

ORIGINAL ARTICLE

Reduction of HIV-1 RNA Levels with Therapy to Suppress Herpes Simplex Virus

Nicolas Nagot, M.D., Abdoulaye Ouédraogo, M.D., Vincent Foulongne, Ph.D., Issouf Konaté, M.D., Helen A. Weiss, Ph.D., Laurence Vergne, Ph.D., Marie-Christine Defer, Ph.D., Didier Djagbaré, M.Sc., Anselme Sanon, M.Sc., Jean-Baptiste Andonaba, M.D., Pierre Becquart, Ph.D., Michel Segondy, Ph.D., Roselyne Vallo, M.Sc., Adrien Sawadogo, M.D., Philippe Van de Perre, M.D., Ph.D., and Philippe Mayaud, M.D., for the ANRS 1285 Study Group*

ABSTRACT

BACKGROUND

From the Centre Muraz (N.N., A.O., I.K., L.V., M.-C.D., D.D., A. Sanon, R.V.) and University Hospital (J.-B.A., A. Sawadogo) —both in Bobo-Dioulasso, Burkina Faso; the London School of Hygiene and Tropical Medicine, London (N.N., H.A.W., P.M.); and Montpellier University Hospital and Research Unit 145, Montpellier, France (V.F., P.B., M.S., P.V.P.). Address reprint requests to Dr. Nagot at the Clinical Research Unit, London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, United Kingdom, or at n_nagot@hotmail.com.

Epidemiologic data suggest that infection with herpes simplex virus type 2 (HSV-2) is associated with increased genital shedding of human immunodeficiency virus type 1 (HIV-1) RNA and HIV-1 transmissibility.

METHODS

We conducted a randomized, double-blind, placebo-controlled trial of HSV suppressive therapy with valacyclovir (at a dose of 500 mg twice daily) in Burkina Faso among women who were seropositive for HIV-1 and HSV-2; all were ineligible for highly active antiretroviral therapy. The patients were followed for 24 weeks (12 weeks before and 12 weeks after randomization). Regression models were used to assess the effect of valacyclovir on the presence and quantity of genital and plasma HIV-1 RNA and genital HSV-2 DNA during treatment, adjusting for baseline values, and to evaluate the effect over time.

RESULTS

A total of 140 women were randomly assigned to treatment groups; 136 were included in the analyses. At enrollment, the median CD4 cell count was 446 cells per cubic millimeter, and the mean plasma viral load was 4.44 log₁₀ copies per milliliter. With the use of summary-measures analysis, valacyclovir therapy was found to be associated with a significant decrease in the frequency of genital HIV-1 RNA (odds ratio, 0.41; 95% confidence interval [CI], 0.21 to 0.80) and in the mean quantity of the virus (log₁₀ copies per milliliter, -0.29; 95% CI, -0.44 to -0.15). However, there was no significant decrease in detection of HIV (risk ratio, 0.93; 95% CI, 0.81 to 1.07). HSV suppressive therapy also reduced the mean plasma HIV-1 RNA level by 0.53 log₁₀ copy per milliliter (95% CI, -0.72 to -0.35). Repeated-measures analysis showed that these effects became significantly stronger during the 3 months of follow-up.

CONCLUSIONS

HSV suppressive therapy significantly reduces genital and plasma HIV-1 RNA levels in dually infected women. This finding may have important implications for HIV control. (ClinicalTrials.gov number, NCT00158509.)

*Members of the Agence Nationale de Recherches sur le SIDA (ANRS) 1285 study group are listed in the Appendix.

N Engl J Med 2007;356:790-9.

Copyright © 2007 Massachusetts Medical Society.

EPIDEMIOLOGIC AND BIOLOGIC DATA SUPPORT a strong association between herpes simplex virus type 2 (HSV-2) and infection with human immunodeficiency virus type 1 (HIV-1).¹⁻⁵ A recent meta-analysis of prospective observational studies showed that patients who were seropositive for HSV-2 had three times the risk of acquiring HIV-1, as compared with those who were seronegative for the virus.⁶ In addition, three of four studies showed that HSV-2 infection may increase the quantity of genital HIV-1 RNA.⁷⁻¹⁰ This association probably arises from both symptomatic¹¹ and asymptomatic genital shedding of HSV-2 DNA, although the evidence for the latter is weaker.¹² The biologic plausibility of HSV-2 as a facilitator for genital HIV-1 RNA is supported by various mechanisms, including local influx of activated CD4+ lymphocytes in HSV-infected lesions¹³ and transactivation of the HIV-1 *tat* protein and long terminal repeat genes by HSV-2 proteins.¹⁴⁻¹⁶ This could result in greater HIV-1 replication at the genital mucosal level and possibly also at the systemic level. In support of the latter hypothesis, symptomatic HSV-2 reactivation has been associated with transient increases in plasma HIV-1 RNA levels¹⁷ and acyclovir treatment with reduced plasma HIV-1 RNA levels.¹⁸

To establish a relationship between HSV-2 and HIV-1 replication, randomized, controlled trials of HSV-2 control strategies are required.^{3,19} Our trial, sponsored by the Agence Nationale de Recherches sur le SIDA under grant number 1285 (ANRS 1285), was a randomized, controlled study to determine whether HSV suppressive therapy reduces HIV-1 replication among dually infected women in Bobo-Dioulasso, Burkina Faso.

PATIENTS AND METHODS

STUDY DESIGN

We conducted a double-blind, placebo-controlled trial of 500 mg of valacyclovir twice daily for 3 months among women who were dually infected with HIV-1 and HSV-2. None of the women in the study were eligible for highly active antiretroviral therapy (HAART), according to recommendations of the World Health Organization (WHO) for developing countries.²⁰ The study protocol was approved by the institutional review board at Centre Muraz and the ethics committees at the Burkina Faso Ministry of Health and the London School of Hygiene and Tropical Medicine. All patients provided written informed consent.

