant rates of HIV-1 infection; the rates were similar in the vaccine and placebo arms during the 36 months of follow-up (figure 5*A*).

### Postinfection Markers of Disease Progression

Among the volunteers who acquired HIV-1 infection, pre-ART viral loads over the course of the 9 visits were similar in the vaccine and placebo arms ($P = .81$). The mean difference (the mean of the vaccine arm minus the mean of the placebo arm) in pre-ART viral load at the visit 2 months after detection was $4.26 - 4.33 = -0.07$ $\log_{10}$ (95% CI, −0.33 to 0.18 $\log_{10}$). The

**Table 1. Baseline demographic characteristics and risk of HIV-1 infection.**

| Category, parameter | Men | | Women | | All | |
|---|---|---|---|---|---|---|
| | Vaccine ($n$ = 3391) | Placebo ($n$ = 1704) | Vaccine ($n$ = 207) | Placebo ($n$ = 101) | Vaccine ($n$ = 3598) | Placebo ($n$ = 1805) |
| Age, years | | | | | | |
| Median | 36 | 35 | 37 | 38 | 36 | 35 |
| Range | 18–62 | 18–62 | 18–55 | 20–55 | 18–62 | 18–62 |
| Race | | | | | | |
| White (non-Hispanic) | 2930 (86) | 1468 (86) | 64 (31) | 27 (27) | 2994 (83) | 1495 (83) |
| Nonwhite | | | | | | |
| Hispanic | 211 (6) | 114 (7) | 28 (14) | 14 (14) | 239 (7) | 128 (7) |
| Black (non-Hispanic) | 121 (4) | 59 (3) | 112 (54) | 57 (56) | 233 (6) | 116 (6) |
| Asian | 56 (2) | 21 (1) | 0 | 0 | 56 (2) | 21 (1) |
| Other | 73 (2) | 42 (3) | 3 (1) | 3 (3) | 76 (2) | 45 (2) |
| Education level[a] | | | | | | |
| Less than a college degree | 1238 (37) | 627 (37) | 171 (83) | 86 (85) | 1409 (39) | 713 (40) |
| College or graduate degree | 2152 (63) | 1077 (63) | 36 (17) | 15 (15) | 2188 (61) | 1092 (60) |
| Baseline behavioral risk score[b] | | | | | | |
| Low risk | 1077 (32) | 538 (32) | 134 (65) | 71 (70) | 1211 (34) | 609 (34) |
| Medium risk | 2156 (64) | 1077 (63) | 73 (35) | 30 (30) | 2229 (62) | 1107 (61) |
| High risk | 158 (5) | 89 (5) | 0 | 0 | 158 (4) | 89 (5) |

**NOTE.** Data are no. (%) of volunteers, unless otherwise noted.

[a] One volunteer was missing education data.

[b] Risk score was defined as the total no. of risk factors reported from the following: (1) unprotected receptive anal sex with an HIV-1–infected male partner; (2) unprotected insertive anal sex with an HIV-1–infected male partner; (3) unprotected receptive anal sex with an HIV-1–uninfected male partner; (4) ⩾5 acts of unprotected receptive anal sex with a male partner of unknown HIV-1 status; (5) ⩾10 sex partners; (6) anal herpes; (7) hepatitis A; (8) use of poppers; and (9) use of amphetamines. Behavioral risk scores ranged from 0 to 7; 0 was categorized as low, 1–3 was categorized as medium, and 4–7 was categorized as high.

rate of initiation of ART was similar in the vaccine (99/225 [44%]) and placebo (53/122 [43%]) arms ($P = .61$, log-rank test). No significant effects of vaccination on any postinfection end points were observed.

### Exploratory Subgroup Analyses

There were no significant interactions with treatment for sex, age, or education level, but interaction tests in Cox proportional hazards models that included both baseline behavioral risk score (low, medium, or high) and race (white or nonwhite) demonstrated that VE significantly differed by behavioral risk level ($P = .041$) and by race ($P = .007$). There was no evidence that the pattern of increasing VE with risk group was restricted to white or nonwhite volunteers, although power was low for assessment of treatment by race by risk interaction. The rerandomization procedure used to account for multiple testing in the 15 subgroups yielded $P = .102$, indicating a nonsignificant trend toward VE being different from 0 in ⩾1 subgroups. Subgroup-specific estimates of VE values with unadjusted 95% CIs and unadjusted and multiplicity adjusted $P$ values are shown in table 3.

