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High-dose Valacyclovir Decreases Plasma HIV-1 RNA More Than Standard-dose Acyclovir in HIV-1, HSV-2 Positive Persons: a Randomized, Crossover Trial

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Abstract

Background—Standard doses of HSV suppressive therapy reduce plasma HIV-1 RNA levels (0.25–0.53 log₁₀ copies/mL) among HIV-1/HSV-2 co-infected persons. Postulated mechanisms for this effect include direct inhibition of HIV-1 by acyclovir or indirect reduction by decreasing HSV-associated inflammation. We hypothesized that high-dose valacyclovir would further reduce plasma HIV-1 RNA, and that the effect would be mediated by greater suppression of HSV shedding.

Methods—34 participants with HIV-1 and HSV-2 not on antiretroviral therapy were enrolled into a randomized, open-label cross-over trial of valacyclovir 1000 mg twice daily or acyclovir 400 mg twice daily for 12 weeks, followed by a two week washout, and then the alternate

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treatment arm for 12 weeks. HSV DNA was measured from daily self-collected genital swabs for the initial 4 weeks of each arm and HIV-1 RNA was quantified from weekly plasma samples.

Results—28 participants provided plasma samples and genital swabs on both acyclovir and valacyclovir. The genital HSV-2 shedding rate was the same on valacyclovir and acyclovir (7.8% vs. 8.2% of days; RR, 0.95; 95% CI, 0.66-1.37; $p=0.78$). Plasma HIV-1 RNA was 0.27 \log_{10} copies/mL lower on valacyclovir compared with acyclovir (95% CI, -0.41 to -0.14 \log_{10} copies/mL; $p<0.001$); this was unchanged after adjustment for genital HSV-2 shedding.

Conclusions—High-dose valacyclovir reduces plasma HIV-1 RNA levels more than standard-dose acyclovir in HIV-1/HSV-2 seropositive persons not receiving antiretroviral therapy. The incremental reduction in plasma HIV-1 RNA achieved is not mediated by greater genital HSV-2 suppression.

Keywords

HIV; viral load; HSV; valacyclovir; acyclovir

Introduction

Decreasing plasma HIV-1 RNA in infected persons can prevent transmission to susceptible partners.¹⁻⁶ However, at the end of 2010, it is estimated that less than half of those who met WHO guidelines for initiation of antiretroviral therapy (ART) were receiving antiretroviral medications.⁷ While the gap between the goal of universal access and the realities of the current levels of antiretroviral coverage exists, alternative strategies to decrease plasma HIV-1 RNA- with the goal of reducing transmission and delaying HIV-1 disease progression- will remain important.

Standard-dose HSV suppressive therapy (acyclovir 400 mg or valacyclovir 500 mg twice daily) among persons coinfecting with HIV-1 and HSV-2 reduced plasma HIV-1 RNA levels by 0.25-0.53 \log_{10} copies/mL in several randomized controlled trials⁸⁻¹⁵ and acyclovir modestly slows HIV-1 progression in patients not receiving ART.^{16,17} Postulated mechanisms by which anti-HSV therapy lowers plasma HIV-1 RNA levels include decreasing pro-inflammatory cytokines,¹⁸ decreasing HIV-1 transcriptional activation by HSV-encoded proteins,^{18,19} and direct inhibition of HIV-1 reverse transcriptase.^{20,21} In one study, high-dose valacyclovir (1.5 g twice daily) was associated with a decline in plasma HIV-1 RNA by 1.23 \log_{10} copies/mL,²² suggesting a dose-response relationship. To investigate whether the reduction in plasma HIV-1 RNA with increasing doses of anti-HSV therapy is mediated by greater HSV suppression, we conducted a randomized, open-label cross-over trial of high-dose valacyclovir compared with standard-dose acyclovir in HSV-2/HIV-1 coinfecting persons in which we measured the effect of therapy on both plasma HIV-1 RNA and genital HSV shedding.

Methods

Study Population

We recruited HIV-1/HSV-2 coinfecting patients in Seattle, WA, between January 2008 and June 2010. Inclusion criteria were an age ≥ 18 years, detectable plasma HIV-1 RNA, the intention to remain in the area, and no current or planned initiation of ART during the study. Exclusion criteria were prior adverse reaction to acyclovir, valacyclovir, or famciclovir, current or planned open-label use of antivirals with anti-HSV activity, including ganciclovir, foscarnet, and cidofovir, active or prior CMV disease, history of seizures, creatinine >1.5 mg/dL, serum transaminases >3 times the upper limit of normal, hematocrit $<30\%$, absolute

neutrophil count (ANC) <1000/ μ L, platelet count <75,000/ μ L, history of thrombotic microangiopathy, and pregnancy.

Study Design

Participants underwent randomization in a 1:1 ratio to receive open-label acyclovir 400 mg twice daily or valacyclovir 1000 mg twice daily. The randomization sequence, developed by the study statistician (AM), was implemented using sealed study kits. After 12 weeks on therapy, participants underwent a two week washout period and then crossed over to the alternative treatment arm for 12 weeks.