PATIENTS

Study patients were recruited from the Yerelon cohort of high-risk women described elsewhere,²¹ from local organizations for people living with HIV/AIDS (PLWHA), and from the University Hospital in Bobo-Dioulasso. Women who were at least 16 years of age, had serum antibodies to both HSV-2 and HIV-1, and were not eligible for HAART could enroll in the study. Exclusion criteria were significant renal impairment (defined as a serum creatinine level of more than twice the normal value) or hypersensitivity to acyclovir; breast-feeding, pregnancy, or a desire to become pregnant in the next 6 months; or a medical indication for HSV suppressive therapy (≥ 6 clinical episodes per year).

TRIAL PROCEDURES

At a screening visit, women were informed of the aims and procedures of the trial and were assessed for eligibility. A blood sample was collected from consenting women for serologic tests, CD4 lymphocyte count, and assessment of renal function. Eligible women underwent an informed-consent process and a 1-week reflection period and then entered the baseline phase, which consisted of six visits every other week. At the scheduled sixth visit, eligibility criteria were reassessed, and after collection of appropriate samples, eligible patients were randomly assigned to two groups by means of a 1:1 allocation scheme with block randomization of 10 patients and following a random-allocation list independently provided by the study drug manufacturer. This list was kept by the statistician for the data and safety monitoring committee. Pre-labeled, sequentially numbered treatment packs were used; investigators and patients were unaware of study-group assignments. The treatment lasted 12 weeks, with six visits every other week starting 2 weeks after randomization.

At each of the 12 visits, a physician performed a gynecologic examination and collected samples. Menstruating women were deferred for genital sampling until 2 days after bleeding had ceased. During the treatment phase, the physician collected empty drug packs for pill counts, recorded and graded adverse events, counseled patients on compliance and safer-sex measures, and provided condoms.

Women who became pregnant discontinued the study drug but were invited to continue regular follow-up procedures. Women with genital ulcers were initially treated with antibiotics ac-

According to national guidelines for the treatment of sexually transmitted infections. After 7 days, nonhealing ulcers were treated with open-label acyclovir (at a dose of 200 mg five times daily for 5 days). The assigned study drug was not discontinued. In addition, both episodic and suppressive therapy with acyclovir remained available to patients after the end of the trial.

SAMPLE COLLECTION

At each visit, swabs were collected for diagnosis of vaginal infections, and cervicovaginal lavage enriched by cervical swabbing was performed for the detection and quantitation of genital HIV-1 RNA and HSV-2 DNA, as previously described.²² At enrollment, additional cervical swabs were collected for the diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Amplicor NG/CT, Roche Diagnostics).

Blood samples were drawn on alternate visits for the quantitation of plasma HIV-1. Urine samples were collected before randomization and once a month during the treatment phase for pregnancy testing (Vikia hCG-S, BioMérieux).

LABORATORY ANALYSES

Serologic Assays and CD4+ Analysis

Depending on the source of recruitment, HIV-1 seropositivity was determined with the use of either an enzyme-linked immunosorbent assay (ELISA) or a rapid-testing strategy with Determine (Abbott), followed by Genie II (BioRad), as recommended by the WHO.²³ The presence of HSV-2 antibodies was detected by means of a specific IgG2 ELISA (Kalon Biologicals) with high sensitivity and specificity in African serum samples.²⁴ Serologic diagnosis of syphilis was based on positive results on the rapid plasma reagin test (RPR, BioMérieux) and *Treponema pallidum* hemagglutination assay (TPHA, Newmarket Laboratories). The CD4+ lymphocyte count was performed with a standard fluorocytometry technique (FACScan, Becton Dickinson).

HIV-1 RNA and HSV-2 DNA

HIV-1 RNA and HSV-2 DNA were quantitated by real-time polymerase-chain-reaction assay with the use of the ABI 7000 system and manual nucleic acid extraction (Qiagen RNA and DNA kits), as described previously.^{22,25,26} Testing of genital HIV-1 RNA was performed in duplicate, and the

mean value of the two measures was used in the analyses. The Centre Muraz laboratory participated in an external quality-control program for HIV-1 RNA quantitation organized by ANRS, and the University of Montpellier Virology Laboratory used a commercial panel (HSV 1/2 Clear QC panel, Argene) as an internal quality control for HSV-2 DNA quantitation.

Vaginal Infections

Trichomonas vaginalis and *Candida albicans* were identified by culture on the InPouch TV (Biomed Diagnostics) and Sabouraud's milieu, respectively. Bacterial vaginosis was diagnosed from Gram-stained vaginal smears with the use of the Nugent standardized scoring system, based on a weighted combination of different morphotypes: lactobacilli, *Gardnerella vaginalis* or bacteroides, and curved gram-variable rods.²⁷

STUDY OUTCOMES

The primary study outcomes were the presence, frequency, and quantity of genital HIV-1 RNA during the treatment phase. Secondary outcomes included the presence and quantity of plasma HIV-1 RNA, as well as the detection of genital HSV-2 DNA and genital ulcers during the treatment phase.

STATISTICAL ANALYSIS

On the basis of data from a pilot study²² and a previous African study,⁷ we estimated that 150 women were required to detect a reduction of 0.4 log₁₀ copy per milliliter of genital HIV-1 RNA between the two study groups, with an estimated variance of 0.5 and a power of 90%, with a 5% type I error. Our calculations allowed for a 15% loss to follow-up.

