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ORIGINAL ARTICLE

Acyclovir and Transmission of HIV-1 from Persons Infected with HIV-1 and HSV-2

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ABSTRACT

BACKGROUND

Most persons who are infected with human immunodeficiency virus type 1 (HIV-1) are also infected with herpes simplex virus type 2 (HSV-2), which is frequently reactivated and is associated with increased plasma and genital levels of HIV-1. Therapy to suppress HSV-2 reduces the frequency of reactivation of HSV-2 as well as HIV-1 levels, suggesting that suppression of HSV-2 may reduce the risk of transmission of HIV-1.

METHODS

We conducted a randomized, placebo-controlled trial of suppressive therapy for HSV-2 (acyclovir at a dose of 400 mg orally twice daily) in couples in which only one of the partners was seropositive for HIV-1 (CD4 count, ≥ 250 cells per cubic millimeter) and that partner was also infected with HSV-2 and was not taking antiretroviral therapy at the time of enrollment. The primary end point was transmission of HIV-1 to the partner who was not initially infected with HIV-1; linkage of transmissions was assessed by means of genetic sequencing of viruses.

RESULTS

A total of 3408 couples were enrolled at 14 sites in Africa. Of the partners who were infected with HIV-1, 68% were women, and the baseline median CD4 count was 462 cells per cubic millimeter. Of 132 HIV-1 seroconversions that occurred after randomization (an incidence of 2.7 per 100 person-years), 84 were linked within couples by viral sequencing: 41 in the acyclovir group and 43 in the placebo group (hazard ratio with acyclovir, 0.92, 95% confidence interval [CI], 0.60 to 1.41; $P=0.69$). Suppression with acyclovir reduced the mean plasma concentration of HIV-1 by 0.25 \log_{10} copies per milliliter (95% CI, 0.22 to 0.29; $P<0.001$) and the occurrence of HSV-2–positive genital ulcers by 73% (risk ratio, 0.27; 95% CI, 0.20 to 0.36; $P<0.001$). A total of 92% of the partners infected with HIV-1 and 84% of the partners not infected with HIV-1 remained in the study for 24 months. The level of adherence to the dispensed study drug was 96%. No serious adverse events related to acyclovir were observed.

CONCLUSIONS

Daily acyclovir therapy did not reduce the risk of transmission of HIV-1, despite a reduction in plasma HIV-1 RNA of 0.25 \log_{10} copies per milliliter and a 73% reduction in the occurrence of genital ulcers due to HSV-2. (ClinicalTrials.gov number, NCT00194519)

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*Other members of the Partners in Prevention HSV/HIV Transmission Study Team are listed in the Supplementary Appendix, available with the full text of this article at NEJM.org.

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THE SEROPREVALENCE OF HERPES SIMPLEX virus type 2 (HSV-2), the most common cause of genital ulcer disease worldwide, is 60 to 90% in populations with human immunodeficiency virus type 1 (HIV-1).¹ Clinical manifestations of HSV-2 range from unrecognized or mild genital symptoms in most persons with HIV-1 infection to severe genital ulcer disease in persons with advanced HIV-1 disease.^{2,3} Genital shedding of the herpes simplex virus occurs on up to 30% of days in persons infected with HIV-1, often when they have no symptoms or observable lesions.^{4,5}

Laboratory and epidemiologic studies suggest that HSV-2 may increase the infectiousness of HIV-1. HSV-encoded proteins bind integrated HIV-1 in coinfecting cells and directly promote the transcription of HIV-1.⁶⁻⁹ In persons who are infected with both HIV-1 and HSV-2, symptomatic and asymptomatic reactivation of HSV-2 has been associated with increased HIV-1 levels in the blood and genital tract.¹⁰⁻¹³ In one study, the risk of transmission of HIV-1 to sexual partners was increased by a factor of 4 among persons with HIV-1 infection who had symptomatic genital ulcer disease, as compared with persons with HIV-1 who did not have genital ulcer disease; the majority of cases of genital ulcer disease were due to HSV-2 infection.¹⁴

Five randomized clinical trials showed that daily therapy for HSV-2 for 8 to 12 weeks reduced plasma HIV-1 levels by 0.25 to 0.50 log₁₀ copies per milliliter.^{4,5,15-17} To evaluate directly whether HSV-2 suppressive therapy could prevent the transmission of HIV-1, we conducted a randomized, double-blind, placebo-controlled trial of acyclovir, administered twice daily, as compared with placebo, among African persons who were infected with both HIV-1 and HSV-2 and their heterosexual partners who were not infected with HIV-1.

METHODS

STUDY POPULATION

We enrolled heterosexual couples in which only one of the partners was seropositive for HIV-1 and that partner was also infected with HSV-2. Couples were recruited at seven sites in southern Africa (Gaborone, Botswana; Gugulethu, Orange Farm, and Soweto in South Africa; and Kitwe, Lusaka, and Ndola in Zambia) and seven sites in East Africa (Eldoret, Kisumu, Nairobi, and Thika in Kenya;

Kigali, Rwanda; Moshi, Tanzania; and Kampala, Uganda) between November 2004 and April 2007, as previously described.¹⁸ The inclusion criteria for couples were three or more episodes of vaginal intercourse in the 3 previous months and the intention to remain together for at least 24 months. The inclusion criteria for partners infected with HIV-1 were an age of at least 18 years, seropositivity for HIV-1 and HSV-2, a CD4 count of 250 or more cells per cubic millimeter, no conditions associated with acquired immunodeficiency syndrome, and no current antiretroviral therapy. Exclusion criteria for persons infected with HIV-1 were previous adverse reactions to acyclovir, current receipt of therapy for HSV, persistent genital ulcers, current participation in another study, plans for extended travel, and current pregnancy. Inclusion criteria for partners who were not infected with HIV-1 were an age of at least 18 years and HIV-1 seronegativity; the partners who were not infected with HIV-1 could be eligible whether they were seropositive or seronegative for HSV-2.

The protocol was approved by the University of Washington Human Subjects Review Committee and the ethics review committees at all local and collaborating organizations. All study participants provided written informed consent in English or their local language. The Bill and Melinda Gates Foundation funded the study but did not assume responsibility for review of the protocol. The authors designed the study, wrote the protocol, had full access to the raw data, performed all analyses, wrote the manuscript, and had final responsibility for the decision to submit the manuscript for publication.

ENROLLMENT

Partners with HIV-1 infection were randomly assigned in a 1:1 ratio to receive acyclovir, at a dose of 400 mg orally twice daily, or matching placebo; a block randomization scheme was used, with stratification according to site with the use of a pseudo-random-number generator. The study drug was manufactured by Ranbaxy Laboratories; matched active and placebo tablets were packaged in bottles that were distributed at monthly visits and were provided for the duration of the couples' participation in the study — up to 24 months. At all visits, participants, individually or as couples, received intensive counseling on risk reduction, were provided with condoms, and received treatment for sexually transmitted infec-

tions according to World Health Organization guidelines.