Clinical and Laboratory Procedures

At the screening visit, HSV-2 seropositivity was determined by Western blot.²³ HIV-1 Western blot, plasma HIV-1 RNA PCR, CD4 count, and routine clinical safety labs were also performed. Testing for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was performed by culture of rectal swabs from men and by nucleic acid amplification testing of urine samples from men and cervical swabs from women; participants who tested positive were treated and became eligible for enrollment 14 days after completing therapy.

Participants provided daily self-collected genital and perianal swabs for HSV PCR using previously described methods,^{24,25} and maintained a diary of genital symptoms for the first four weeks of each treatment period.²⁶ DNA from 200 μ l of PCR buffer surrounding each swab was extracted with a QIAamp 96 DNA Blood Kit (Qiagen) following the manufacturer's recommendations. HSV gB specific Taqman quantitative real-time PCR was performed using QuantiTect multiplex PCR master mix (Qiagen) on a 7900HT sequencing detection system. The sequences of HSV gB primers and probe were previously described.²⁷ An internal control was included in each reaction to monitor for false negative results due to inhibition of the PCR assay.²⁸ We reported as positive those samples in which 150 copies of HSV DNA/mL were detected.²⁹ Weekly plasma samples were collected for HIV-1 RNA quantification by a TaqMan RT-PCR assay.³⁰ The lower limit of quantification was 50 copies of HIV-1 RNA/mL. Participants who, on screening, had detectable plasma HIV-1 RNA below the limit of quantification were retained in the analysis. Plasma CMV DNA was also quantified by a CMV-specific TaqMan PCR assay; we reported samples in which 50 copies of CMV DNA/mL were detected as positive.²⁸ Laboratory staff were not aware of treatment assignments. At each visit we assessed symptoms of genital herpes reactivation, medication tolerance, and adherence by pill counts. At the end of each study arm, participants' creatinine, CD4 count, and complete blood count were measured. Throughout the study, condoms, risk reduction, and adherence counseling were provided.

To more precisely assess the kinetics of plasma HIV-1 RNA decline after initiation of valacyclovir, beginning in April 2010, we invited participants, including those who already completed the study, to participate in a substudy which measured plasma HIV-1 RNA one day prior to, at initiation, and at 6, 24, 48, and 72 hours after initiating valacyclovir.

Sample Size Calculation and Statistical Analysis

We estimated that a sample size of 29 participants would be required to detect a 0.25 log₁₀ copies/mL difference^{10,11} in plasma HIV-1 RNA between the study arms and a sample size of 38 participants, with four weeks of daily genital swabs per treatment arm, would be required to detect a decrease in the HSV shedding rate from 4%, the rate expected on standard suppressive therapy,¹³ to 1%, with 80% power, at a two-sided type I error rate of 5%. We have recently validated a more accurate method of sample size computation for crossover shedding studies.³¹ This rigorously validated calculation, which accounts for

person-to-person variability in shedding over time, required 26 participants to detect a 50% reduction in genital HSV shedding.

The co-primary endpoints of the study were the plasma HIV-1 RNA level and genital HSV shedding rate during high-dose valacyclovir compared with standard-dose acyclovir administration. Secondary endpoints included days with genital herpes lesions and the quantity of HSV-2 DNA from genital swabs during shedding episodes on each arm. Plasma HIV-1 RNA and genital HSV DNA levels were \log_{10} transformed to stabilize variance. The genital shedding rate was defined as the number of swabs with HSV divided by the number of swabs collected. A genital shedding episode was defined as a consecutive series of positive genital swabs separated by at least 2 negative swabs. Analyses were modified intent-to-treat, including those participants who contributed at least one specimen on both arms. The first day of treatment and all washout days were excluded from analysis. Participants who initiated ART were censored at that time.

Linear mixed models were used to compare plasma HIV-1 RNA and HSV-2 DNA quantity from genital swabs by treatment arm. We explored whether the effect of valacyclovir compared with acyclovir on plasma HIV-1 RNA levels was modified, in separate models, by baseline plasma HIV-1 RNA level, CD4 count, or detection of CMV viremia by testing the significance of interaction terms. Genital HSV-2 shedding rates were compared by treatment arm using random effects poisson regression. The Wilcoxon signed-rank test was used to compare the total number of genital shedding episodes, days with genital HSV-2 lesions, and drug adherence rate by treatment arm. Cox regression analysis, accounting for multiple episodes per participant,³² was used to compare episode duration by treatment arm. Period effects on plasma HIV-1 RNA and genital HSV-2 shedding rates were assessed by testing the significance of session adjusted for treatment arm. Carryover effects were evaluated by testing the interaction of treatment and session. Correlation of drug adherence on each treatment arm with change in mean plasma HIV-1 RNA levels from baseline and genital HSV-2 shedding rates was evaluated with Spearman correlation coefficients.

First-phase viral decay slopes were determined for substudy participants, for whom plasma HIV-1 RNA levels were assessed more frequently over the first three days of valacyclovir treatment. Previous studies of HIV-1 viral decay after antiretroviral administration have observed an initial static phase prior to the exponential decline in the first phase of viral decay.³³⁻³⁵ Viral decay slopes, determined by linear regression using robust variance estimates in clusters, consequently included data points beginning at 24 hours.