Analyses were conducted with Stata statistical software, version 9.0 (StataCorp) with the use of a modified intention-to-treat approach, in which the data for pregnant women were censored at the time of the first positive urine test. An a priori decision was made to adjust for the pre-randomization, baseline-phase genital HIV-1 RNA and HSV-2 DNA levels to allow for the variability of viral shedding for each of these outcomes respectively.²⁸ A value equal to half the threshold was allocated to values below the threshold, including undetectable samples, and all viral loads were log₁₀-transformed before analysis.

Primary analyses evaluated the effect per woman, using summary measures to combine the measurements for each woman into a single value. The effect on the presence of HIV-1 RNA or HSV-2 DNA at any time during the treatment phase (detection) was estimated as a risk ratio, using Poisson regression with robust standard errors,²⁹ adjusted for the presence of the outcome measure during the baseline phase. The frequency of genital HIV-1 RNA and HSV-2 DNA (the proportion of visits during which the virus was detected, per woman) was estimated by a proportional-odds-ordered logistic-regression model adjusted for baseline frequency. Linear regression was used to compare the mean levels of genital or plasma HIV-1 RNA (in log₁₀ copies per milliliter) between the study groups, adjusted for mean baseline values. Unadjusted measures of effect are also shown, and all reported P values are two-sided.

Further analyses were carried out on a per-visit basis with the use of repeated-measures analysis. Risk ratios were estimated by means of Poisson regression models fitted with generalized estimating equations with an exchangeable correlation matrix. The effect of the intervention on the presence of genital HIV-1 RNA and HSV-2 DNA was assessed by including an interaction term for the baseline or treatment phase and the study group. The trend of the treatment effect with time was evaluated by including an interaction term for study group and time. The effect of valacyclovir on the quantity of genital HIV-1 RNA was assessed with the use of random-effects linear regression among visits with detectable HIV-1 RNA only, owing to the high proportion of patients with no detectable shedding of the virus. The effect on the quantity of plasma HIV-1 RNA was assessed during all visits.

RESULTS

Of 195 women who underwent screening, 150 were eligible to participate in the study; 148 were enrolled in the baseline phase of the study between August 2004 and January 2005. Of these, 140 women were randomly assigned to study groups, and 136 (68 in each group) were included in the analyses (Fig. 1). The mean age of participants was 32 years (range, 16 to 50), the median

