# Valganciclovir for Suppression of Human Herpesvirus 8 Replication: A Randomized, Double-Blind, Placebo-Controlled, Crossover Trial

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## Abstract

**Background**—Human herpesvirus 8 (HHV-8) replication is critical in the induction and maintenance of Kaposi sarcoma, primary effusion lymphoma and some cases of Castleman disease. *In vitro* and observational studies suggest that ganciclovir inhibits HHV-8 replication, but no randomized clinical trials have been conducted.

**Methods**—26 HHV-8-infected men were randomized to receive 8 weeks of oral valganciclovir (900 mg once daily) or matching placebo. After a 2 week washout period, participants received the other study drug for 8 additional weeks. Oral swabs for HHV-8 and CMV DNA quantification by real-time PCR were collected daily during the study.

**Results**—16 HIV-positive and 10 HIV-negative men enrolled and completed the study. 3029(88%) of 3439 anticipated swabs were available for analysis. HHV-8 was detected on 44% of days on placebo versus 23% on valganciclovir (relative risk [RR] 0.54, 95% confidence interval (CI) 0.33-0.90, p=0.02). Valganciclovir reduced CMV oral shedding by 80% (RR 0.20 95% CI 0.08-0.48, p<0.001). HHV-8 and CMV shedding were independent. Hematologic, renal or hepatic toxicities were no more common on active drug versus placebo, though valganciclovir recipients reported more days of diarrhea.

**Conclusions**—Once daily oral valganciclovir is well tolerated and significantly reduces HHV-8 replication.

## Introduction

Human herpesvirus 8 (HHV-8) is the etiologic agent of Kaposi sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman disease (MCD)[1]. While KS incidence has declined where highly active antiretroviral therapy (HAART) use is widespread[2], it remains the most common AIDS-associated malignancy in the US[3], the primary malignancy following transplantation in some geographic regions[4], and the most common population-wide cancer in many parts of Africa[5]. The response of KS to treatment with chemotherapy or HAART is often incomplete[6], and no therapy for MCD or PEL has been proven effective in comparative trials.

The study of antiviral therapy for HHV-8 infection has been hampered by the lack of a traditional *in vitro* system to model HHV-8 replication. The virus does not sustain lytic replication in cell culture, prohibiting methods of antiviral susceptibility testing which rely on cytopathic effect, and no animal model of HHV-8 infection has been established. *In vitro* susceptibility of HHV-8 to antivirals has been supported by two findings. First, ganciclovir is phosphorylated in the presence of both the HHV-8 thymidine kinase at open reading frame (ORF) 21, and phosphotransferase at ORF36[7]. Second, ganciclovir, cidofovir and foscarnet inhibit the production of HHV-8 from latently-infected cell lines upon stimulation, while antivirals such as acyclovir have shown little or no activity[8-12]. These preclinical studies offer strong support for the antiviral activity of valganciclovir against HHV-8.

We hypothesized that valganciclovir will reduce HHV-8 replication *in vivo*. Ganciclovir therapy reduces symptoms and signs of HHV-8-associated MCD in parallel with reductions in HHV-8 plasma viral load[13]. Early observations made during studies of ganciclovir therapy for cytomegalovirus (CMV) retinitis in HIV-positive patients found that ganciclovir reduces the rate of new KS development by 40[14]-75%[15]. These studies suggest that inhibition of HHV-8 replication may be associated with clinical benefit, but data on HHV-8 replication were not collected. Additionally, HHV-8 replication may be activated by CMV[16], so it remains unclear whether the effect of ganciclovir on KS observed in prior trials was due to a direct antiviral activity against HHV-8 or indirectly through ganciclovir's suppression of CMV replication. To date, no randomized clinical trial has assessed the effect of antiviral medications on HHV-8 replication.