The protocol was approved by the University of Washington Human Subjects Review Committee and participants gave written informed consent. The study was funded by a grant to the University of Washington from GlaxoSmithKline; additional funding came from grants from the National Institutes of Health. The University of Washington investigators wrote the protocol, conducted the study, analyzed the data, and prepared the manuscript for publication.

Results

Of forty-nine persons screened, fifteen were found to be ineligible for reasons including lack of HSV-2 infection (n=5), plans to initiate ART (n=3), and undetectable plasma HIV-1 RNA (n=3). Of the thirty-four persons enrolled, three (8.8%) participants were treated for Chlamydia, including one participant who was treated for both Chlamydia and Gonorrhea, prior to enrollment. Six participants did not contribute to both arms of the study. Thus, the final analysis set included twenty-eight participants, of whom twelve (43%) initiated acyclovir and sixteen (57%) initiated valacyclovir first (Figure 1). The mean age of

participants was 44 (range, 24-46); eighteen (64%) were Caucasian and twenty-three (82%) were men (Table 1). Overall, from participants during treatment with acyclovir or valacyclovir, we obtained and analyzed 594 plasma samples for HIV-1 RNA quantification, for 570 of which we were also able to measure CMV DNA, and 1425 genital swabs for HSV DNA quantification.

Effect of Study Drug on Plasma HIV-1 RNA

Twenty-seven participants had plasma HIV-1 RNA levels available for analysis, as samples for one participant were persistently inhibited. Of these participants, twenty-three (85%) had a mean plasma HIV-1 RNA level lower on valacyclovir than acyclovir (Figure 2 and 3). Plasma HIV-1 RNA during treatment was 0.27 log₁₀ copies/mL lower (95% CI, -0.41 to -0.14 log₁₀ copies/mL; p<0.001) on valacyclovir compared with acyclovir (Table 2). Sixteen (59%) of twenty-seven participants on valacyclovir and five (20%) of twenty-five participants on acyclovir achieved a decrease in mean plasma HIV-1 RNA by 0.25 log₁₀ copies/mL relative to baseline. No association between genital HSV shedding and plasma HIV-1 RNA was detected. When the genital HSV shedding rate on drug was included in the model, there was no change in the results: plasma HIV-1 RNA during treatment was 0.27 log₁₀ copies/mL lower on valacyclovir compared with acyclovir (95% CI, -0.41 to -0.14 log₁₀ copies/mL; p<0.001). We found no evidence that the baseline HIV-1 RNA level, CD4 count, or detection of CMV viremia modified the effect of valacyclovir compared with acyclovir on plasma HIV-1 RNA (Table 2). Because the mean plasma HIV-1 RNA was higher in participants who started in the valacyclovir arm, evaluation for period effect was adjusted for baseline plasma HIV-1 RNA on each drug arm; no period (p=0.27) or carry-over (p=0.39) effects were found. One participant received interferon and ribavirin during the acyclovir arm for Hepatitis C treatment. When this participant was excluded from the analysis, plasma HIV-1 RNA during treatment was 0.31 log₁₀ copies/mL lower (95% CI, -0.43 to -0.19 log₁₀ copies/mL; p<0.001) on valacyclovir compared with acyclovir. Two participants in the analysis had plasma HIV-1 RNA below the limit of quantification (<50 copies/ml); when these participants were excluded the results were similar.

Effect of Study Drug on Genital HSV Shedding

Twenty-eight participants collected swabs of genital skin and mucosa during both study arms. The HSV shedding rate did not differ between study arms: HSV was detected on 7.8% of days (55 of 707) on valacyclovir and 8.2% of days (59 of 718) on acyclovir (RR, 0.95; 95% CI, 0.66-1.37; p=0.78). There were 19 HSV shedding episodes on acyclovir and 18 on valacyclovir. No significant difference was seen in episode duration by treatment arm (p=0.68). The median quantity of HSV-2 DNA for positive samples was 3.0 log₁₀ copies/mL on both arms (range, acyclovir, 2.2 to 6.4, valacyclovir, 2.2 to 6.8 log₁₀ copies/mL; p=0.67). Genital lesions were present on 7 (1.0%) of 772 days, occurring in only one participant during the valacyclovir arm, compared with 27 (4.0%) of 750 days, occurring in two participants during the acyclovir arm (p=0.16). 93% of participants did not report any genital lesion throughout the study and 94% of all shedding episodes were asymptomatic. There was a significant period effect such that rates of HSV-2 shedding were 76% (range, 60 - 85%) lower during the second treatment arm compared to the first treatment arm regardless of treatment given (p<0.001). There was no significant drug carry-over (p=0.58). When the participant receiving interferon and ribavirin was excluded from analysis, we still found no significant difference in the rate of HSV detection on valacyclovir compared with acyclovir (RR: 0.82; 95% CI, 0.55-1.23; p=0.33).