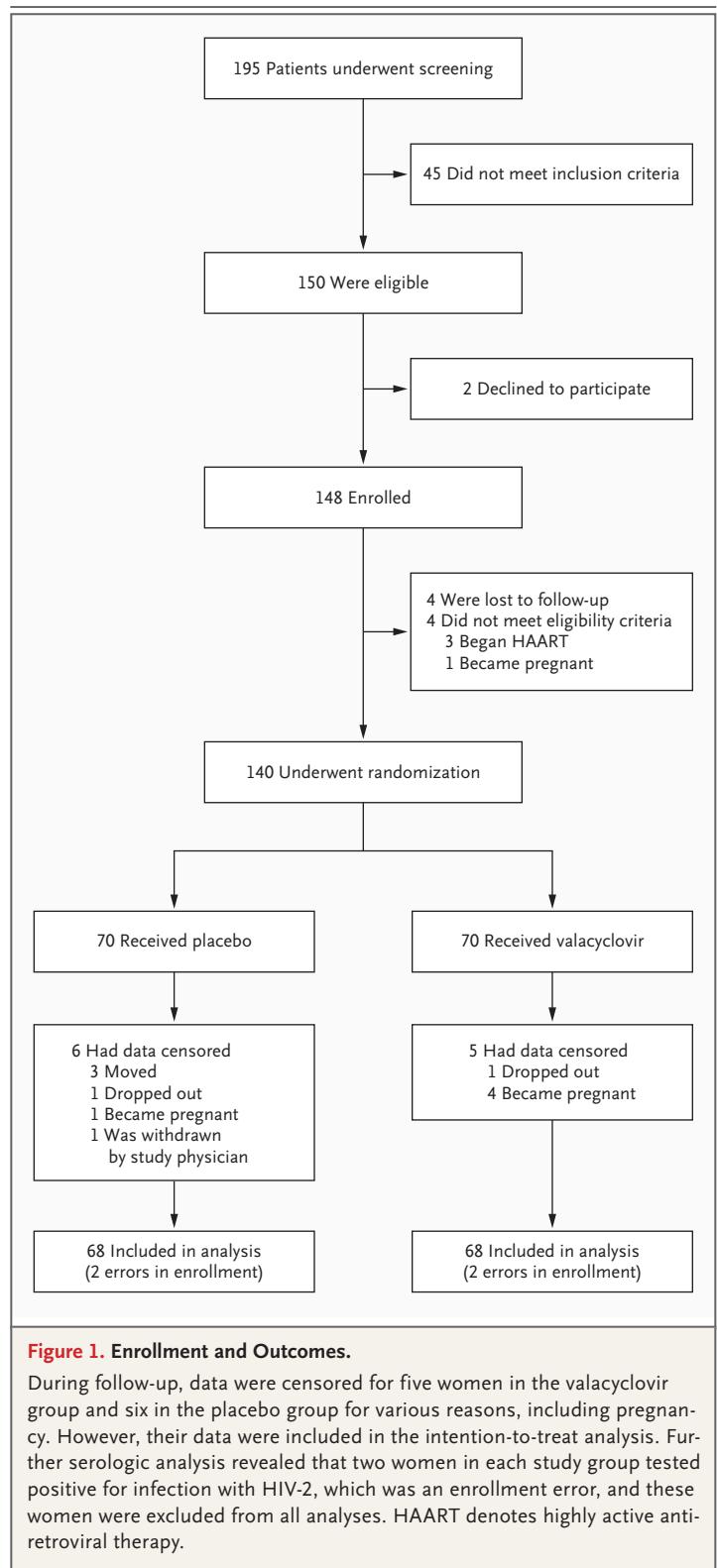


Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Placebo (N=68)	Valacyclovir (N=68)
Age — no. (%) †		
16–24 yr	10 (14.9)	12 (18.5)
25–34 yr	33 (49.3)	32 (49.2)
≥35 yr	24 (35.8)	21 (32.3)
Source of recruitment — no. (%)		
Sex worker	48 (70.6)	39 (57.4)
PLWHA organizations	13 (19.1)	25 (36.8)
Hospital outpatient	7 (10.3)	4 (5.9)
Age when first had sex — yr		
Median	16	16
Interquartile range	15–17	15–18
No. of sex acts in previous week — no. (%)		
None	22 (32.4)	26 (38.2)
1–5	37 (54.4)	31 (45.6)
≥6	9 (13.2)	11 (16.2)
Contraceptive use — no. (%)		
None	26 (38.2)	31 (45.6)
Hormonal contraception	9 (13.2)	10 (14.7)
Intrauterine device	0	1 (1.5)
Condom	33 (48.5)	26 (38.2)
Frequency of vaginal douching — no. (%)		
Never	7 (10.3)	9 (13.2)
Once a day or less	33 (48.5)	35 (51.5)
More than once a day	28 (41.2)	24 (35.3)
Condom use with last regular partner — no. (%)		
No. of patients	46	38
No	2 (4.3)	2 (5.3)
Yes	44 (95.7)	36 (94.7)
Stage of HIV infection — no. (%) ‡		
I	33 (48.5)	35 (51.5)
II or III	35 (51.5)	32 (47.1)
Unknown	0	1 (1.5)
CD4 lymphocyte count per mm ³		
Median	435	451
Interquartile range	319–637	339–683
Plasma HIV-1 RNA — log ₁₀ copies per ml		
Mean	4.66	4.29
95% CI	4.48–4.84	4.02–4.54
Serologic syphilis (RPR+/TPHA+) — no. (%)	0	1 (1.5)
<i>Neisseria gonorrhoeae</i> — no. (%)	0	0
<i>Chlamydia trachomatis</i> — no. (%) §	2 (3.2)	1 (1.6)
<i>Trichomonas vaginalis</i> — no. (%)	4 (5.9)	1 (1.5)
<i>Candida albicans</i> — no. (%)	7 (10.3)	4 (5.9)
Bacterial vaginosis — no. (%)	23 (33.8)	24 (35.3)

* PLWHA denotes people living with HIV/AIDS, RPR rapid plasma reagin, and TPHA *Treponema pallidum* hemagglutination assay. Percentages may not total 100 because of rounding.

† Data were missing for three patients in the valacyclovir group and one patient in the placebo group.

‡ Infections were staged according to the criteria of the World Health Organization.

§ Data were missing for seven women in the valacyclovir group and six women in the placebo group.

CD4 cell count was 446 cells per cubic millimeter (interquartile range, 334 to 628), and the mean plasma HIV-1 RNA level was 4.44 log₁₀ copies per milliliter (95% confidence interval [CI], 4.25 to 4.62) at enrollment. There were no major differences at enrollment between the study groups with regard to predefined demographic, behavioral, and clinical characteristics (Table 1). However, the mean quantity of plasma HIV-1 RNA and the proportion of visits with detectable genital HIV-1 were higher in the placebo group during the entire baseline phase (Table 2).

During the treatment phase, data from 11 women were censored (5 in the valacyclovir group and 6 in the placebo group) owing to pregnancy, dropout, or travel (Fig. 1). Overall, of 408 possible visits, 376 (92.2%) were completed in the valacyclovir group and 374 (91.7%) in the placebo group. On the basis of the pill count, the average treatment compliance was 97.2% (95% CI, 94.0 to 100.0) in the valacyclovir group and 96.7% (95% CI, 94.6 to 98.9) in the placebo group. No serious adverse events or cases of hepatic or renal impairment associated with valacyclovir were reported, and the frequency of mild-to-moderate side effects was similar in the two groups (Table 3).

As compared with women who never shed HSV-2 during the baseline phase, women with detectable HSV-2 DNA at least once during the baseline phase were found to have significantly higher mean viral loads (in log₁₀ copies per milliliter) of genital HIV-1 RNA (3.04 for those with some HSV-2 shedding vs. 2.81 for those without shedding, $P=0.05$) and plasma HIV-1 RNA levels (4.75 for those with some HSV-2 shedding vs. 4.24 for those without shedding, $P=0.001$) during the baseline phase.

EFFECT OF TREATMENT

Genital HIV-1 RNA

Valacyclovir had a nonsignificant effect on the proportion of women who had detectable HIV-1 RNA at least once (risk ratio, 0.93; 95% CI, 0.81 to 1.07). However, treatment was associated with a significant reduction in the frequency of genital HIV-1 RNA (odds ratio, 0.41; 95% CI, 0.21 to 0.80) (Table 2 and Fig. 2), adjusted for corresponding values in the baseline phase. In addition, the mean quantity of genital HIV-1 RNA was significantly lower in the valacyclovir group (mean reduction adjusted for baseline-phase values, -0.29 log₁₀ copy per milliliter; 95% CI, -0.44 to -0.15 ; $P<0.001$).