Replicating HHV-8 is found most frequently in the oropharynx, and the daily collection of saliva for the quantification of HHV-8 DNA provides a simple and accurate measure of HHV-8 replication[17,18]. We therefore sought to determine the safety and efficacy of valganciclovir on oropharyngeal HHV-8 replication in HIV-seropositive and negative persons asymptomatically infected with HHV-8 in a randomized, double-blind, placebo-controlled, crossover trial.

## Methods

### Study participants

To define the potential antiviral effects of ganciclovir on HHV-8 replication, we selected for this study 26 men who were observed in previous trials to shed HHV-8 from the oropharynx on ≥40% of days sampled[18]. Subjects were recruited at the University of Washington Virology Research Clinic in Seattle between February 2003 and 2005. Persons receiving medications with known activity against human herpesviruses or bone marrow suppression, a history of CMV disease, hypersensitivity to ganciclovir, or evidence of renal or hepatic dysfunction were excluded. Participants were eligible regardless of HIV status, but HIV-positive patients receiving antiretroviral therapy (ART) could not change medications during

the course of the study. The first 26 men who agreed to participate and met the inclusion and exclusion criteria were enrolled in the study.

## Study Procedures

Upon enrollment, participants were randomly assigned to initial administration of valganciclovir, 900 mg once daily, or matching placebo, for 8 weeks (Figure 1). Participants were randomized with the use of a computerized random number generator, stratified by HIV status. Participants, clinicians, laboratory personnel and biostatisticians were blinded to treatment assignment throughout the trial. After a two week washout period during which no study medications were taken, participants then received the other treatment for another 8 weeks. Participants collected oropharyngeal swabs daily and kept a symptom diary at home as previously described[18]. The diary surveyed common gastrointestinal complaints (nausea, vomiting, diarrhea, abdominal pain, cramping), constitutional symptoms (fever, night sweats, chills, swollen glands), as well as sore throat, rash, headache, days missed from school or work, and visits to health care professionals. Participants returned to clinic every two weeks for assessment of safety (complete blood count with differential, hepatic and renal function tests), ascertainment of medication adherence by pill counts, and return of oropharyngeal samples. A data safety monitoring board reviewed all clinical and laboratory data when 50% of participants completed the first arm of the study.

## Laboratory Assessments

Serum was tested for antibodies to HIV and CMV using enzyme immunoassays (Abbott Laboratories). DNA was extracted from oropharyngeal samples for the quantification of HHV-8 by real-time polymerase chain reaction (PCR) using primers to *orf73*[19], and CMV with a double-primer set to UL55 and UL123-exon 4[20]. All samples with ≥500 copies of HHV-8 or ≥100 copies of CMV DNA / mL were characterized as positive[18,20]. Several negative and positive controls were run with each reaction, including two reaction mixtures without DNA (negative control) as well as at least one sample with a known quantity of HHV-8 DNA. An internal control was amplified with each specimen to assure that negative results were not attributable to inhibition of PCR reaction[18,20]. Specimens were run in batches of 560 swabs. They were grouped into these batches depending on when the specimens were received in the lab. Most participants submitted 14 days of swabs at a visit; consequently, each run typically contained a mix of participants in both active treatment and placebo arms of the trial.

**Statistical Analysis—**The primary endpoint was the reduction in HHV-8 oropharyngeal replication associated with valganciclovir use as measured by HHV-8 shedding frequency, defined by the number of days with HHV-8 detected by PCR divided by the total days with PCR samples; the secondary endpoint was the safety of valganciclovir in persons with asymptomatic HHV-8 infection. In this crossover study design, each person acted as their own control to minimize the effect of variability in HHV-8 shedding on treatment efficacy measures and optimize study power. Assuming a background shedding rate of 40% and allowing for a 20% dropout rate, we calculated that 26 participants would be required to have an 80% chance of detecting ≥ 50% reduction in the rate of oropharyngeal HHV-8 detection.