FOLLOW-UP

Visits for partners infected with HIV-1 were scheduled monthly to dispense the study drug or placebo, collect any unused drug from the preceding month, and provide counseling on adherence. Monthly adherence to the assigned treatment was assessed by means of pill counts and self-reporting of 100% adherence or less than 100% adherence. Interviews were conducted to obtain information regarding high-risk sexual practices in the previous month and symptoms of genital herpes in the previous 7 days. Participants with genital ulceration that was consistent with genital herpes were given open-label acyclovir at a dose of 400 mg three times daily for 5 days, in addition to the study drug; ulcers were swabbed and the specimens were sent for polymerase-chain-reaction (PCR) testing for HSV.

At quarterly visits, all participants underwent a genital examination, and urine samples were obtained from female participants for pregnancy testing. Women infected with HIV-1 who became pregnant were referred to antenatal clinics for services relating to the prevention of mother-to-child transmission, and the study medication was discontinued until a follow-up pregnancy test was negative. Participants with HIV-1 infection who met national guidelines for the initiation of antiretroviral therapy during the follow-up period were referred to local HIV-1 clinics. Partners who were not infected with HIV-1 were interviewed on a quarterly basis about high-risk sexual practices and symptoms of sexually transmitted infections and underwent antibody testing for HIV-1.

The laboratory procedures have been described previously¹⁸ and are summarized in the Supplementary Appendix, available with the full text of this article at NEJM.org. Study data were recorded on case-report forms and were submitted electronically to a database (DataFax, Clinical Datafax Systems) at DF/Net Research. All investigators except for one statistician and two data managers remained unaware of the randomization assignments until the completion of the study.

END POINTS

The primary end point was the acquisition of HIV-1 in partners who had not previously been infected; the diagnosis was made on the basis of

a positive HIV-1–antibody test after a previously negative test. For participants who were found to have undergone seroconversion to HIV-1 at the first quarterly visit, plasma samples that had been obtained at the time of enrollment were tested for HIV-1 RNA; if the samples were positive, the couple was excluded from the analyses because HIV-1 infection occurred before randomization.

HIV-1 in the initially uninfected partner was classified as likely to have been acquired from the person's study partner (linked transmission) or likely to have been acquired from someone other than the person's study partner (unlinked transmission); the classification was based on the sequencing of plasma samples from the source partner and the newly infected partner for C2-V3-C3 regions of *env* (and, if needed, p17–p24 regions of *gag*), phylogenetic analysis, and posterior probability of linkage with the use of pairwise nucleotide distances between sequences (see the Supplementary Appendix). Members of an adjudication committee who were experts in HIV-1 sequencing and were unaware of the treatment assignments reviewed the sequencing data and provided a consensus classification of linkage (see the section on Determination of HIV-1 Transmission Linkage and Fig. 1 in the Supplementary Appendix).

STATISTICAL ANALYSIS

We estimated that with a sample of 3646 couples who were discordant for HIV-1 serologic status and a follow-up period of 12 months, and with the assumption that 85% of the transmissions of HIV-1 would be linked, 88 linked transmissions of HIV-1 would occur and the study would have 90% power to detect a 50% reduction in the incidence of HIV-1 infection in the acyclovir group as compared with the placebo group (from an estimated incidence of 4.0 per 100 person-years to 2.0 per 100 person-years), at a two-sided type I error rate of 5%. In December 2005, owing to a rate of enrollment that was slower than that projected, the protocol was modified to call for the enrollment of 3000 couples for up to 24 months of follow-up. A total of 96% of the partners infected with HIV-1 and 95% of the partners not infected with HIV-1 consented to participate in the extended follow-up. The study was reviewed by an independent data safety and monitoring board; the statistical analyses were monitored on the basis of the Lan–DeMets group sequential method with O'Brien–Fleming stopping boundaries.^{19,20}