Table 2. Analysis of Genital and Plasma HIV-1 RNA, Genital HSV-2 DNA, and Genital Ulcerations According to Study Phase and Group.*

Variable	Baseline Phase		Treatment Phase		Unadjusted Measure of Effect (95%CI)	Adjusted Measure of Effect† (95%CI)	P Value for Adjusted Analysis
	Placebo (N=68)	Valacyclovir (N=68)	Placebo (N=68)	Valacyclovir (N=68)			
Summary-measures analysis							
Genital HIV-1 RNA detected							
At least once — no. (%)	61 (89.7)	54 (79.4)	59 (86.8)	51 (75.0)	RR=0.86 (0.73 to 1.02)	RR=0.93 (0.81 to 1.07)	0.30
At no visit — no. (%)	7 (10.3)	14 (20.6)	9 (13.2)	17 (25.0)	OR=0.34‡ (0.18 to 0.65)	OR=0.41‡ (0.21 to 0.80)	0.009
At <50% of visits — no. (%)	11 (16.2)	17 (25.0)	10 (14.7)	23 (33.8)			
At 50–99% of visits — no. (%)	40 (58.8)	27 (39.7)	31 (45.6)	19 (27.9)			
At all visits — no. (%)	10 (14.7)	10 (14.7)	18 (26.5)	9 (13.2)			
Mean quantity — log ₁₀ copies/ml	2.97	2.87	3.02	2.65	-0.37 (-0.59 to -0.14)	-0.29 (-0.44 to -0.15)	<0.001
95% CI	2.83 to 3.11	2.68 to 3.05	2.86 to 3.19	2.50 to 2.81			
Plasma HIV-1 RNA detected							
At least once — no. (%)§	68 (100.0)	63 (92.6)	67 (100.0)	61 (92.4)	RR=0.92 (0.86 to 0.99)	RR=0.98 (0.95 to 1.02)	0.32
Mean quantity — log ₁₀ copies/ml	4.66	4.29	4.81	3.94	-0.87 (-1.19 to -0.55)	-0.53 (-0.72 to -0.35)	<0.001
95% CI	4.48 to 4.84	4.02 to 4.54	4.61 to 5.01	3.67 to 4.20			
Genital HSV-2 DNA detected							
At least once — no. (%)	31 (45.6)	30 (44.1)	37 (54.4)	13 (19.1)	RR=0.35 (0.20 to 0.60)	RR=0.35 (0.21 to 0.60)	<0.001
At no visit — no. (%)	37 (54.4)	38 (55.9)	31 (45.6)	55 (80.9)	OR=0.18‡ (0.08 to 0.39)	OR=0.17‡ (0.07 to 0.38)	<0.001
At <50% of visits — no. (%)	26 (38.2)	29 (42.6)	28 (41.2)	13 (19.1)			
At 50–99% of visits — no. (%)	5 (7.4)	1 (1.5)	8 (11.8)	0			
At all visits — no. (%)	0	0	1 (1.5)	0			
At least one episode of vesicle or genital ulceration — no. (%)	19 (27.9)	20 (29.4)	19 (27.9)	3 (4.4)	RR=0.16 (0.05 to 0.51)	RR=0.16 (0.05 to 0.49)	0.002
Repeated-measures analysis							
Genital HIV-1 RNA							
Virus detected — no./total no. of visits (%)	235/391 (60.1)	190/393 (48.3)	232/364 (63.7)	142/366 (38.8)	RR=0.62 (0.49 to 0.80)	RR=0.77 (0.64 to 0.93)	0.006
Mean quantity — log ₁₀ copies/ml	3.54	3.70	3.60	3.54	-0.11 (-0.32 to -0.09)	-0.28 (-0.46 to -0.09)	0.003
95% CI	3.46 to 3.63	3.59 to 3.81	3.51 to 3.70	3.40 to 3.67			
Plasma HIV-1 RNA							
Mean quantity — log ₁₀ copies/ml	4.65	4.33	4.76	3.93	-0.86 (-1.18 to -0.54)	-0.45 (-0.62 to -0.29)	<0.001
95% CI	4.53 to 4.77	4.17 to 4.49	4.64 to 4.89	3.76 to 4.10			
Genital HSV-2 DNA detected — no./total no. of visits (%)	50/393 (12.7)	38/394 (9.6)	66/368 (17.9)	15/368 (4.1)	RR=0.22 (0.12 to 0.40)	RR=0.29 (0.14 to 0.58)	0.001

* Mean quantities of virus were compared with the use of a regression coefficient. CI denotes confidence interval, RR risk ratio, and OR odds ratio. Percentages may not total 100 because of rounding.

† Values are adjusted for the outcome measure in the baseline phase.

‡ The odds ratio is for the last four categories in the variable.

§ Data regarding plasma HIV-1 RNA were missing for two women in the valacyclovir group and one woman in the placebo group during the treatment phase.

Table 3. Adverse Events.*

Event	Placebo (N=68)	Valacyclovir (N=68)	P Value
	no. (%)		
Nausea	7 (10.3)	11 (16.2)	0.31
Vomiting	6 (8.8)	4 (5.9)	0.51
Diarrhea	7 (10.3)	3 (4.4)	0.19
Dysphagia	3 (4.4)	1 (1.5)	0.31
Constipation	10 (14.7)	5 (7.4)	0.17
Dyspnea	3 (4.4)	2 (2.9)	0.65
Anxiety	2 (2.9)	3 (4.4)	0.65
Headache	27 (39.7)	20 (29.4)	0.21
Hypersensitivity reactions	14 (20.6)	10 (14.7)	0.37
Renal colic	1 (1.5)	0	0.32
Paresthesia	11 (16.2)	4 (5.9)	0.06
Myalgia	2 (2.9)	4 (5.9)	0.40
Fatigue	17 (25.0)	10 (14.7)	0.13
Arthralgia	3 (4.4)	6 (8.8)	0.30

* All adverse events were mild to moderate (category 1 or 2 on a 4-point scale).