All data were collected and managed at the University of Washington, and categorized and initially analyzed without knowledge of treatment assignment. The first day of each study arm and the days of the washout period were excluded from treatment efficacy analyses. Generalized linear mixed models using the Poisson distribution and log link with person-level random effects tested the efficacy of valganciclovir in reducing the frequency of HHV-8 and CMV replication. HHV-8 quantity was analyzed among days with HHV-8 detected and $log_{10}$-transformed prior to analysis. Linear mixed effects models with person-level random

intercepts compared mean quantity of HHV-8 in the active treatment and placebo arms. Covariates for time period and the interaction between time period and treatment were created to test for period and carryover effects[21]. To summarize both frequency and quantity data graphically, mean $\log_{10}$ copy of HHV-8 detected was calculated using all days, assigning the value 0 to days without HHV-8 detected.

Adherence rates, calculated as the number of unreturned pills divided by the total pills each participant should have taken, were compared between active treatment and placebo arms using generalized linear mixed models. The correlation between adherence rates and treatment efficacy, and the relationship between treatment efficacy for HHV-8 and CMV were estimated using the Spearman rank correlation coefficient.

Safety was monitored by clinical and laboratory evaluation of the participants. Transaminitis was defined by a serum alanine aminotransferase (ALT) > 250 U / dL, renal insufficiency by a serum creatinine ≥1.5 mg/dL, anemia by a serum hematocrit < 30%, thrombocytopenia by a platelet count of <20,000 per mL and neutropenia by < 1500 cells per mL. Person-level comparisons of adverse events on active drug and placebo used McNemar's test and day-level comparisons of adverse events used generalized linear mixed effects models. SAS statistical software (version 9.1 Cary, NC) was used for all analyses.

## Results

### Participant Characteristics

The median age of study participants was 42 years (range 24-66), and 17 of 26 participants (65%) reported their race as white (Table 1). All participants identified themselves as men who have sex with men (MSM). Sixteen of 26 (62%) participants were HIV-positive, with a median CD4 T-cell count of 434 cells / mm$^3$ (49-936) and a mean $\log_{10}$ HIV RNA copy of 3.8 per mL of plasma (range 2.2-5.3). Eight of the 16 HIV-positive participants were on ART during the course of the study, including 7 on HAART, defined by any combination of ≥3 antiretroviral agents including at least one non-nucleoside reverse transcriptase or protease inhibitor.

### Effect of Valganciclovir on the HHV-8 Detection in the Oropharynx

Overall, 3029 (88%) of 3439 oropharyngeal swabs were available for analysis. Valganciclovir reduced the frequency and quantity of HHV-8 in saliva. HHV-8 was detected on 583 (44%) of 1333 total days on the placebo arm, and on 318 (23%) of 1360 days on the valganciclovir arm. Thus the use of valganciclovir was associated with a 46% reduction in the detection of HHV-8 in the oropharynx, with a relative risk (RR) of 0.54 (95% confidence interval (CI) 0.33-0.90, p=0.02) for detecting HHV-8 during valganciclovir administration versus placebo.

Next, we analyzed the quantity of HHV-8 detected in the oropharynx on days with HHV-8 detected. The mean $\log_{10}$ copies detected on placebo was 5.0 per mL (range 2.7-7.9) compared with 4.7 on valganciclovir (range 2.7-7.6). Valganciclovir significantly reduced the quantity of HHV-8 by 0.44 logs (95% CI 0.12 to 0.75 logs, p=0.007).

Reductions in HHV-8 oropharyngeal shedding were seen among both HIV-positive and negative participants. On a person level, 15 (94%) of 16 HIV-positive participants had HHV-8 detected on at least one day of the placebo arm versus 11 (69%) of 16 during valganciclovir use. We also observed a reduction in day-level rates of HHV-8 detection in HIV-positive participants, with HHV-8 detected on 438 (53%) of 822 days on the placebo arm versus 246 (29%) of 854 days on the valganciclovir arm. Among HIV negative participants, 9 (90%) of 10 HIV-negative participants had HHV-8 detected in saliva on at least one day of the placebo arm versus 8 (80%) of 10 during valganciclovir use. However, HHV-8 was detected on 145

(28%) of 511 days during placebo use versus 72 (14%) of 506 days during valganciclovir use for the HIV negative subgroup.