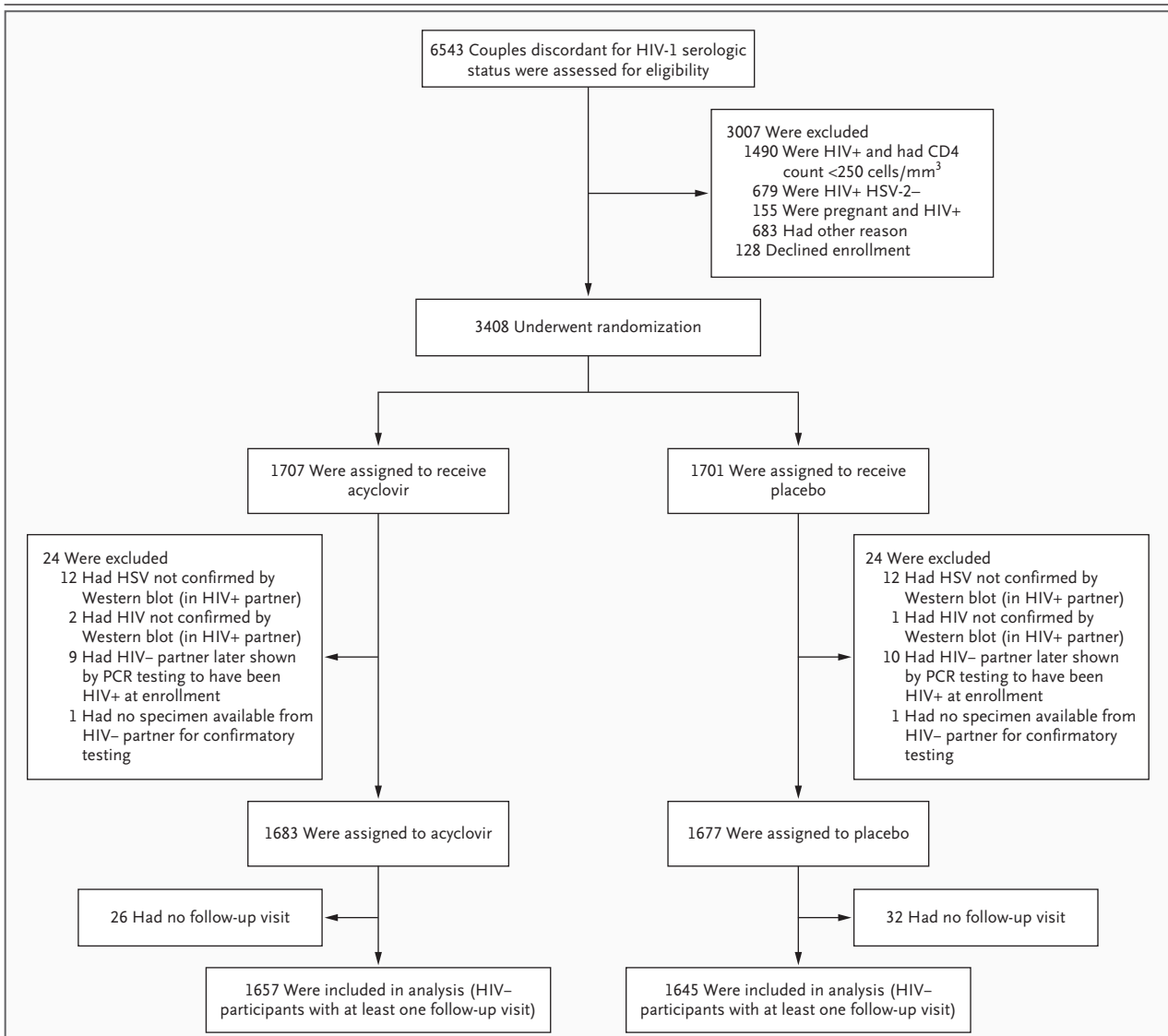


Figure 1. Enrollment and Follow-up of Participants.

Data are shown for the intention-to-treat cohort, from which the modified intention-to-treat analysis was derived. Seropositivity for HSV-2 at enrollment was determined with the use of the HerpeSelect-2 immunoassay (Focus Technologies) at the site laboratory (a cut-off index value for seropositivity of >3.4 was used to improve specificity [see Laboratory Procedures in the Supplementary Appendix]); inclusion in the primary analysis was based on positive results of Western blotting for HSV-2 performed at the University of Washington (see the Supplementary Appendix). The numbers for follow-up visits include only participants who attended scheduled visits; in the case of partners not infected with HIV-1, visits with HIV-1 tests up to and including the first visit in which a positive HIV-1 test result was recorded were included. The total study populations include all participants who were expected to attend visits (in the case of partners not infected with HIV-1, this included visits up to and including the first visit in which a positive HIV-1 test result was recorded) and reflect a staged close-out of the sites after 12 months. During the follow-up period, three participants who were seropositive for HIV-1 were given a drug kit that was incorrect for their randomization group; the follow-up time for their HIV-1-uninfected partner was censored at that point.

The primary analysis was a modified intention-to-treat analysis of linked transmissions of HIV-1; unlinked transmissions, seroconversions that occurred among men when their female partners who were infected with HIV-1 were pregnant and not taking the study drug, and seroconversions that occurred after the death of the HIV-1-infected partner were excluded. The secondary analysis

was an intention-to-treat analysis of all HIV-1 transmissions that occurred after randomization, regardless of whether the transmission was linked or whether the HIV-infected person had been exposed to the study drug before seroconversion of his or her partner.

A proportional-hazards regression model, stratified according to site, was used to compare the time to the first positive HIV-1 test between the two study groups, and the Kaplan–Meier method was used to estimate the cumulative probability of infection with HIV-1. Subgroup analyses, defined a priori, were performed with the modified intention-to-treat data set according to the characteristics of the partner who was infected with HIV-1 (sex, presence or absence of genital ulcer disease before enrollment, baseline plasma HIV-1 RNA level, and average level of adherence to the study drug) and the partner who was not infected with HIV-1 (baseline HSV-2 serologic status and circumcision status in the case of men). Tests for differential treatment effects according to these characteristics were based on likelihood-ratio comparisons between models with appropriate interaction terms and those without appropriate interaction terms.

A linear mixed-effects model, with a random intercept, was used to evaluate the effect of the intervention on plasma concentrations of HIV-1 (\log_{10} copies per milliliter) before the initiation of antiretroviral therapy, adjusted for the baseline concentrations of HIV-1.²¹ Generalized estimating equations with Poisson errors and robust variances were used to examine temporal trends in high-risk sexual practices. To evaluate the effect of treatment on genital ulcer disease in partners infected with HIV-1, the numbers of episodes of genital ulcer disease that were observed on examination over the course of the follow-up period were analyzed by log-linear regression, with (log-transformed) duration of follow-up as the offset term in the regression models, negative binomial errors, and robust variances. Reported P values are two-sided and were not adjusted for multiple comparisons.

RESULTS

STUDY PARTICIPANTS

A total of 6543 couples who were discordant for HIV-1 serologic status were screened for the study; 3408 of these couples were enrolled. The partners who had HIV-1 infection were randomly assigned

to receive acyclovir, at a dose of 400 mg twice daily, or placebo (Fig. 1). After 24 couples in each group were excluded on the basis of confirmatory testing for HIV-1 and HSV-2, the final analysis included 3360 couples (1683 in the acyclovir group and 1677 in the placebo group).

The two groups had similar baseline characteristics (Table 1). In the case of 68% of the couples, the partner who was infected with HIV-1 was the female partner. A total of 76% of the couples were married, and 90% lived together; among the couples living together, the mean duration of the partnership was 5 years. Partners with HIV-1 infection reported a median of six sexual acts with their partner in the month before enrollment. In partners infected with HIV-1, the median baseline CD4 count was 462 cells per cubic millimeter, and median HIV-1 plasma RNA levels were 3.9 \log_{10} copies per milliliter in women and 4.3 \log_{10} copies per milliliter in men. At the time of enrollment, 23% of the partners with HIV-1 infection reported having had symptoms of genital ulcer disease in the previous 3 months, and genital ulcers were observed on examination in 3% of all HIV-1-infected partners. Among the partners who did not have HIV-1 infection at enrollment, 68% were seropositive for HSV-2.

FOLLOW-UP, PREGNANCY, AND ADHERENCE TO THE STUDY TREATMENT

Of the couples who were eligible for 24 months of follow-up, 92% of the partners infected with HIV-1 and 84% of the partners without HIV-1 infection remained in the study for 24 months. Participants who were not infected with HIV-1 accounted for 4868 person-years of follow-up for the assessment of the incidence of HIV-1 infection.