Effect of Study Drug on Plasma CMV DNA

CMV DNA was detected in six plasma samples from five participants during treatment with acyclovir and six samples from two participants during treatment with valacyclovir. The

mean quantity of CMV DNA for positive samples was 2.00 log₁₀ copies/mL during acyclovir and 1.99 log₁₀ copies/mL during valacyclovir treatment.

Valacyclovir Substudy

Ten participants completed the substudy, during which plasma was collected for HIV-1 RNA more frequently for the first 72 hours after valacyclovir initiation. Two participants had plasma HIV-1 RNA <50 copies/mL at the time of valacyclovir initiation and were excluded from analysis. The remaining eight participants were all men. The mean age was 47 years (range, 29-66 years). At enrollment, the mean CD4 count was 410 cells/μL (range, 242-714 cells/μL) and the mean plasma HIV-1 RNA level was 4.10 log₁₀ copies/mL (range, 3.57 to 4.97 log₁₀ copies/mL). Seven of the eight participants experienced a decline in plasma HIV-1 RNA between 24 and 72 hours (Figure 4; see Supplemental Digital Content). On average, plasma HIV-1 RNA decreased by -0.20 log₁₀ copies/mL/day (95% CI, -0.38 to -0.19 log₁₀ copies/mL/day).

Adherence to Study Drug

The median adherence rate was 93% on valacyclovir (range, 34-100%) and 96% on acyclovir (range, 75-100%; p=0.23). We did not find a correlation between drug adherence and genital HSV shedding on valacyclovir (r=0.02) or acyclovir (r=-0.16), nor between adherence and the change in mean plasma HIV-1 RNA from baseline on valacyclovir (r = -0.10) or acyclovir (r = 0.06). One participant took additional doses of acyclovir for three days during the acyclovir arm. Two participants used valacyclovir during the acyclovir arm, one for twelve days and the other for nine days. Both acyclovir and valacyclovir were taken by one participant during the washout period, stopping the day before starting the valacyclovir arm. All participants were included in the analysis according to their assigned treatment group.

Adverse Reactions

One participant developed neutropenia during the acyclovir arm, with a nadir ANC of 570 cells/μL, thought to be related to concomitant interferon use. With cessation of interferon therapy, the ANC normalized and remained normal during 12 weeks of high-dose valacyclovir. One participant developed urticaria after 2.5 weeks of valacyclovir and was discontinued from the study; this participant was not included in the analysis since he did not participate in the acyclovir arm. Two participants with depression developed exacerbations during the valacyclovir arm, one of whom was hospitalized for two weeks and did not follow-up during this period. Both participants were included in the analysis.

Discussion

In our study, valacyclovir, dosed at 1000 mg twice daily, reduced plasma HIV-1 RNA levels by 0.27 log₁₀ copies/mL more than standard-dose acyclovir (400 mg twice daily) in HIV-1/HSV-2 seropositive persons not on ART. We did not, however, find a difference in the rate of genital HSV shedding between the two arms and the decrease in plasma HIV-1 RNA was unchanged after adjusting for genital HSV reactivation, suggesting that the differential effect of high-dose valacyclovir on HIV-1 RNA is not mediated by greater HSV-2 suppression.

The absence of differential effect of high-dose valacyclovir compared with standard-dose acyclovir on genital HSV shedding contrasts with the suggestion of greater efficacy of valacyclovir 500 mg twice daily compared with standard-dose acyclovir in another study of HIV-1 infected patients.³⁶ It is consistent, however, with a prior study of immunocompetent hosts that showed no difference in HSV suppression by valacyclovir 500 mg twice daily compared with standard-dose acyclovir.²⁶

The median adherence rate was 93% during the valacyclovir arm and 96% during the acyclovir arm. Imperfect adherence would be expected to attenuate the effect size; the genital HSV shedding rate found in this study, however, was comparable to the rates of 4-14% found in prior studies of HIV-1/HSV-2 coinfecting persons treated with HSV suppressive therapy.^{13,37,38} No correlation was found between adherence and HSV shedding or reduction in plasma HIV-1 RNA.

Recent studies have raised the question of whether acyclovir directly inhibits HIV-1 replication. After phosphorylation of acyclovir by kinases of human herpesviruses (HHV)²⁰ and possibly non-HHV kinases,³⁹ and subsequent phosphorylation by cellular kinases, acyclovir triphosphate may competitively inhibit HIV-1 reverse transcriptase as a guanosine-5' triphosphate analog chain terminator.^{20,21} Previous studies of HSV suppressive therapy with standard-dose acyclovir and valacyclovir have demonstrated a similar decrease in plasma HIV-1 RNA (0.25-0.53 log₁₀ copies/mL) compared with the response seen with 24 weeks of AZT monotherapy.⁴⁰ If acyclovir is a reverse transcriptase inhibitor, the development of reverse transcriptase resistance mutations with acyclovir monotherapy would be expected. Such resistance has been seen in vitro with the development of V75I, T69N, and M184I reverse transcriptase mutations.²¹ These mutations have not been recognized in patients treated with prolonged administration of suppressive doses of acyclovir and valacyclovir in the absence of ART.^{41,42} The decrease in plasma HIV-1 RNA as a result of suppressive acyclovir therapy has also been sustained over 24 months,⁴¹ which would be unexpected with acyclovir monotherapy, or any other reverse transcriptase inhibitor when given alone. A potential explanation for this discrepancy is that, in vivo, the fitness cost associated with these resistance mutations may outweigh the survival advantage of acyclovir resistance when acyclovir is provided at standard suppressive doses.^{41,42}