Both overall and in subgroups, multivariate analyses in which either baseline covariates (sex, age, race, education level, geographic region, and risk behavior) or risk behavior over time was controlled for yielded covariate adjusted point and CI estimates of VE that were nearly identical to the unadjusted values (data not shown). Because only 6 female volunteers acquired HIV-1 infection (4 black placebo recipients, 1 black vaccinee, and 1 Hispanic vaccinee), the above analyses of risk and race were repeated for men only; these analyses gave subgroup-specific point estimates of VE and 95% CIs that were very similar to those obtained for both sexes combined. Because site of enrollment could confound estimates of VE, the analyses of VE were repeated with stratification by site. Generally, the results were very similar to the unstratified results, except that estimates of VE decreased appreciably for the high behavioral risk subgroup (from 43% to 19%).

### Antibody Responses, Viral Sequencing, and Selective VE

All vaccinees assessed demonstrated HIV-1–specific antibody responses [22]. The vaccinees with higher peak levels of MN CD4–blocking, GNE8 CD4–blocking, or MN-neutralizing responses tended to have a lower rate of HIV-1 infection; these analyses are described and interpreted elsewhere [22].

The subtype B consensus sequence at the tip of the gp120 V3 domain, GPGRAF, which is present in both the MN and GNE8 vaccine antigens, was selected as the main region for detection of the effects of vaccine on virus population dynamics. Overall, there was no evidence of selective efficacy on the basis of virus type. VE was estimated as 0% for viruses with