Acyclovir or matching placebo was dispensed at 88% of the monthly visits. The reasons for not dispensing the study medication included missed visits (in 48% of cases), pregnancy (36%, accounting for 4% of the person-years of follow-up among all participants with HIV-1 infection and 6% of the person-years of follow-up among female participants, with similar rates in the two groups), death of the HIV-1-infected partner while the partner who was not infected with HIV-1 remained in the study (10%), and other reasons (5%). An assessment of pill counts suggested that participants took 96% of the dispensed doses. The product of the percentage of drug taken and the percentage of drug dispensed (85%) provides an overall mea-

Table 1. Baseline Characteristics of the Participants, According to Study Group.				
Characteristic	Partners Who Were Infected with HIV-1		Partners Who Were Not Infected with HIV-1	
	Acyclovir (N=1683)	Placebo (N=1677)	Acyclovir (N=1683)	Placebo (N=1677)
Demographic				
Female sex — no. (%)	1127 (67)	1145 (68)	556 (33)	532 (32)
Age — no. (%)				
18–25 yr	309 (18)	334 (20)	243 (14)	224 (13)
26–35 yr	809 (48)	760 (45)	730 (43)	722 (43)
≥36 yr	565 (34)	583 (35)	710 (42)	731 (44)
Education — no. of years of schooling				
Median	8	8	8	8
Interquartile range	6–11	7–11	7–12	7–11
Any monthly income — no. (%)	619 (37)	593 (35)	818 (49)	828 (49)
Married to study partner — no. (%)*	1270 (75)	1267 (76)	1273 (76)	1270 (76)
Living with study partner — no. (%)*	1514 (90)	1513 (90)	1516 (90)	1520 (91)
Duration of partnership — yr*				
Median	5	5	5	5
Interquartile range	3–10	2–10	3–10	2–10
No. of children				
Median	2	2	2	2
Interquartile range	1–4	1–4	1–4	1–4
Sexual activity				
Sexual contacts in previous month — no.*				
Median	5	6	8	9
Interquartile range	3–10	4–10	4–13	4–13
Unprotected sexual acts in previous month — no.*				
Median	0	0	0	0
Interquartile range	0–1	0–1	0–1	0–1
Any unprotected sex in previous month — no. (%)*	488 (29)	502 (30)	501 (30)	516 (31)
Outside sexual partnerships in previous month — no./total no. (%)*†	59/1683 (4)	62/1677 (4)	47/814 (6)	56/798 (7)
Clinical				
CD4 count — cells/mm ³				
Median	470	455	—	—
Interquartile range	350–637	343–625	—	—
Plasma HIV RNA — log ₁₀ copies/ml				
Women				
Median	3.96	3.92	—	—
Interquartile range	3.23–4.57	3.20–4.50	—	—
Men				
Median	4.31	4.35	—	—
Interquartile range	3.69–4.86	3.63–4.92	—	—

Table 1. (Continued.)				
Characteristic	Partners Who Were Infected with HIV-1		Partners Who Were Not Infected with HIV-1	
	Acyclovir (N=1683)	Placebo (N=1677)	Acyclovir (N=1683)	Placebo (N=1677)
Genital ulcer disease — no./total no. (%)				
Women				
Reported symptoms in previous 3 months	241/1127 (21)	257/1145 (22)	9/556 (2)	6/532 (1)
Detected on examination	38/1127 (3)	35/1145 (3)	3/556 (1)	6/532 (1)
Men				
Reported symptoms in previous 3 months	144/556 (26)	119/532 (22)	24/1127 (2)	21/1145 (2)
Detected on examination	12/556 (2)	11/532 (2)	18/1127 (2)	12/1145 (1)
Male circumcision — no./total no. (%)	188/556 (34)	180/532 (34)	613/1127 (54)	629/1145 (55)
Any laboratory-confirmed diagnosis of STI — no./total no. (%)‡				
Women	208/1127 (18)	217/1145 (19)	69/556 (12)	73/532 (14)
Men	43/556 (8)	39/532 (7)	117/1127 (10)	112/1145 (10)

* These data were obtained separately from each member of the couple; therefore, responses between the partner infected with HIV-1 and the partner not infected with HIV-1 sometimes vary.

† Data on outside partnerships during the previous month were obtained for HIV-negative participants only after case-report forms were modified to collect this information.

‡ Sexually transmitted illness (STI) included syphilis, gonorrhea, chlamydial infection, and trichomoniasis.

sure of study-drug coverage during the follow-up period. Overall, 71% of participants with HIV-1 infection had at least 90% coverage, 26% had less than 90% coverage, and 3% had missing data at some visits. Acyclovir was not associated with severe side effects; only one person discontinued acyclovir owing to side effects (headaches).

EFFECT OF ACYCLOVIR ON TRANSMISSION OF HIV-1

Seroconversion to HIV-1 occurred in 132 initially uninfected partners, representing an incidence of 2.7 per 100 person-years (95% confidence interval [CI], 2.3 to 3.2) (Fig. 2). A total of 38 transmissions (29%) were determined to be not genetically linked, as assessed by viral sequencing, probably reflecting HIV-1 infection from a person other than the study partner; 3 transmissions (2%) could not be classified. Of 91 genetically linked HIV-1 transmissions (69%), 6 were excluded because they occurred while women with HIV-1 infection were pregnant and were not taking the study drug, and 1 was excluded because the incorrect drug was dispensed at month 12. Thus, 84 linked transmissions of HIV-1 were included in the modified intention-to-treat analysis. The incidence of HIV-1 infection, calculated on the basis of linked HIV-1

transmissions, was 1.8 per 100 person-years (95% CI, 1.5 to 2.3) overall, 2.5 per 100 person-years (95% CI, 1.8 to 3.4) for male-to-female transmission, and 1.5 per 100 person-years (95% CI, 1.1 to 2.0) for female-to-male transmission (hazard ratio for male-to-female transmission as compared with female-to-male transmission, 1.65; 95% CI, 1.06 to 2.56; $P=0.02$). The difference between the rate of male-to-female transmission and the rate of female-to-male transmission was attenuated after adjustment for plasma HIV-1 RNA levels (hazard ratio, 1.33; 95% CI, 0.77 to 2.29).

Of the 84 linked transmissions included in the primary modified intention-to-treat analysis, 41 occurred in the acyclovir group and 43 in the placebo group (hazard ratio with acyclovir, 0.92; 95% CI, 0.60 to 1.41; $P=0.69$) (Fig. 3). In the secondary intention-to-treat analysis of all transmissions, 67 occurred in the acyclovir group and 64 in the placebo group (hazard ratio with acyclovir, 0.99; 95% CI, 0.71 to 1.40; $P=0.97$) (Fig. 2 in the Supplementary Appendix).