The rate of decline of plasma HIV-1 RNA in the first week of treatment with high-dose valacyclovir is smaller than for a potent reverse transcriptase inhibitor such as tenofovir, where the first phase viral decay slope is 0.32-0.39 log₁₀ copies/mL/day when provided as monotherapy.³³ We also found considerable variability among participants in the decline in plasma HIV-1 RNA achieved with valacyclovir and acyclovir, in contrast to patients treated with antiretrovirals. Consequently, the mechanism of in vivo anti-HIV-1 effect has yet to be clarified.

A recent study found that HIV progression was delayed preferentially among patients receiving suppressive acyclovir with plasma HIV-1 RNA $\geq 50,000$ copies/mL.¹⁷ Although we did not find that baseline plasma HIV-1 RNA $\geq 50,000$ vs. $<50,000$ log₁₀ copies/mL modified the effect of valacyclovir compared with acyclovir on plasma HIV-1 RNA, inference is limited by the small number of participants in our study with plasma HIV-1 RNA $\geq 50,000$ log₁₀ copies/mL. If persons with a high plasma HIV-1 viral load do experience a greater decline in plasma HIV-1 RNA with anti-HSV therapy, it is possible that the relatively low mean baseline plasma HIV-1 RNA in this study (3.89 log₁₀ copies/mL compared with 4.0 to 4.6 log₁₀ copies/mL in other studies of the effect of anti-HSV therapy on plasma HIV-1 RNA)^{8-13,22,43} may have contributed to the decreased effect size observed in comparison to previous studies.⁸⁻¹³ As most previous trials occurred in developing countries, however, many other differences between the populations may impact the effect of anti-HSV therapy on plasma HIV-1 RNA.

Despite decreasing plasma HIV-1 RNA by 0.25 log₁₀ copies/mL, standard-dose acyclovir was not found to decrease HIV-1 transmission.¹⁰ We do not know the optimal dose of anti-HSV therapy to decrease plasma HIV-1 RNA, but it appears that there is a dose-response based on this study and the recent publication by Mugwanya et al.,²² where a 50% higher dose of valacyclovir (1.5 g twice daily) was associated with a 0.62 log₁₀ copies/mL

reduction in plasma HIV-1 RNA compared with standard-dose acyclovir. The higher dose of valacyclovir may be required for it to act as a more potent antiretroviral²¹ or for it to adequately suppress the effect of other herpesviruses on up-regulating HIV-1 transcription. We explored whether the difference in plasma HIV-1 RNA achieved with high-dose valacyclovir vs. acyclovir was greater among the participants in whom CMV viremia was detected compared to participants in whom it was not; the difference was not statistically significant. Inference is limited by the small number of participants with CMV viremia during the study and reliance on CMV viremia as a surrogate for CMV reactivation. To further investigate the potential role of CMV, plasma HIV-1 RNA on acyclovir and high-dose valacyclovir could be compared in CMV seronegative and CMV seropositive persons; but we acknowledge that enrollment into such a study would be challenging.

Whether the greater decline in HIV-1 RNA achieved by high-dose valacyclovir would translate into a more substantial delay of HIV progression than was observed with acyclovir,^{16,17} or would provide sufficiently potent reduction that could reduce HIV-1 transmission, is not known. Mathematical modeling has suggested that a decrease in mean plasma HIV-1 RNA by 0.74 log₁₀ copies/mL could decrease heterosexual HIV-1 transmission by 50%.⁴⁴ While the provision of ART to all HIV-positive persons irrespective of CD4 count is the ideal approach to reduce HIV-1 transmission since treatment decreases transmission by at least 96%,¹ at the end of 2010, it is estimated that less than half of those who met WHO guidelines for initiation of ART (CD4<350 cells/μL) were receiving antiretroviral medications⁷ and few national HIV treatment guidelines currently recommend universal ART.⁴⁵ Until we can successfully expand ART access, high-dose valacyclovir, if demonstrated to decrease HIV transmission by persons not yet eligible for ART, could offer a provisional means to decrease the number of new HIV infections. Future studies, in persons on ART with suppressed plasma HIV-1 RNA, could also assess whether high-dose anti-HSV therapy could reduce genital HIV-1 RNA shedding, which may be a potential transmission risk in persons with suppressed plasma viral load.⁴⁶