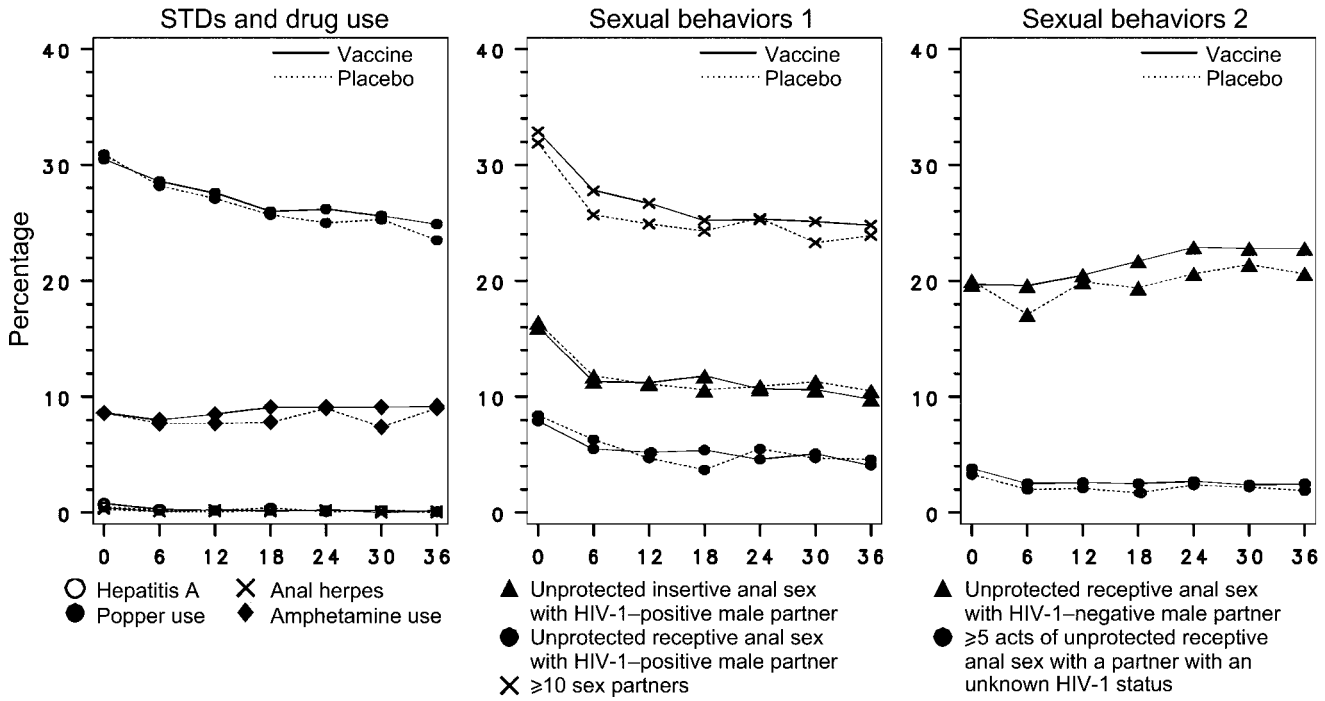


**Figure 2.** Self-reported risk behaviors by treatment arm and month of visit. STDs, sexually transmitted diseases.

the GPGRAF sequence and 19% for viruses without the GPGRAF sequence. In exploratory analyses, there was no evidence of differential efficacy in any behavioral risk group between viruses with and those without the GPGRAF sequence. A nonsignificant trend was found for nonwhite volunteers, with an estimated VE of 73% (95% CI, 35% to 88%) for viruses with the GPGRAF sequence versus an estimated VE of 24% (95% CI, −59% to 63%) for viruses without the GPGRAF sequence ($P = .077$).

## DISCUSSION

VAX004 was the first phase 3 placebo-controlled efficacy study of a vaccine to prevent HIV-1 infection [20]. More than 5000 MSMs were enrolled, in whom the predominant site of infection was rectal. A relatively small number (308) of women at high risk for heterosexual transmission of HIV-1 were also enrolled. Because only 6 women acquired HIV-1 infection during follow-up, compared with 362 men, the study had very little power to assess VE in women. Every analysis of VE gave very similar results, regardless of whether both sexes or only men were evaluated.

Despite producing neutralizing and CD4-blocking antibody responses in all vaccinees assessed for immunogenicity [22], the vaccine was ineffective in preventing HIV-1 infection or in modifying postinfection markers of disease progression. This failure to protect likely derived from the lack of induction of antibodies capable of neutralizing genetically diverse primary HIV-1 isolates. Additionally, results from a phase 3 trial of a B/E rgp120 vaccine in Thailand showed no evidence of efficacy, although the presumed mode of transmission in that study differed in that it was intravenous [27].

The rgp120 vaccine used in the present trial appeared to be safe; other than the rate of local reactogenicity, no other rates of adverse events were meaningfully increased in vaccinees versus placebo recipients. Furthermore, although it has been hypothesized that a more rapid disease progression due to "immune enhancement" is a possible risk for vaccinees [28, 29], pre-ART viral loads and time to initiation of ART in the 368 volunteers who acquired HIV-1 infection provided no evidence of such a phenomenon.

The findings of the present study should reassure those who have been worried about the difficulties of conducting a phase 3 trial of an HIV-1 vaccine [30–32]—concerns with regard to recruiting, retaining, and reducing the pool of participants for future trials [30]; the potential for increased high-risk behavior by participants [31]; and conducting such a trial ethically should be allayed [32]. Also, this trial was conducted with the understanding that it is possible to inflict social harm on individuals who volunteer for HIV-vaccine trials. To minimize the risk of social harm, advice and training were given to staff and volunteers; in the end, minimal harm occurred [20]. In addition, at least with this rgp120 vaccine, the chance of false-positive serologic test results was minimal [33].

The findings with regard to risk-reduction counseling are less reassuring. Volunteers received comprehensive counseling by trained counselors at each study visit. Self-reported baseline risk behavior was a good predictor of subsequent infection,

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

**Figure 3.** Self-reported risk behaviors by race, treatment arm, and month of visit. STDs, sexually transmitted diseases.

with infection rates at least 10-fold greater in the high-risk subgroup than in the low-risk subgroup, and overall self-reported risk behavior decreased over the course of the trial, although amphetamine use remained constant and unprotected receptive anal sex with an HIV-1–uninfected partner increased slightly. Despite the intensive counseling, the HIV-1 infection rate in the study population (which predominantly consisted of well-educated MSM) remained high and was steady during the 3 years of follow-up. In the absence of more-effective counseling, an effective HIV-1 vaccine, or other preventive methods, the HIV-1 epidemic may continue unchecked and might, in some populations, approximate the current prevalence in sub-Saharan African adults.