In prespecified subgroup analyses performed with the modified intention-to-treat data set, there were no significant differences in the treatment effect according to sex, baseline plasma concen-

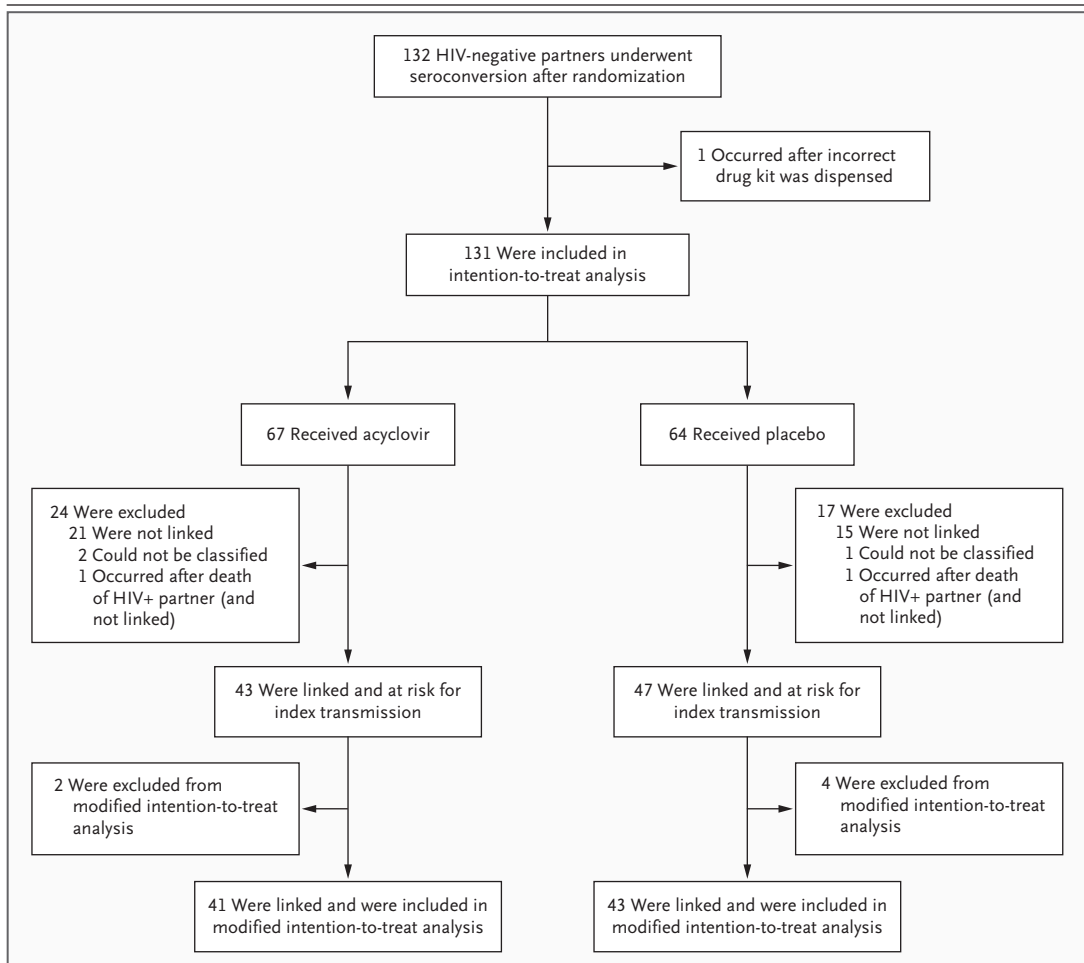


Figure 2. End Points with Respect to Transmission of HIV-1, According to the Study Group, in the Modified Intention-to-Treat and the Intention-to-Treat Populations.

The 132 seroconversions to HIV-1 excluded 19 in partners who, during confirmatory testing, were found to be seronegative for HIV-1 (i.e., negative for HIV-1 antibodies) but who were infected with HIV-1 (i.e., positive for HIV-1 RNA) at study enrollment and were infected therefore with HIV-1 before their HIV-1-infected partner received acyclovir or placebo. Determination of linkage for transmission pairs was based on viral sequencing in the partner who was originally infected with HIV-1 and that person's partner who underwent seroconversion to HIV-1; linkage data were reviewed by an expert adjudication committee. The modified intention-to-treat analysis excluded HIV-1 transmissions among male partners who underwent seroconversion when their HIV-1-infected female partners were pregnant and not taking the study drug as specified by the protocol (two in the acyclovir group and four in the placebo group).

tration of HIV-1, presence or absence of symptoms of genital ulcer disease in the partner with HIV-1 infection in the 3 months before enrollment, level of adherence to the assigned treatment (<90% or ≥90%), baseline HSV-2 serologic status of the partner who was not infected with HIV-1, or circumcision status of male partners who were not infected with HIV-1 (Fig. 4). Partners without HIV-1 infection who were HSV-2-seropositive were at increased risk for acquiring HIV-1, as compared

with those who were HSV-2-seronegative (hazard ratio, 2.02; 95% CI, 1.15 to 3.57), corroborating previous epidemiologic observations.²²

EFFECT OF ACYCLOVIR IN REDUCING PLASMA HIV-1 CONCENTRATIONS

The mean plasma concentration of HIV-1 during the follow-up period was 0.25 log₁₀ copies per milliliter lower in the acyclovir group than in the placebo group (95% CI, 0.22 to 0.29; P<0.001)

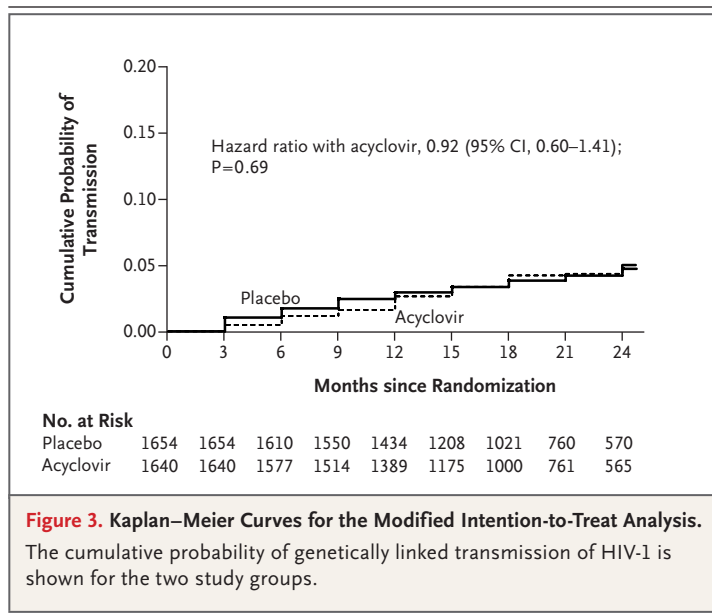
(Fig. 5). The reductions were similar in men and women (0.30 and 0.23 log₁₀ copies per milliliter, respectively; $P=0.10$ for the interaction between sex and treatment). An effect of acyclovir was seen across all strata of baseline plasma viral loads. The reduction in plasma HIV-1 concentrations was greater among participants who had baseline plasma HIV-1 concentrations of less than 100,000 copies per milliliter than among those who had baseline concentrations of 100,000 or more copies per milliliter (0.27 vs. 0.10 log₁₀ copies per milliliter; $P=0.001$ for the difference in acyclovir effect between the two viral-load strata). There was a significant association between the mean rate of adherence to treatment in the previous quarter and the reduction in plasma HIV-1 levels with acyclovir: the reduction in the plasma HIV-1 level was 0.16 log₁₀ copies per milliliter when the adherence rate was less than 75%, 0.19 log₁₀ copies per milliliter when the adherence rate was 75 to 89%, and 0.27 log₁₀ copies per milliliter when the adherence rate was 90% or more ($P=0.002$).