Our findings are limited by high loss to follow-up, which is especially problematic in cross-over studies and decreases power. HIV-1 infected patients with detectable plasma HIV-1 RNA are becoming increasingly difficult to enroll in studies in developed countries where antiretrovirals are available and prescribed early in the course of HIV-1 disease to decrease associated morbidity and mortality, prevent transmission to sexual partners, and reduce community viral load.⁴⁷ Since persons who remain viremic and not on treatment are often those with psychosocial comorbidities, such as serious mental illness, substance abuse, and poverty,⁴⁸⁻⁵⁰ maintaining high adherence to study protocol has become challenging in this population. As we collected swabs on a daily basis, we may have underestimated the frequency of shedding by missing episodes <24 hours in duration,²⁵ although this would be expected to similarly affect both arms. The cross-over design of this trial, with each participant serving as their own control, increased efficiency and decreased potential confounding. This design also mitigated potential ramifications of the period effect that we found for HSV-2 shedding rates, since in the second treatment period, each group received a different drug.

By simultaneous measurement of daily genital HSV DNA in each participant, this study demonstrates that the incremental reduction in plasma HIV-1 RNA achieved by high-dose valacyclovir compared with standard-dose acyclovir is not mediated by greater genital HSV suppression. Further research is needed to determine the characteristics that modify the effect of anti-HSV therapy on HIV-1 RNA and to determine the potential population-level impact of high-dose valacyclovir on HIV-1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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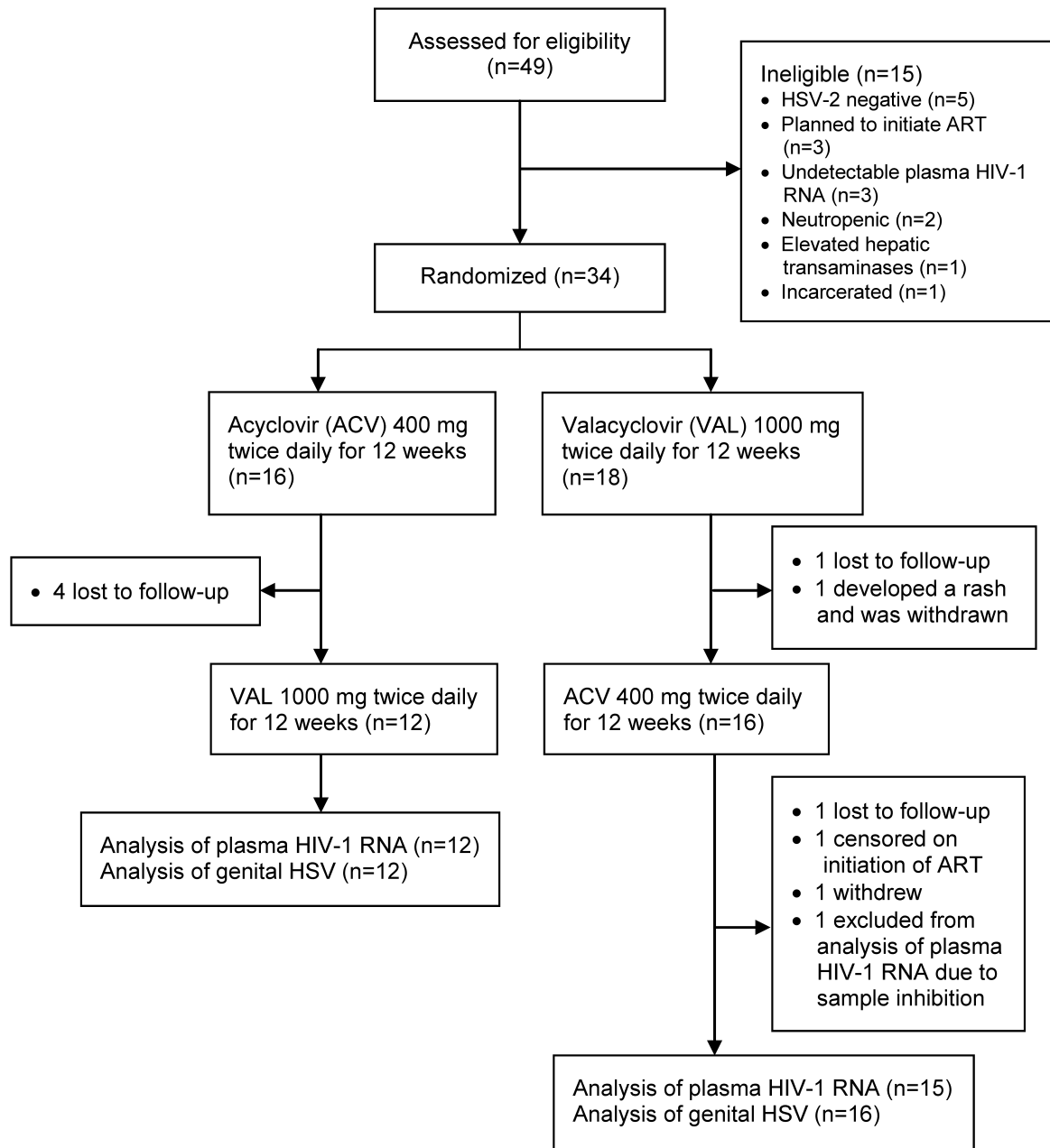


Figure 1. Enrollment and follow-up of participants. ART: Antiretroviral therapy.