On the basis of interaction tests, VE estimates differed significantly by behavioral risk level and race. This result motivated exploratory subgroup analyses, which indicated possible efficacy of the vaccine in certain subgroups, such as in the high behavioral risk subgroup (VE, 43%) and in nonwhite volunteers (VE, 47%). However, the largest of these subgroups (the nonwhite volunteers) comprised only 17% of the study population, and the VE estimates for these 2 subgroups were not significantly different from 0% after adjustment for the multiplicity of tests performed. Because there was evidence of effect modification and because the high behavioral risk and nonwhite subgroups each had a substantial number of infections (58 and 59, respectively), we here discuss 4 possible explanations for the findings of the exploratory subgroup analyses. There is precedent for the possibility that VE can differ by demographic factors: a similar recombinant glycoprotein vaccine has been reported to confer protection against genital herpes infection in women but not in men [34].

The first possible explanation is that the variation in VE estimates across subgroups could simply be attributable to statistical variation and, therefore, not reflect any underlying pattern in the true VE values. Second, a finding of VE within a subgroup could have been caused by greater exposure to HIV-1 in the placebo arm because of possible imbalances in risky behavior or other host or virologic factors. However, our multivariate analyses, which took baseline attributes into account, suggested that imbalances between the 2 treatment arms (if there were any) did not account for observed VE, and risk behaviors over time were similar in the vaccine and placebo arms. Within the racial subgroups and within the behavioral low- and medium-risk subgroups, the rate of loss to follow-up was well balanced between the treatment arms, and the behavioral risk factors of volunteers who were lost to follow-up were well balanced. Within the high behavioral risk subgroup, placebo recipients had a higher dropout rate than did vaccinees. Also, for volunteers who dropped out in this subgroup, placebo recipients reported higher rates of unprotected receptive anal sex with an HIV-1–uninfected partner (81%) than did vaccine recipients (43%). However, neither of these differences would explain the higher observed VE in the high behavioral risk subgroup.

Third, the finding of an apparently higher VE in the high behavioral risk subgroup could be the result of synergy between the vaccine-induced immune response and a natural "priming" of the immune response by frequent exposure to HIV-1 without infection, which has been proposed as a possible explanation for the phenomenon of highly exposed yet persistently uninfected sex workers [4–6]. Although there was no evidence of increased antibody responses in the high behavioral risk subgroup in the present study [22], there may have been priming of cellular or humoral immune responses undetected by any of the assays carried out to date.

Fourth, biological differences, such as differences in immune responses or in genetic markers of resistance to HIV-1 infection [4–9], could explain why the vaccine appeared to be effective only in nonwhite volunteers. Differences in immune responses by sex and race have been reported [35, 36]. In the present study, lower vaccine-induced antibody responses correlated with higher infection rates in all racial subgroups [22]. Given that the overall VE estimate (6%) was near 0%, this result cannot be interpreted to imply that higher antibody responses were the cause of protection. Although it may be implausible to group Asian and black volunteers on the basis of genetic similarities, possible differences in exposure among racial subgroups to environmental factors or other infecting pathogens that could increase [37, 38] or decrease susceptibility [39–43] to HIV-1 infection might help to account for differing VE estimates. For example, some studies have demonstrated that coinfection with GB virus C (GBV-C)—a flavivirus whose prevalence varies widely and appears to correlate with injection drug use, high-risk sexual activity, and certain geographic areas—has an apparently beneficial effect on progression of HIV-1 disease [42, 43]. Proposed mechanisms include GBV-C–mediated reduction in expression of CCR5, induction of anti–HIV-1 cytokines, and enhancement of natural immunity [43], any of which could work synergistically with a vaccine-induced antibody response.

What conclusions can be drawn from the present phase 3

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

**Figure 4.** Self-reported risk behaviors by behavioral risk group, treatment arm, and month of visit. STDs, sexually transmitted diseases.