PROTECTIVE EFFECT OF ACYCLOVIR AGAINST GENITAL ULCER DISEASE

A total of 217 episodes of genital ulcer disease were observed over the course of the follow-up period in the acyclovir group, as compared with 550 episodes in the placebo group (risk ratio, 0.39; 95% CI, 0.32 to 0.48; $P<0.001$). The protective effect of acyclovir against genital ulcer disease was greater when the analysis was restricted to the cases of genital ulcer disease that were positive for HSV DNA as assessed by PCR testing (92 in the acyclovir group vs. 336 in the placebo group; risk ratio, 0.27; 95% CI, 0.20 to 0.36, $P<0.001$). There was no significant difference in the protective effect of acyclovir against genital ulcer disease according to sex or status with respect to self-reported symptoms of genital ulcer disease during the 3 months before enrollment ($P=0.55$ and $P=0.77$, respectively). Among participants infected with HIV-1, episodic acyclovir therapy was provided at 1.8% of the visits in the acyclovir group and 4.7% of the visits in the placebo group.

SERIOUS ADVERSE EVENTS

A total of 381 serious adverse events, including 77 deaths, were reported; the number of events and the reported causes were similar in the two study groups (see the Supplementary Appendix). No serious adverse events were reported as having been possibly or probably related to acyclovir therapy.



SEXUAL PRACTICES

High-risk sexual practices reported by participants decreased significantly during the follow-up period. A total of 29% of the partners infected with HIV-1 reported having had unprotected sex during the month before enrollment, as compared with an average of 7% during the follow-up visits ($P<0.001$); the reductions were similar in the two groups ($P=0.31$). The proportion of participants not infected with HIV-1 who reported having had sex with a partner other than their study partner increased from 5.4% at baseline to 15.5% at 24 months ($P<0.001$), with no significant difference between the groups ($P=0.36$). Among participants who underwent seroconversion to HIV-1, transmissions that were not genetically linked within the partnership were significantly more common among those who reported having had sex with persons other than their study partner in the 6 months before seroconversion than among those who did not (79% vs. 23%, $P<0.001$).

DISCUSSION

A standard dose of the HSV-2 suppressive drug acyclovir (400 mg twice daily) given for up to 24 months to persons who were infected with both HIV-1 and HSV-2 and who had CD4 counts of 250 or more cells per cubic millimeter, did not reduce transmission of HIV-1 to sexual partners, despite significant reductions in plasma HIV-1 concentrations and in the incidence of genital ulcer disease caused by HSV-2. Thus, the lack of efficacy

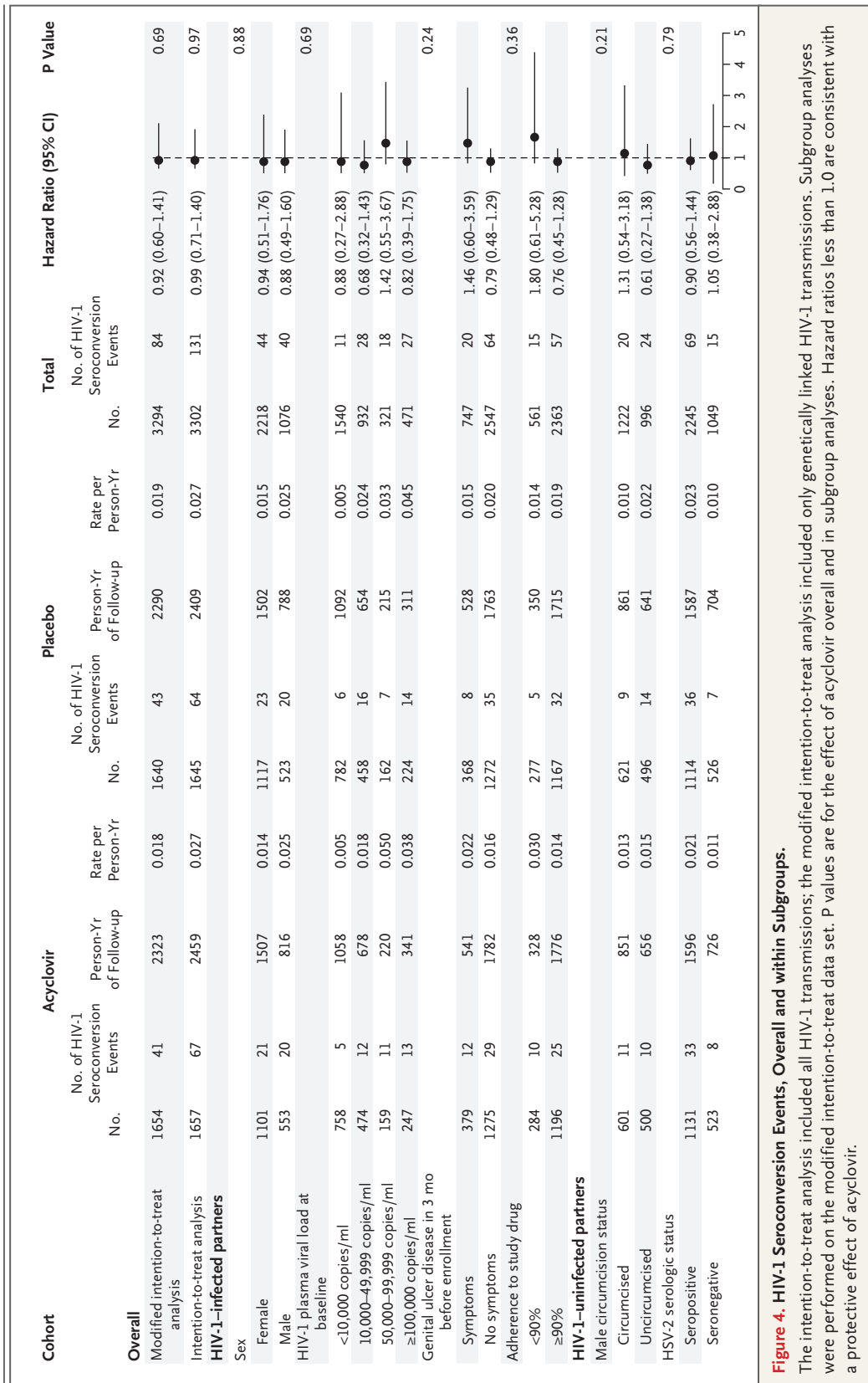


Figure 4. HIV-1 Seroconversion Events, Overall and within Subgroups.