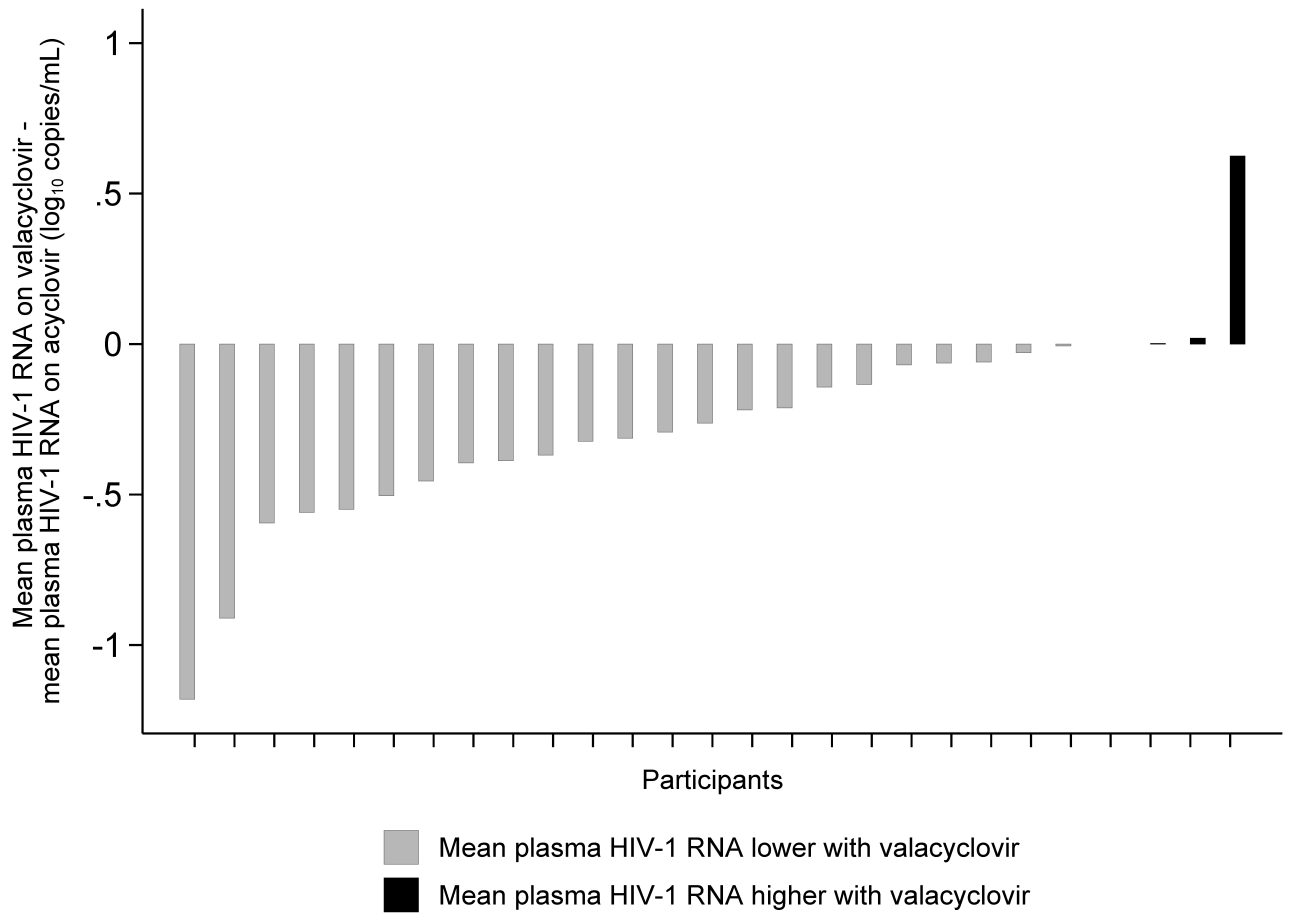


Figure 2. Difference in mean plasma HIV-1 RNA level between valacyclovir and acyclovir per participant, ordered by the magnitude of difference.