Analyses adjusting for HIV and HAART status showed that valganciclovir significantly reduced the risk of detecting HHV- 8 in the oropharynx by 46% (RR 0.54, 95% CI 0.33-0.90, p=0.02) and the HHV-8 copy number by a mean of 0.44 logs (95% CI 0.12 to 0.76 logs, p=0.007) (Table 2). Small numbers precluded detailed analyses of the effect of valganciclovir within subgroups defined by HIV infection status and HAART use, but modeling suggested differential effects of valganciclovir within HIV and HAART categories (p=0.053 for interaction), with the greatest treatment effect occurring among the HIV positive participants on HAART (RR=0.30, 95% CI 0.19-0.47, p<0.001).

In analyses adjusting for valganciclovir use, HIV positive participants not on HAART (RR=2.36, 95% CI 1.00-5.53) and those receiving HAART (RR=1.59, 95% CI 0.58- 4.34) tended to shed HHV-8 more frequently than HIV-negative men, although this comparison was not statistically significant overall (p=0.14). HHV-8 shedding was more common among HIV-positive persons with higher CD4 T-cell counts (RR 6.0 for participants with CD4 > 200 versus <200 cells / mm$^3$, 95% CI 2.0-17.7, p=0.003). The effect of valganciclovir on the quantity of HHV-8 did not vary greatly by HIV and HAART categories (p=0.98 for interaction).

### Kinetics of HHV-8 Inhibition with Valganciclovir

During the valganciclovir arm, minimal HHV-8 shedding (<10%) was observed in approximately half of the participants (Figure 2). On average, lower levels of HHV-8 shedding were observed after the first 1-2 weeks of valganciclovir treatment (Figure 3). As participants switched to the washout and placebo arms, viral shedding rebounded. We did not find significant period (the effect of valganciclovir was the same whether it was given before or after placebo) or carryover (treatment with valganciclovir first did not effect shedding in the subsequent placebo arm) effects for either the shedding frequency (p=0.83, p=0.76, respectively) or quantity (p=0.90, p=0.59, respectively) (Figure 3a compared with 3b).

### Effect of Valganciclovir on CMV Replication and Relation to HHV-8 Replication

Twenty five (96%) of 26 men were CMV seropositive, and 14 (56%) shed CMV from the oropharynx on at least 1 day (9 HIV-positive and 5 HIV-negative persons). Valganciclovir reduced CMV shedding by 80% (RR 0.20, 95% CI 0.08-0.48, p<0.001); CMV was detected on 163 (13%) of 1255 days of placebo administration versus 33 (3%) of 1215 days of valganciclovir use. Valganciclovir's effect on CMV was seen in both HIV-positive (109 (14%) CMV positive days of 798 days on placebo versus 23 (3%) of 821 days on valganciclovir) and HIV-negative participants (54 (12%) CMV positive days of 457 on placebo versus 10 (3%) of 394 days on valganciclovir).

Among the 11 participants who never shed CMV, valganciclovir still reduced HHV-8 shedding (56% shedding rate during placebo use versus 29% shedding rate during valganciclovir use, RR 0.53, 95% CI 0.29-0.97, p=0.04). We found no significant correlation between the effect of valganciclovir on HHV-8 and the effect of valganciclovir on CMV (r=0.15, p=0.47).

### Adherence with Study Procedures and Safety of Valganciclovir

5274 out of 5560 total pills (94.9%) were not returned, for an estimated median adherence of 97.1% (range 73%-100%). Adherence rates were not significantly different during placebo versus active drug arms (p=0.68), and were not related to the effect of valganciclovir on HHV-8 shedding (r=-0.16, p=0.44).