The intention-to-treat analysis included all HIV-1 transmissions; the modified intention-to-treat analysis included only genetically linked HIV-1 transmissions. Subgroup analyses were performed on the modified intention-to-treat data set. P values are for the effect of acyclovir overall and in subgroup analyses. Hazard ratios less than 1.0 are consistent with a protective effect of acyclovir.

of HSV-2 suppressive therapy in preventing the transmission of HIV-1 among participants in this study does not appear to have been caused by poor activity of acyclovir against HSV-2 or by poor adherence to treatment, as assessed by monthly pill counts and by serum acyclovir testing in a subgroup of participants (Supplementary Appendix).

Epidemiologic and laboratory data accumulated over the course of more than 20 years suggest that genital HSV-2 infection increases the infectiousness of persons with HIV-1 infection. Genital ulcer disease increased female-to-male transmission of HIV-1 by a factor of nearly 5 among clients of female sex workers,²³ and symptomatic genital ulcer disease in partners infected with HIV-1 increased the per-contact risk of HIV-1 transmission by a factor of 4 among monogamous HIV-1-serodiscordant couples.¹⁴ Elevated HIV-1 levels in plasma and genital secretions have been associated with symptomatic and asymptomatic reactivation of genital HSV-2 infection.^{10-13,24} Moreover, in five placebo-controlled, randomized trials, plasma HIV-1 RNA levels were reduced by 0.25 to 0.5 log₁₀ copies per milliliter when persons who were infected with both HIV-1 and HSV-2 but were not receiving antiretroviral therapy received standard doses of HSV-2 suppressive agents (valacyclovir at a dose of 500 mg twice daily or acyclovir at a dose of 400 to 800 mg twice daily) for 1 to 3 months.^{4,5,15-17} Although the effects of acyclovir therapy on HIV-1 replication were thought to be indirectly mediated through HSV-2, two recent *in vitro* studies suggest that acyclovir may also have direct activity in reducing the replication of HIV-1.^{25,26}

We selected acyclovir and the 400-mg twice-daily dose for our study on the basis of the similar efficacy of acyclovir at this dose to a dose of 500 mg of valacyclovir twice daily in reducing symptomatic genital ulcer disease and asymptomatic reactivation of genital HSV-2²⁷ and on the basis of the availability of acyclovir in a low-cost generic formulation. In addition, the reductions in plasma HIV-1 levels observed with this dose of acyclovir are similar to those seen with valacyclovir when it is used to suppress HSV-2 infection in persons infected with both HIV-1 and HSV-2.^{4,5,15-17} The reduction in plasma HIV-1 levels that we observed was similar to that seen in previous studies; therefore, the lack of efficacy in reducing the transmission of HIV-1 does not appear to have been due to lack of adherence to treatment or to

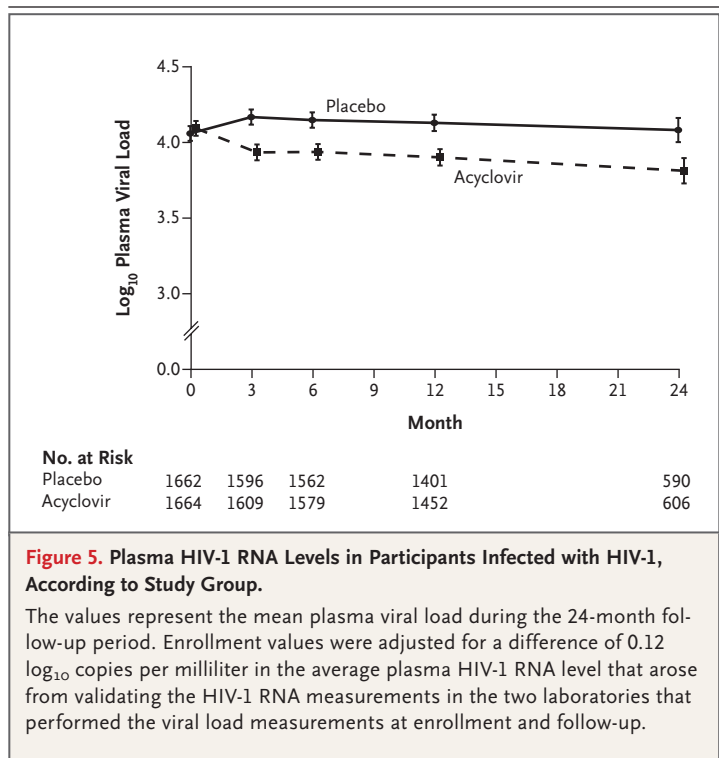


Figure 5. Plasma HIV-1 RNA Levels in Participants Infected with HIV-1, According to Study Group.

The values represent the mean plasma viral load during the 24-month follow-up period. Enrollment values were adjusted for a difference of 0.12 log₁₀ copies per milliliter in the average plasma HIV-1 RNA level that arose from validating the HIV-1 RNA measurements in the two laboratories that performed the viral load measurements at enrollment and follow-up.

the formulation of the study drug, nor is it likely that the efficacy would have been improved if standard doses of valacyclovir rather than acyclovir had been used.

The lack of efficacy of acyclovir in reducing the transmission of HIV-1 in our study suggests that a greater reduction in HIV-1 levels is needed to reduce the risk of transmission. This indication that a greater reduction in the plasma viral load may have to be achieved provides information that can be useful in the development of other biomedical strategies for the prevention of HIV-1, such as the treatment of coexisting infections (e.g., malaria or helminthic infection), and in the development of HIV-1 vaccines directed at reducing the HIV-1 viral load.^{28,29}

Most transmissions of HIV-1 infection in Africa are thought to occur within stable, cohabiting HIV-1-serodiscordant couples.³⁰ Our study shows the feasibility of conducting trials involving such couples^{18,31} to directly evaluate the efficacy of preventive strategies in decreasing the infectiousness of persons infected with HIV-1. The rate of HIV-1 transmission in our study population was 2.7 cases per 100 person-years overall, which is substantially lower than the rate in previous observational studies of HIV-1-serodiscordant cou-

ples.^{32,33} It is likely that the lower rate in our study was due largely to our having provided monthly counseling on risk reduction, free condoms, and other preventive services. Since approximately 30% of HIV-1 transmissions in this cohort of stable, cohabiting couples were attributable to outside partnerships, couples in which one of the partners is seropositive and the other is seronegative for HIV-1 should be counseled on potential misconceptions about the risk from sexual activity with other partners whose HIV-1 status is unknown.