The repeated-measures analysis confirmed these results (Table 2) and found that the number of visits in which women had detectable genital HIV-1 RNA was significantly lower in the valacyclovir group (risk ratio, 0.77; 95% CI, 0.64 to 0.93; $P=0.006$). Further analyses showed an increase in treatment effect over time for both the presence of detectable genital HIV-1 RNA (risk ratio for every 2-week period during treatment phase, 0.93; 95% CI, 0.88 to 0.97; $P=0.001$) and the quantity of genital HIV-1 RNA (average reduction in mean quantity every 2 weeks during the treatment phase, 0.08 \log_{10} copy per milliliter; 95% CI, 0.04 to 0.11; $P<0.001$) (Fig. 2).

Plasma HIV-1 RNA

The mean quantity of plasma HIV-1 RNA among women in the valacyclovir group was significantly lower than in the placebo group ($-0.53 \log_{10}$ copy per milliliter; 95% CI, -0.72 to -0.35 , adjusted for mean baseline-phase value; $P<0.001$). A similar result was found with the use of a repeated-measures analysis (Table 2). In addition, the treatment effect on plasma HIV-1 RNA increased with time ($P<0.001$), with an average reduction of 0.09 \log_{10} copy per milliliter every 2 weeks (95% CI, 0.05 to 0.12; $P<0.001$).

Genital HSV-2 DNA

As expected, valacyclovir was highly effective in lowering the detection and frequency of genital HSV-2 DNA. Significantly fewer women in the valacyclovir group shed HSV-2 at all during the treatment phase: 19.1% in the valacyclovir group vs. 54.4% in the placebo group (risk ratio, 0.35; 95% CI, 0.21 to 0.60; $P<0.001$). In addition, the frequency of detection of genital HSV-2 DNA was strongly reduced in the valacyclovir group (odds ratio, 0.17; 95% CI, 0.07 to 0.38; $P<0.001$) (Table 2). Results were confirmed by repeated-measures analysis (Table 2). In a similar way, the proportion of women with at least one genital ulcer declined from baseline in the valacyclovir group, from 29.4% in the baseline phase to 4.4% in the treatment phase, as compared with no change in the placebo group (risk ratio, 0.16; 95% CI, 0.05 to 0.49; $P=0.002$).

DISCUSSION

Daily treatment with valacyclovir for 3 months significantly diminished the shedding of HIV-1 RNA, reduced plasma HIV-1 RNA levels, and reduced genital HIV-1 RNA levels when shedding was present in women dually infected with HIV-1 and HSV-2. This effect steadily increased over time, which suggested that a longer duration of treatment might have led to an even greater reduction in HIV-1 RNA levels. These findings should be verified through clinical trials of longer duration. The effect of acyclovir on plasma HIV-1 RNA has been observed previously among 12 patients who were seropositive for HSV-2 and HIV-1.¹⁸ Acyclovir is unlikely to have a direct pharmacologic effect on HIV-1,³⁰ and hence the most plausible explanation is that HSV suppressive therapy prevents clinical and subclinical reactivations responsible for an increased HIV-1 viral load. Indeed, HSV reactivation generates an influx of activated CD4+ T lymphocytes³¹ and significantly up-regulates HIV-1 replication.^{14-16,32} Transient HIV-1 systemic reactivation has been demonstrated during clinical HSV-2 episodes.^{17,18} In addition, subclinical HSV-2 episodes have been associated with mucosal disruption and lymphocytic infiltration similar to that found in clinical genital herpetic lesions,³³ which suggests that asymptomatic HSV-2 shedding may act similarly and maintain HIV-1 replication at a high level. Data from our baseline phase support this hypothesis,

with higher quantities of genital and plasma HIV-1 RNA among women shedding HSV-2.

Plasma HIV-1 RNA is the strongest predictor of genital HIV-1 RNA,³⁴ although some compartmentalization of HIV-1 shedding may exist at the genital level.³⁴⁻³⁶ Thus, the effect of HSV suppressive therapy on HIV-1 shedding may simply be a consequence of a reduced systemic viral load. A complementary explanation would be that direct viral interaction occurs at the genital level. A trial of similar design conducted among women whose disease characteristics qualified them for HAART showed that valacyclovir could have a further effect on the residual shedding of HIV-1 despite good systemic control, which supports an effect of HSV-2 on independent mucosal HIV-1 replication.³⁷

As expected, suppressive therapy with 500 mg of valacyclovir twice daily among women with HIV-1 infection proved very effective in reducing genital ulcerations and genital HSV-2 DNA. The effect on clinical episodes was similar to that reported in previous suppressive-treatment trials among coinfecting women.^{38,39}

Our study had several strengths, including a high level of compliance with study procedures and study drugs. Moreover, repeated-measures analysis of viral load allowed for adjustment for within-person variability of HSV-2 and HIV-1 shedding, as seen during the baseline phase.

Although our trial had no HIV clinical outcomes, its results may be relevant to HIV-1 prevention and management. It is estimated that a large proportion of persons who are seropositive for HIV-1 are also HSV-2 seropositive.^{7,40} The strong and significant reduction in plasma and genital HIV-1 RNA levels associated with valacyclovir treatment suggests that sustained forms of HSV-2 control (either antiviral therapy or effective vaccination) may reduce HIV-1 transmission, assuming that a reduction in genital and plasma HIV-1 RNA levels is a proxy for decreased transmissibility.^{19,41} This finding could be especially relevant among populations likely to play an important role in the dynamics of HIV transmission and among couples with discordant HIV status.¹⁹ At the individual level, the reduction in plasma HIV-1 RNA levels (with the likely reduction in CD4+ activation, which is responsible for T-lymphocyte depletion⁴²) may lead to immunologic benefit over a longer duration of valacyclovir treatment, thereby slowing the course of HIV-1