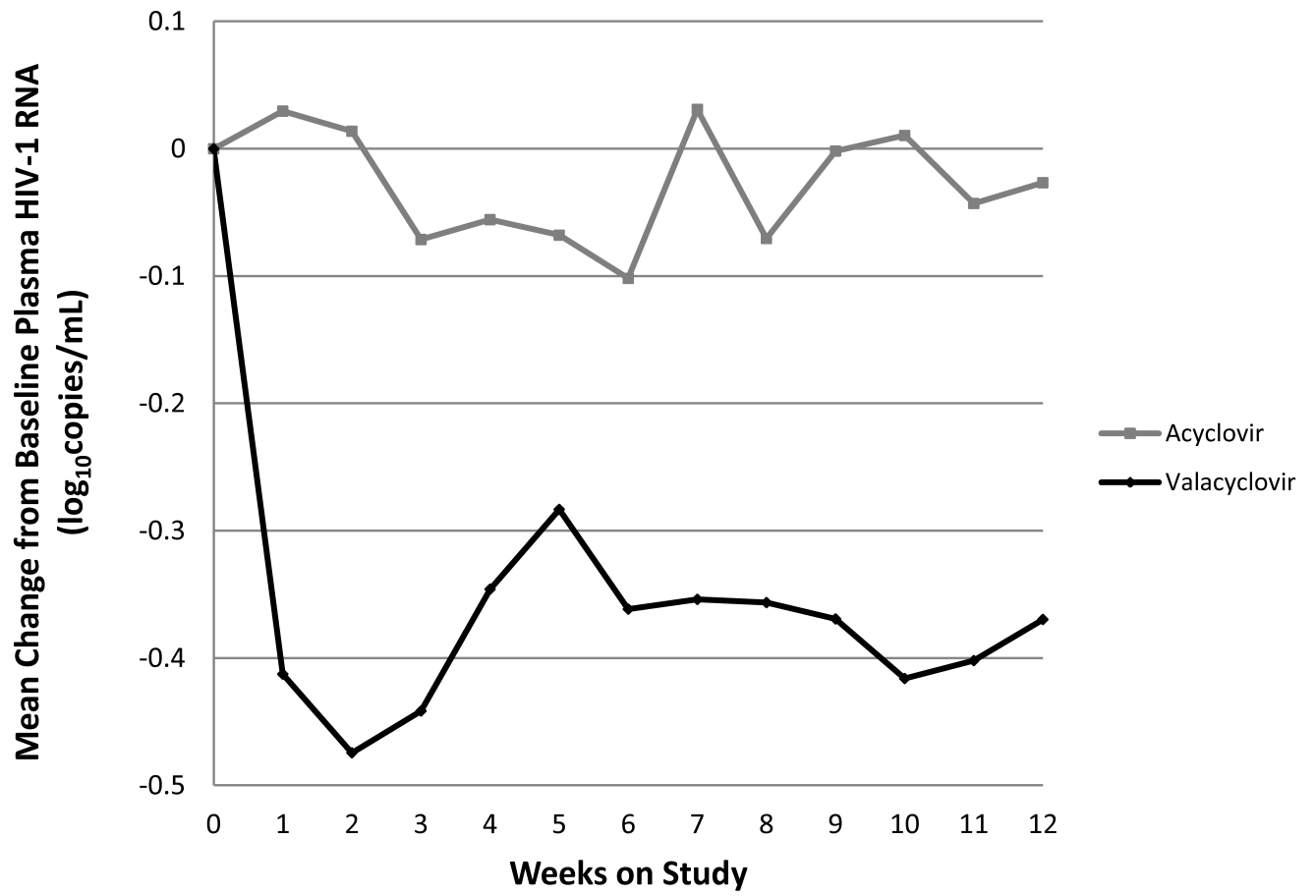


Figure 3. Mean change from baseline plasma HIV-1 RNA over time on valacyclovir compared with acyclovir.

Table 1

Demographic and clinical characteristics of the study population.

Characteristic	Acyclovir, followed by valacyclovir (N=12)	Valacyclovir, followed by acyclovir (N=16)	Total (N=28)
Age, mean (range), years	43 (24-66)	45 (29-66)	44 (24-66)
Sex, N (%)			
Male	8 (67)	15 (94)	23 (82)
Female	4 (33)	1 (6)	5 (18)
Race, N (%)			
Caucasian	8 (67)	10 (63)	18 (64)
African-American	3 (25)	5 (31)	8 (29)
Other	1 (8)	1 (6)	2 (7)
HSV Serology, N (%)			
HSV-2+	3 (25)	6 (37)	9 (32)
HSV-1/2+	9 (75)	10 (63)	19 (68)
CD4 Count at Screening, Mean (Range), cells/ μ L	627 (359-894)	424 (199-1055)	511 (199-1055)
Plasma HIV-1 RNA at Screening, Mean (Range), log ₁₀ copies/mL	3.3 (1.2-5.5)	4.3 (3.5-5.1)	3.9 (1.2-5.5)

Table 2

Difference in plasma HIV-1 RNA during treatment with valacyclovir and acyclovir.

	N	Plasma HIV-1 RNA during treatment with valacyclovir – plasma HIV-1 RNA during treatment with acyclovir (log ₁₀ copies/mL)	P- value
All participants	27	-0.27 (-0.41, -0.14)	<0.001
CD4 count (cells/μL)			0.75
<350	5	-0.24 (-0.56, 0.08)	
350-499	11	-0.23 (-0.44, -0.01)	
500	11	-0.35 (-0.79, 0.10)	
Baseline HIV-1 RNA (copies/mL)			0.80
<50,000	21	-0.26 (-0.42, -0.10)	
50,000	6	-0.30 (-0.57, -0.03)	
Any CMV viremia during study			0.34
No	22	-0.24 (-0.39, -0.09)	
Yes	5	-0.41 (-0.72, -0.09)	