No participant on either active study drug or placebo experienced anemia, thrombocytopenia, or renal insufficiency. No serious adverse events were observed during the study. One participant had a transient elevation in ALT during administration of placebo. Three participants were neutropenic during the placebo arm (2 HIV-positive and one HIV-negative), compared with 2 during the valganciclovir arm (both HIV-positive). Among a subset of 22 participants providing data on gastrointestinal symptoms during the study, nausea was reported by 2 HIV-positive participants while taking valganciclovir and 3 while taking placebo, and abdominal cramps were reported by 3 HIV-positive participants during the valganciclovir arm and 4 during the placebo arm. Diarrhea was reported by 6 HIV-positive and 1 HIV-negative participant while taking valganciclovir and 3 HIV-positive and 1 HIV-negative participant while taking placebo, though this person-level difference was not significant (p=0.50). When examined at a visit or day level, participants reported a total of 132 days of diarrhea, including 9% of days on valganciclovir and 2% of days while on placebo (p=0.02).

## Discussion

We found that oral valganciclovir effectively inhibits mucosal HHV-8 replication. The frequency and quantity of HHV-8 detected in the oropharynx were significantly reduced during valganciclovir therapy. The antiviral effect was reasonably prompt, and appeared independent of the drug's substantial reduction in CMV replication. HHV-8 replication returned upon discontinuation of the drug, indicating the need to use daily dosing to achieve effective virologic control. This study also corroborates previous findings that oral shedding of HHV-8 is frequent among asymptomatically-infected HIV-positive persons (especially those with high CD4 T-cell counts)[19,22], as well as men without HIV infection[18].

The role that direct antiviral chemotherapy will play in preventing HHV-8-associated infection or disease remains to be determined. The reduced incidence of KS observed in the aforementioned studies of HIV-positive persons treated with ganciclovir suggested that the prevention of KS with antivirals was possible. While latent HHV-8 is the predominant form of viral infection in KS tissue, HHV-8 replication is essential to the initiation or maintenance of HHV-8-associated disease. Lytic HHV-8 replication is necessary for the persistence of KS lesions[23,24], and the detection of HHV-8 in the plasma has been associated with the subsequent development of KS in prospective studies of patients with HIV[25-30]. Additionally, HHV-8 elaborates angiogenic and inflammatory factors during lytic replication, including an interleukin-6 analogue and a viral G-protein coupled receptor[31]. These data suggest the potential of antiviral in mitigating some of these viral replication-related sequelae. We felt it was important to establish the antiviral activity of valganciclovir prior to embarking on a study demonstrating its clinical efficacy. Given the current expense of valganciclovir and the potential toxicities associated with long-term use, its role in the prevention of KS in endemic areas remains to be explored. One potential strategy would be intermittent administration to persons predicted to be at high risk for developing KS based on previously described risk factors, such as HHV-8 viremia[25-30], absence of neutralizing antibodies to HHV-8[32], or profound immunosuppression[33]. Alternatively, valganciclovir may be useful for the prevention of KS in high-risk transplant patients, as it has been shown to be used safely for the prevention of CMV disease in this setting. While lytic replication may play a role in some aspects of KS, antiviral therapy is not likely to be of benefit when used as a single agent for the treatment KS, as the role of persistent viral replication after malignant transformation is less clear[34].

Antiviral therapy may also be useful in the treatment of some HHV-8-related disease. For example, almost all cells infected with HHV-8 in MCD harbor lyticallyreplicating virus[35], and the quantity of HHV-8 in the peripheral blood closely parallels the acuity of disease[36]. The results of this randomized trial lend support to our previous clinical observations

documenting reductions in HHV-8 viremia and concomitant clinical improvement among MCD patients receiving valganciclovir[13]. The degree of HHV-8 replication in patients with PEL is intermediate between KS and MCD, but evidence for an adjunctive role for antivirals in the treatment of PEL offers hope that the treatment of this highly mortal disease could be improved[37]. Studies to optimize the dose and duration of valganciclovir in MCD and PEL should be undertaken.