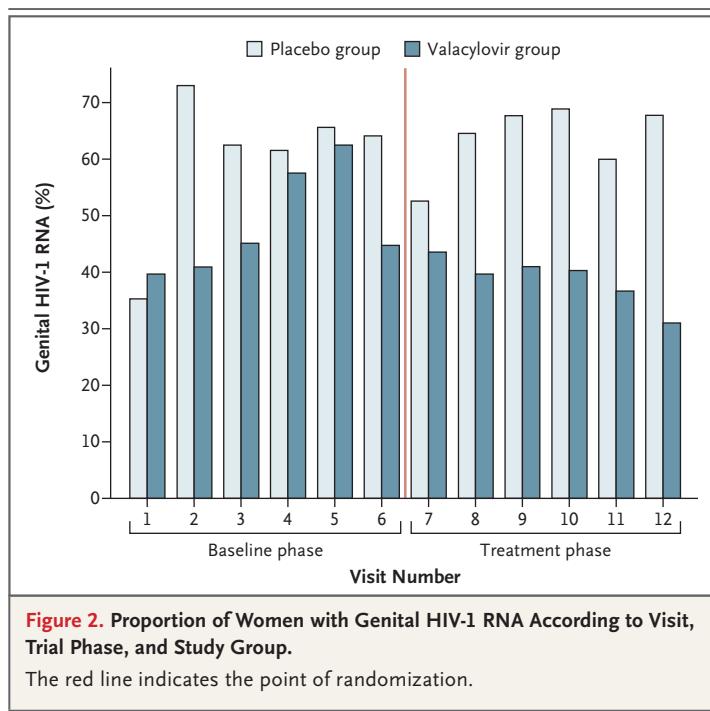


Figure 2. Proportion of Women with Genital HIV-1 RNA According to Visit, Trial Phase, and Study Group.

The red line indicates the point of randomization.

disease. In addition to individual benefit regarding clinical recurrence, HSV suppressive therapy could also have an effect on HSV-2 transmission.⁴³

The link between HSV-2 and HIV-1 should stimulate the development and evaluation of HSV-2 control methods. Several ongoing randomized, controlled trials will provide further evidence of the effect of HSV suppressive therapy on HIV-1 transmission and acquisition.¹⁹ Other trials are planned to evaluate the individual benefits, using immunologic and clinical outcomes. In particular, research to develop an HSV vaccine should rank high on the international research agenda for the prevention of HIV and HSV-2. Such a vaccine would represent a long-lasting form of HSV-2 control and possibly an important tool in HIV prevention.

Supported by the French Agence Nationale de Recherches sur le SIDA (ANRS, contract 2003-149).

Dr. Mayaud reports receiving research support from Glaxo-SmithKline for other projects. No other potential conflict of interest relevant to this article was reported.

This article is dedicated to the memory of Laurence Vergne, who died tragically in January 2007.

We thank the women and the organizations for persons living with HIV/AIDS who participated in this study; the staff from Service d'Hygiène of Bobo-Dioulasso; Brigitte Bazin, Jean-François Delfraissy, and Michel Kazatchkine (of ANRS) for their constant support and encouragement; members of the data and safety monitoring committee, including chair Prof. Simon Cousens of

the London School of Hygiene and Tropical Medicine, Prof. Adama Traoré of Centre Hospitalier Universitaire Ouagadougou in Burkina Faso, and Prof. Jean-Marie Hureau of Hôpital Pitié-Salpêtrière in Paris; Rodolphe Thiebaut of INSERM E0338 Biostatistics

in Bordeaux, France, and Profs. David Mabey and Richard Hayes of the London School of Hygiene and Tropical Medicine for their review of the manuscript; and the United Kingdom Department for International Development.

APPENDIX

Investigators in the ANRS 1285 study group were as follows: *Centre Muraz, Bobo-Dioulasso, Burkina Faso* — E. Bahemba, A. Berthé, M. Coulibaly, M.-C. Defer, R. Diallo, D. Djagbaré, I. Konate, F. Ky-Dama, G.T. M'Boutiki, N. Meda, I. Millogo, N. Nagot, A. Ouédraogo, D. Ouedraogo, F. Rouet, A. Sanon, H. Sawadogo, R. Vallo, L. Vergne; *London School of Hygiene and Tropical Medicine, London* — P. Mayaud, N. Nagot, H.A. Weiss; *Montpellier University Hospital and Research Unit 145, Institute for Research and Development and University of Montpellier 1, Montpellier, France* — P. Becquart, V. Foulongne, M. Segondy, P. Van de Perre; *University Hospital of Bobo-Dioulasso, Burkina Faso* — J.-B. Andonaba, A. Sawadogo.