**Table 2. Rates of immunization and study completion.**

| Category | Men | | Women | | All | |
|---|---|---|---|---|---|---|
| | Vaccine ($n$ = 3391) | Placebo ($n$ = 1704) | Vaccine ($n$ = 207) | Placebo ($n$ = 101) | Vaccine ($n$ = 3598) | Placebo ($n$ = 1805) |
| Dose no.[a] | | | | | | |
| Dose 1 (month 0) | 3391 (100) | 1704 (100) | 207 (100) | 101 (100) | 3598 (100) | 1805 (100) |
| Dose 2 (month 1) | 3344 (99) | 1681 (99) | 196 (95) | 99 (98) | 3540 (99) | 1780 (99) |
| Dose 3 (month 6) | 3202 (96) | 1609 (96) | 180 (87) | 95 (94) | 3382 (95) | 1704 (96) |
| Dose 4 (month 12) | 3051 (92) | 1511 (92) | 162 (79) | 86 (87) | 3213 (91) | 1597 (91) |
| Dose 5 (month 18) | 2920 (89) | 1450 (89) | 158 (77) | 81 (82) | 3078 (89) | 1531 (88) |
| Dose 6 (month 24) | 2811 (87) | 1379 (85) | 147 (72) | 78 (79) | 2958 (86) | 1457 (85) |
| Dose 7 (month 30) | 2720 (85) | 1323 (83) | 139 (68) | 74 (76) | 2859 (84) | 1397 (83) |
| Received all scheduled immunizations[b] | 2851 (84) | 1397 (82) | 130 (63) | 75 (74) | 2981 (83) | 1472 (82) |
| Final visit[c] | | | | | | |
| HIV-1 uninfected at final visit | 2632 (78) | 1292 (76) | 151 (73) | 78 (77) | 2783 (77) | 1370 (76) |
| HIV-1 infected before or at final visit | 239 (7) | 123 (7) | 2 (1) | 4 (4) | 241 (7) | 127 (7) |
| HIV-1 status unknown at final visit (lost to follow-up) | 520 (15) | 289 (17) | 54 (26) | 19 (19) | 574 (16) | 308 (17) |

**NOTE.** Data are no. (%) of volunteers.

[a] Rates of immunization were calculated as the no. vaccinated during each visit divided by the no. who were uninfected (i.e., not diagnosed with HIV-1 infection before or during the indicated visit).

[b] No. who received either all 7 doses or all doses before infection divided by the no. enrolled. Volunteers, once diagnosed with HIV-1 infection, were not scheduled for further vaccination visits.

[c] The final visit was at month 36.

study about the use rgp120 as a preventive vaccine? The lack of protection demonstrates that monomeric rgp120 is insufficiently immunogenic against field HIV-1 isolates and that improved or different rgp120 constructs, or different approaches, will be required. The trends toward efficacy observed in the exploratory subgroup analyses, if real [24, 44], raise the possibility that improved rgp120 immunogens can protect in certain circumstances; these trends also may provide clues that can inform the design of new HIV-1 vaccines, whether they are based on rgp120 or other approaches. Improvement of an rgp120 vaccine might require additional, more representative, or modified subtype envelope antigens (e.g., oligomeric vs. monomeric forms); newer adjuvants that enhance innate immunity or promote a Th1-biased response [45–48]; or combination with vaccines that promote cellular immunity to HIV-1 [3, 49, 50]. A phase 3 trial using the latter approach was recently initiated in Thailand; in the trial, immunization with rgp120 is combined with a canarypox vector vaccine [51].

To further aid the interpretation of the results of VAX004 and to provide information that is helpful to the HIV-1 vaccine field, additional analyses are either ongoing or planned, including analyses of the ability of participant serum to neutralize a spectrum of primary HIV-1 isolates, of T cell responses, of the occurrence of genetic polymorphisms of infecting strains, and of the prevalence of GBV-C coinfection.

## THE RGP120 HIV VACCINE STUDY GROUP

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**Table 3. Attack rates of HIV-1 infection and vaccine efficacy (VE) against HIV-1 infection.**