Our study design was an efficient approach to define the antiviral activity of oral valganciclovir against HHV-8 replication *in vivo*. However, this study design also had a number of important limitations. First, the population was highly selected, having been previously observed to have HHV-8 frequently detected in the oropharynx. Future studies using less selected patient populations, and defining the antiviral effects in other body sites should be pursued. Similarly additional trials to define the optimal dose for preventing of HHV-8-associated diseases are needed. Valganciclovir is initially administered at 900 mg twice daily for the treatment of cytomegalovirus ("induction"), after which it is often reduced to 900 mg daily ("maintenance"). Given that the toxicity of valganciclovir is in part dose-related, and that this study was an exploratory trial, we elected to use the lower dose. It was gratifying that we were able to demonstrate an effective antiviral effect with minimal toxicity, suggesting that trials of longer duration that might result in true clinical benefit appear possible. The observed reductions in HHV-8 shedding were limited in both magnitude and duration. It is unclear whether higher doses would have resulted in greater efficacy, greater toxicity, or both.

In conclusion, we found valganciclovir to be the first antiviral agent shown to reduce HHV-8 replication in a randomized clinical trial. Antivirals have been shown to be useful in the prevention of other viral-associated malignancies, including hepatocellular carcinoma[38] and post-transplant lymphoproliferative disease[39]. The prevention of KS with valganciclovir in persons found to be high risk based on immunosuppression and the presence of frequent and sustained HHV-8 replication could be an important strategy in KS-endemic areas. Additional research is needed to define the optimal use of antiviral drugs persons with HHV-8-infection, but this study offers hope that the prevention of HHV-8-associated malignancies might be feasible.