REFERENCES

- Weiss H. Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes* 2004;11:Suppl 1:24A-35A.
- Malkin JE. Epidemiology of genital herpes simplex virus infection in developed countries. *Herpes* 2004;11:Suppl 1:2A-23A.
- Corey L, Wald A, Celum CL, Quinn TC. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *J Acquir Immune Defic Syndr* 2004;35:435-45.
- Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. *J Infect Dis* 2002;185:45-52.
- Herpes simplex virus type 2: programmatic and research priorities in developing countries. Report of a WHO/UNAIDS/LSHTM workshop (London, 14-16 February 2001). London: WHO/UNAIDS, 2001.
- Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* 2006;20:73-83.
- Mbopi-Keou FX, Gresenguet 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.
- McClelland RS, Wang CC, Overbaugh J, et al. Association between cervical shedding of herpes simplex virus and HIV-1. *AIDS* 2002;16:2425-30.
- 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.
- Cowan FF, Pascoe SJ, Barlow KL, et al. Association of genital shedding of herpes simplex virus type 2 and HIV-1 among sex workers in rural Zimbabwe. *AIDS* 2006;20:261-7.
- 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.
- Celum CL. The interaction between herpes simplex virus and human immunodeficiency virus. *Herpes* 2004;11:Suppl 1:36A-45A.
- Koelle DM, Liu Z, McClurkan CM, et al. Expression of cutaneous lymphocyte-associated antigen by CD8(+) T cells specific for a skin-tropic virus. *J Clin Invest* 2002;110:537-48.
- Mosca JD, Bednarik DP, Raj NB, et al. Activation of human immunodeficiency virus by herpesvirus infection: identification of a region within the long terminal repeat that responds to a trans-acting factor encoded by herpes simplex virus 1. *Proc Natl Acad Sci U S A* 1987;84:7408-12.
- Margolis DM, Rabson AB, Straus SE, Ostrove JM. Transactivation of the HIV-1 LTR by HSV-1 immediate-early genes. *Virology* 1992;186:788-91.
- Golden MP, Kim S, Hammer SM, et al. Activation of human immunodeficiency virus by herpes simplex virus. *J Infect Dis* 1992;166:494-9.
- 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.
- 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.
- Celum CL, Robinson NJ, Cohen MS. Potential effect of HIV type 1 antiretroviral and herpes simplex virus type 2 antiviral therapy on transmission and acquisition of HIV type 1 infection. *J Infect Dis* 2005;191:Suppl 1:S107-S114.
- Safe and effective use of antiretroviral treatments in adults with particular reference to resource limited settings. Geneva: World Health Organization, 2000.
- Nagot N, Ouedraogo A, Ouangre A, et al. Is sexually transmitted infection management among sex workers still able to mitigate the spread of HIV infection in West Africa? *J Acquir Immune Defic Syndr* 2005;39:454-8.
- Nagot N, Foulongne V, Becquart P, et al. Longitudinal assessment of HIV-1 and HSV-2 shedding in the genital tract of West African women. *J Acquir Immune Defic Syndr* 2005;39:632-4.
- Joint United Nations Programme on HIV/AIDS (UNAIDS)-WHO. Revised recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec* 1997;72:81-7.
- van Dyck E, Buve A, Weiss HA, et al. Performance of commercially available enzyme immunoassays for detection of antibodies against herpes simplex virus type 2 in African populations. *J Clin Microbiol* 2004;42:2961-5.
- Rouet F, Ekouevi DK, Chaix ML, et al. Transfer and evaluation of an automated, low-cost real-time reverse transcription-PCR test for diagnosis and monitoring of human immunodeficiency virus type 1 infection in a West African resource-limited setting. *J Clin Microbiol* 2005;43:2709-17.
- Legoff J, Bouhhal H, Gresenguet G, et al. Real-time PCR quantification of genital shedding of herpes simplex virus (HSV) and human immunodeficiency virus (HIV) in women coinfecting with HSV and HIV. *J Clin Microbiol* 2006;44:423-32.
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol* 1991;29:297-301.
- Coombs RW, Reichelderfer PS, Landay AL. Recent observations on HIV type-1 infection in the genital tract of men and women. *AIDS* 2003;17:455-80.
- Zou G. A modified Poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;159:702-6.
- Collier AC, Schoenfeld D, Hirsch M, et al. Lack of effect on survival of high-dose acyclovir and zidovudine compared with zidovudine alone for acquired immunodeficiency syndrome. *Antiviral Ther* 1998;3:25-32.
- Cunningham AL, Turner RR, Miller AC, Para ME, Merigan TC. Evolution of recurrent herpes simplex lesions: an immunohistologic study. *J Clin Invest* 1985;75:226-33.
- Fauci AS. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* 1988;239:617-22.

33. Patel R, Barton SE. Antiviral chemotherapy in genital herpes simplex virus infections. *Int J STD AIDS* 1995;6:320-8.
34. Kovacs A, Wasserman SS, Burns D, et al. Determinants of HIV-1 shedding in the genital tract of women. *Lancet* 2001;358:1593-601. [Erratum, *Lancet* 2002;359:630.]
35. Philpott S, Burger H, Tsoukas C, et al. Human immunodeficiency virus type 1 genomic RNA sequences in the female genital tract and blood: compartmentalization and intrapatient recombination. *J Virol* 2005;79:353-63.
36. Kemal KS, Foley B, Burger H, et al. HIV-1 in genital tract and plasma of women: compartmentalization of viral sequences, coreceptor usage, and glycosylation. *Proc Natl Acad Sci U S A* 2003;100:12972-7.
37. Ouedraogo A, Nagot N, Vergne L, et al. Impact of suppressive herpes therapy on genital HIV-1 RNA among women taking antiretroviral therapy: a randomized controlled trial. *AIDS* 2006;20:2305-13.
38. DeJesus E, Wald A, Warren T, et al. Valacyclovir for the suppression of recurrent genital herpes in human immunodeficiency virus-infected subjects. *J Infect Dis* 2003;188:1009-16. [Erratum, *J Infect Dis* 2003;188:1404.]
39. Schacker T, Hu HL, Koelle DM, et al. Famciclovir for the suppression of symptomatic and asymptomatic herpes simplex virus reactivation in HIV-infected persons: a double-blind, placebo-controlled trial. *Ann Intern Med* 1998;128:21-8.
40. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J Infect Dis* 2002;186:Suppl 1:S3-S28.
41. 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.
42. Grossman Z, Meier-Schellersheim M, Sousa AE, Victorino RM, Paul WE. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat Med* 2002;8:319-23.
43. Corey L, Wald A, Patel R, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. *N Engl J Med* 2004;350:11-20.

Copyright © 2007 Massachusetts Medical Society.