| Category, parameter | Rate of HIV-1 infection | | VE (95% CI) | *P* | |
|---|---|---|---|---|---|
| | Vaccine | Placebo | | Unadjusted[a] | Adjusted[b] |
| All volunteers | 241/3598 (6.7) | 127/1805 (7.0) | 6 (−17 to 24) | .59 | >.5 |
| Men | 239/3391 (7.0) | 123/1704 (7.2) | 4 (−20 to 23) | .73 | >.5 |
| Women | 2/207 (1.0) | 4/101 (4.0) | 74 (−42 to 95) | .093 | .41 |
| Race | | | | | |
| White (non-Hispanic) | 211/2994 (7.0) | 98/1495 (6.6) | −6 (−35 to 16) | .60 | >.5 |
| Men | 211/2930 (7.2) | 98/1468 (6.7) | −6 (−35 to 16) | .61 | … |
| Women | 0/64 (0) | 0/27 (0) | … | … | … |
| Hispanic | 14/239 (5.9) | 9/128 (7.0) | 15 (−96 to 63) | .70 | >.5 |
| Men | 13/211 (6.2) | 9/114 (7.9) | 20 (−88 to 66) | .61 | … |
| Women | 1/28 (3.6) | 0/14 (0) | … | … | … |
| Black (non-Hispanic) | 6/233 (2.6) | 9/116 (7.8) | 67 (6 to 88) | .028 | .24 |
| Men | 5/121 (4.1) | 5/59 (8.5) | 54 (−61 to 87) | .21 | … |
| Women[c] | 1/112 (0.9) | 4/57 (7.0) | 87 (−19 to 98) | .033 | … |
| Asian (all men) | 3/56 (5.4) | 3/21 (14.3) | 66 (−70 to 93) | .17 | >.5 |
| Other | 7/76 (9.2) | 8/45 (17.8) | 50 (−39 to 82) | .18 | >.5 |
| Men | 7/73 (9.6) | 8/42 (19.0) | 51 (−34 to 82) | .16 | … |
| Nonwhite | 30/604 (5.0) | 29/310 (9.4) | 47 (12 to 68) | .012 | .13 |
| Men | 28/461 (6.1) | 25/236 (10.6) | 43 (3 to 67) | .036 | … |
| Women | 2/143 (1.4) | 4/74 (5.4) | 74 (−43 to 95) | .10 | … |
| Age | | | | | |
| ⩽30 years | 84/971 (8.7) | 43/504 (8.5) | −1 (−46 to 30) | .95 | >.5 |
| >30 years | 157/2627 (6.0) | 84/1301 (6.5) | 8 (−19 to 30) | .51 | >.5 |
| Education level[d] | | | | | |
| Less than a college degree | 95/1409 (6.7) | 52/713 (7.3) | 8 (−29 to 34) | .63 | >.5 |
| College or graduate degree | 146/2188 (6.7) | 75/1092 (6.9) | 4 (−27 to 27) | .77 | >.5 |
| Baseline behavioral risk score[e] | | | | | |
| Low risk | 32/1211 (2.6) | 11/609 (1.8) | −48 (−193 to 26) | .26 | >.5 |
| Medium risk | 177/2229 (7.9) | 90/1107 (8.1) | 3 (−25 to 25) | .82 | >.5 |
| High risk | 32/158 (20.3) | 26/89 (29.2) | 43 (4 to 66) | .032 | .29 |

**NOTE.** Data are no. of infected volunteers/no. of total volunteers (%) in category. CI, confidence interval.

[a] Two-sided *P* values from a log-rank test.

[b] Two-sided *P* values from a nonparametric bootstrap procedure that was conducted with 10,000 resampled data sets; Wald statistics from univariate Cox proportional hazards models were used [25].

[c] All 5 infected black women were from 1 site. gp120 sequence analysis of the 5 isolates from these women indicated that 3 of the isolates (all from placebo recipients) were clustered together in a phylogenetic tree, which suggests at least a phylogenetic linkage. The 3 phylogenetically linked infections occurred during 3 separate calendar years.

[d] One volunteer was missing education data.

[e] Risk score was defined as the total no. of risk factors reported from the following: (1) unprotected receptive anal sex with an HIV-1–infected male partner; (2) unprotected insertive anal sex with an HIV-1–infected male partner; (3) unprotected receptive anal sex with an HIV-1–uninfected male partner; (4) ⩾5 acts of unprotected receptive anal sex with a male partner of unknown HIV-1 status; (5) ⩾10 sex partners; (6) anal herpes; (7) hepatitis A; (8) use of poppers; and (9) use of amphetamines. Behavioral risk scores ranged from 0 to 7; 0 was categorized as low, 1–3 was categorized as medium, and 4–7 was categorized as high.

and Peter B. Gilbert, Michael G. Hudgens, Barbara J. Metch, and Steven G. Self have received consulting fees from VaxGen in the past.