In summary, our study of heterosexual HIV-1-serodiscordant couples showed that among the partners who were infected with both HIV-1 and HSV-2 and who had CD4 counts of 250 or more cells per cubic millimeter, treatment with standard doses of acyclovir for the suppression of HSV-2 infection did not decrease the incidence of transmission of HIV-1 to the uninfected partners, despite significant reductions in plasma HIV-1 levels and in the incidence of genital ulcer disease. New strategies to reduce the risk of transmission of HIV-1 are needed for HIV-1-serodiscordant couples.

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APPENDIX

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REFERENCES

- Weiss H. Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes* 2004;11:Suppl 1:24A-35A.
- Siegal FP, Lopez C, Hammer GS, et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med* 1981;305:1439-44.
- Strick LB, Wald A, Celum C. Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. *Clin Infect Dis* 2006;43:347-56.
- Zuckerman RA, Lucchetti A, Whittington WL, et al. Herpes simplex virus (HSV) suppression with valacyclovir reduces rectal and blood plasma HIV-1 levels in HIV-1/HSV-2-seropositive men: a randomized, double-blind, placebo-controlled crossover trial. *J Infect Dis* 2007;196:1500-8.
- Baeten JM, Strick LB, Lucchetti A, et al. Herpes simplex virus (HSV)-suppressive therapy decreases plasma and genital HIV-1 levels in HSV-2/HIV-1 coinfected women: a randomized, placebo-controlled, cross-over trial. *J Infect Dis* 2008;198:1804-8.
- Kucera LS, Leake E, Iyer N, Raben D, Myrvik QN. Human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 2 (HSV-2) can coinfect and simultaneously replicate in the same human CD4+ cell: effect of coinfection on infectious HSV-2 and HIV-1 replication. *AIDS Res Hum Retroviruses* 1990;6:641-7.
- Diaz JJ, Dodon MD, Schaefer-Uthurtal N, et al. Post-transcriptional transactivation of human retroviral envelope glycoprotein expression by herpes simplex virus Us11 protein. *Nature* 1996;379:273-7.
- Heng MC, Heng SY, Allen SG. Co-infection and synergy of human immunodeficiency virus-1 and herpes simplex virus-1. *Lancet* 1994;343:255-8.
- Mosca JD, Bednarik DP, Raj NB, et al. Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. *Nature* 1987;325:67-70.
- Schacker T, Ryncarz AJ, Goddard J, Diem K, Shaughnessy M, Corey L. Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1-infected men. *JAMA* 1998;280:61-6.
- Schacker T, Zeh J, Hu H, Shaughnessy M, Corey L. Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. *J Infect Dis* 2002;186:1718-25.
- Mbopi-Kéou F-X, Grésenguet G, Mayaud P, et al. Interactions between herpes simplex virus type 2 and human immunodeficiency virus type 1 infection in African women: opportunities for intervention. *J Infect Dis* 2000;182:1090-6.
- Baeten JM, McClelland RS, Corey L, et al. Vitamin A supplementation and genital shedding of herpes simplex virus among HIV-1-infected women: a randomized clinical trial. *J Infect Dis* 2004;189:1466-71.
- Gray RH, Wawer MJ, Brookmeyer R, et al. Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda. *Lancet* 2001;357:1149-53.
- Nagot N, Ouédraogo A, Foulongne V, et al. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus. *N Engl J Med* 2007;356:790-9.
- Dunne EF, Whitehead S, Sternberg M, et al. Suppressive acyclovir therapy reduces HIV cervicovaginal shedding in HIV- and HSV-2-infected women, Chiang Rai, Thailand. *J Acquir Immune Defic Syndr* 2008;49:77-83.
- Delany S, Mlaba N, Clayton T, et al. Impact of aciclovir on genital and plasma HIV-1 RNA in HSV-2/HIV-1 co-infected women: a randomized placebo-controlled trial in South Africa. *AIDS* 2009;23:461-9.
- Lingappa JR, Kahle E, Mugo N, et al. Characteristics of HIV-1 discordant couples enrolled in a trial of HSV-2 suppression to reduce HIV-1 transmission: the Partners Study. *PLoS One* 2009;4(4):e5272.
- Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. *Biometrika* 1983;70:659-63.
- O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979;35:549-56.
- Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982;38:963-74.
- Glynn JR, Biraro S, Weiss HA. Herpes simplex virus type 2: a key role in HIV incidence. *AIDS* 2009;23:1595-8.
- Cameron DW, Simonsen JN, D'Costa LJ, et al. Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men. *Lancet* 1989;2:403-7.
- Mole L, Ripich S, Margolis D, Holodniy M. The impact of active herpes simplex virus infection on human immunodeficiency virus load. *J Infect Dis* 1997;176:766-70.
- Lisco A, Vanpouille C, Tchesnokov EP, et al. Acyclovir is activated into a HIV-1 reverse transcriptase inhibitor in herpesvirus-infected human tissues. *Cell Host Microbe* 2008;4:260-70.
- McMahon MA, Siliciano JD, Lai J, et al. The antiherpetic drug acyclovir inhibits HIV replication and selects the V75I reverse transcriptase multidrug resistance mutation. *J Biol Chem* 2008;283:31289-93.
- Gupta R, Wald A, Krantz E, et al. Valacyclovir and acyclovir for suppression of shedding of herpes simplex virus in the genital tract. *J Infect Dis* 2004;190:1374-81.
- Walson JL, Otieno PA, Mbuchi M, et al. Albendazole treatment of HIV-1 and helminth co-infection: a randomized, double-blind, placebo-controlled trial. *AIDS* 2008;22:1601-9.
- Gilbert PB, DeGruttola VG, Hudgens MG, Self SG, Hammer SM, Corey L. What constitutes efficacy for a human immunodeficiency virus vaccine that ameliorates viremia: issues involving surrogate end points in phase 3 trials. *J Infect Dis* 2003;188:179-93.
- Dunkle KL, Stephenson R, Karita E, et al. New heterosexually transmitted HIV infections in married or cohabiting couples in urban Zambia and Rwanda: an analysis of survey and clinical data. *Lancet* 2008;371:2183-91.
- Lingappa JR, Lambdin B, Bukusi EA, et al. Regional differences in prevalence of HIV-1 discordance in Africa and enrollment of HIV-1 discordant couples into an HIV-1 prevention trial. *PLoS One* 2008;3(1):e1411.
- Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. *N Engl J Med* 2000;342:921-9.
- Fideli US, Allen SA, Musonda R, et al. Virologic and immunologic determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa. *AIDS Res Hum Retroviruses* 2001;17:901-10.

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