## Acknowledgments

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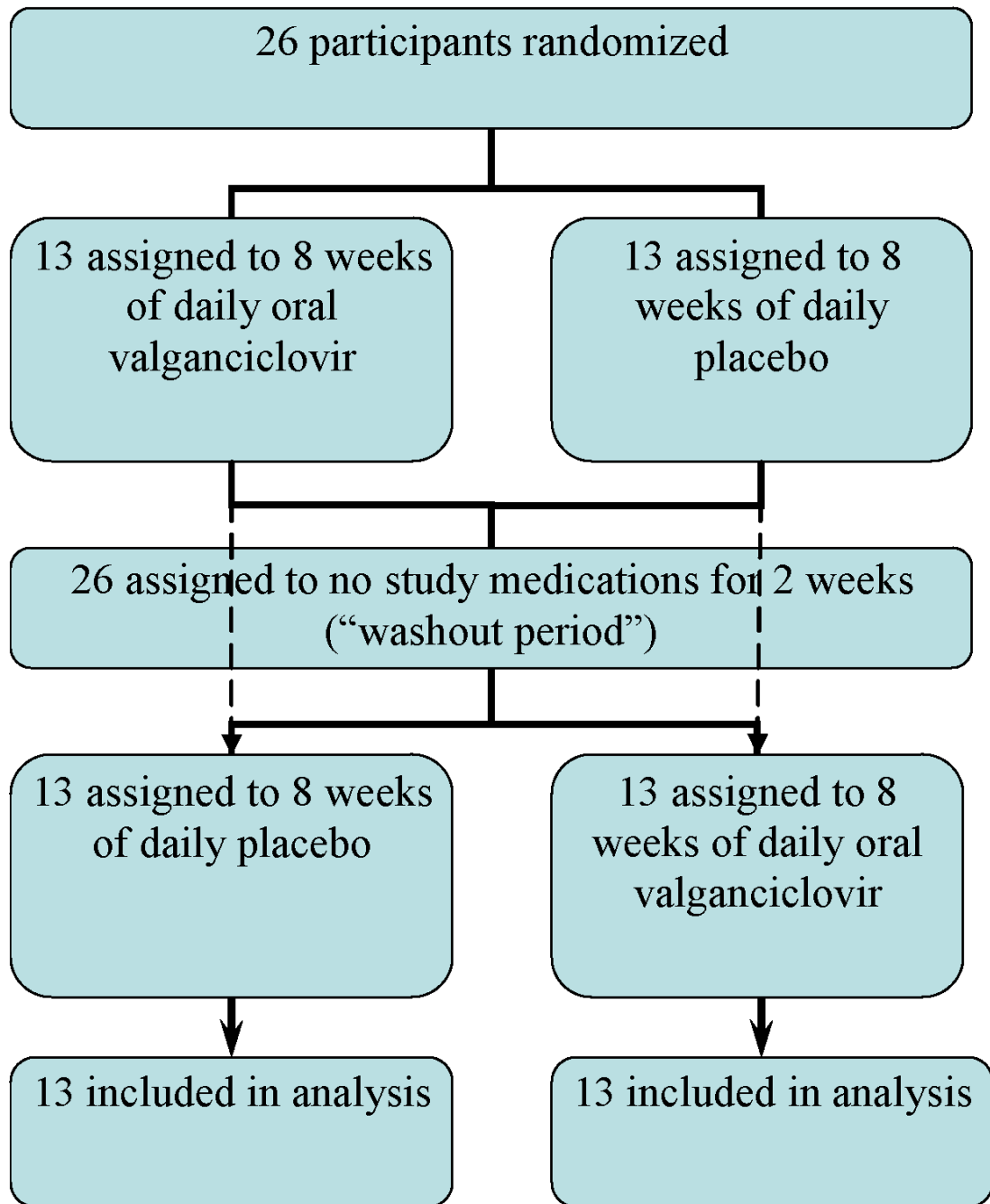
**Figure 1.**
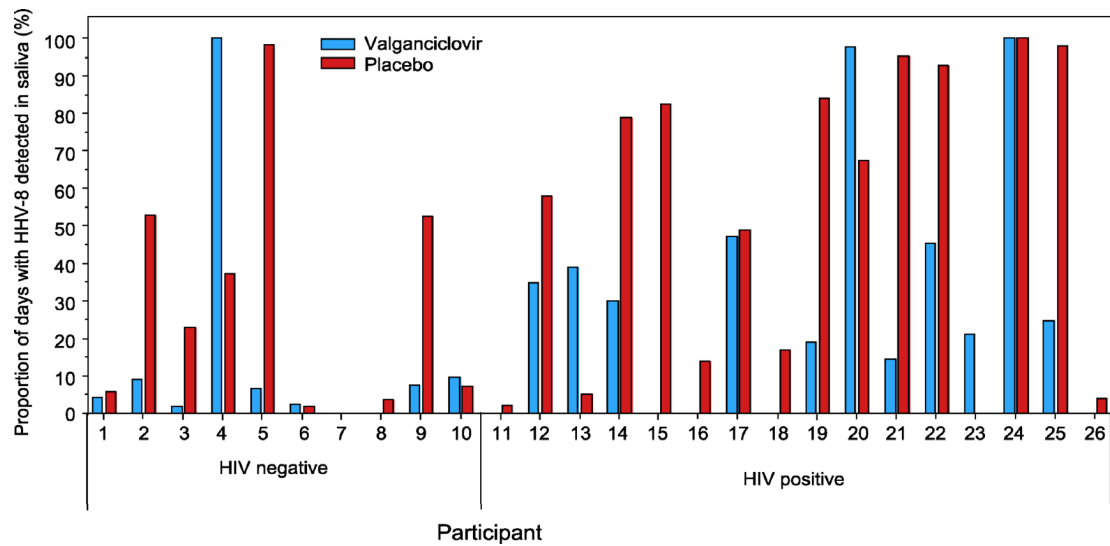Study Design of Trial.