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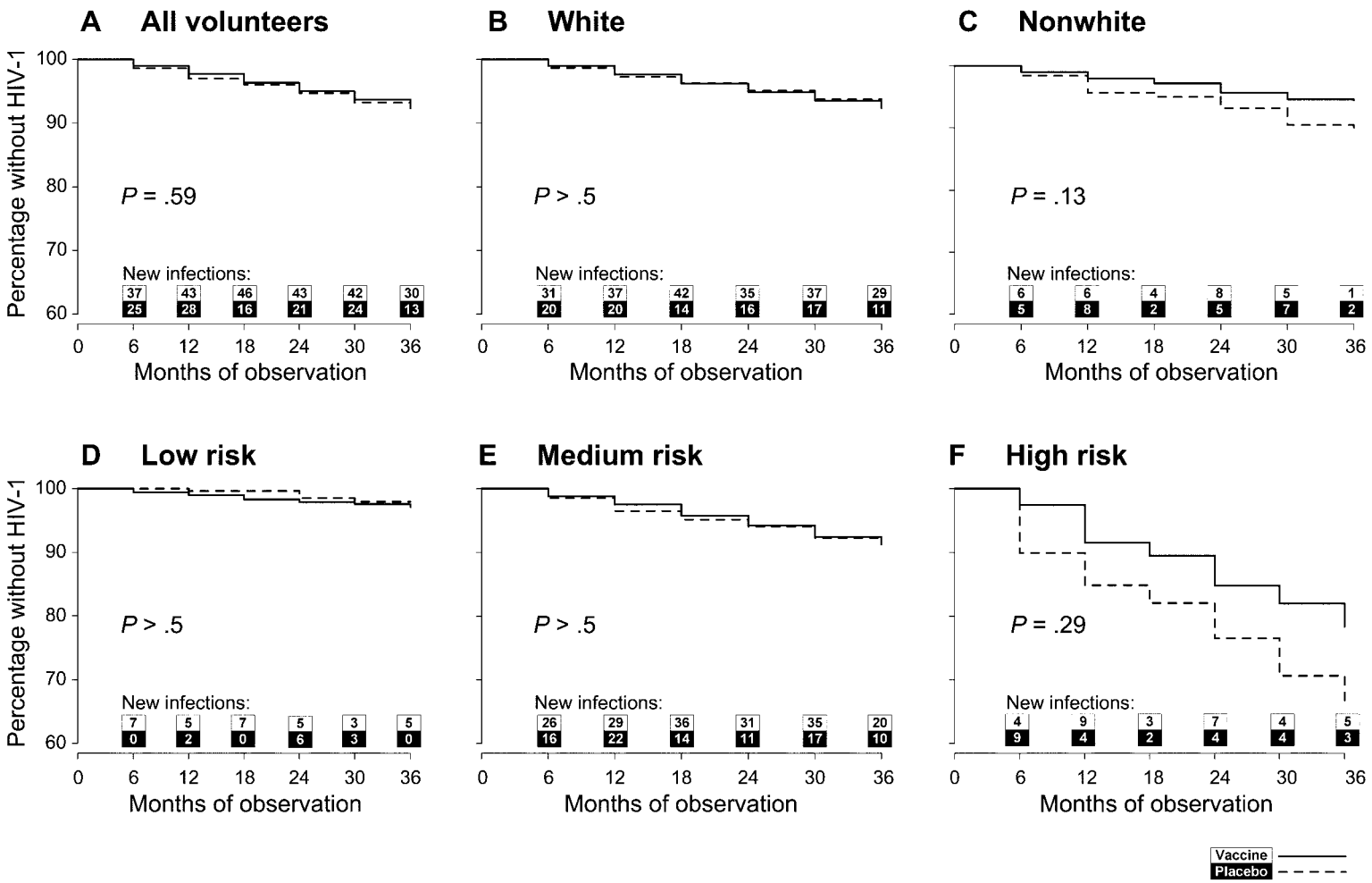


**Figure 5.** Kaplan-Meier curves showing time to HIV-1 infection, with adjusted *P* values

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