**Figure 2.**
Comparison of HHV-8 Oropharyngeal Shedding Rates on Valganciclovir versus Placebo,
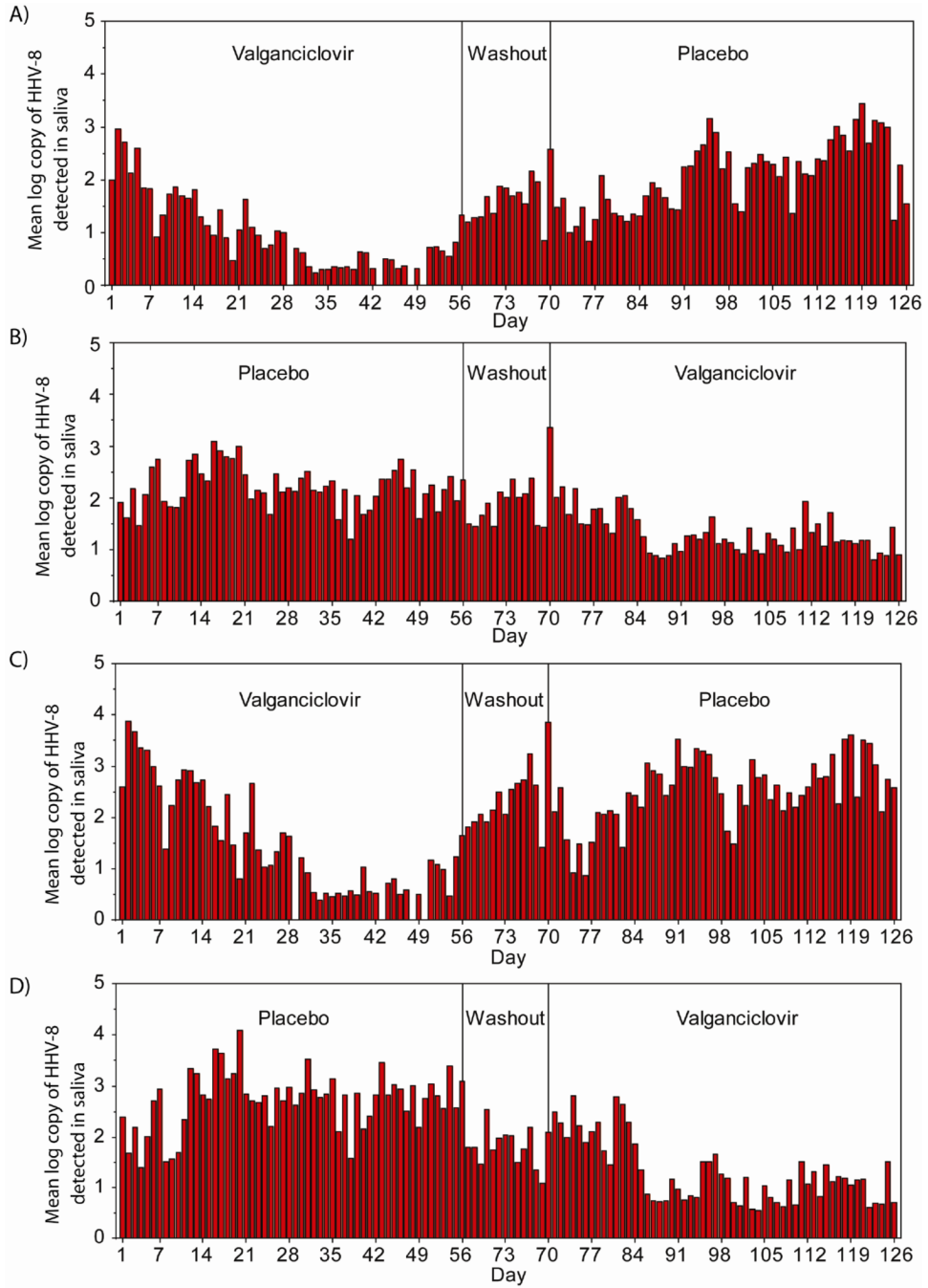Divided by Participant HIV Infection Status.

**Figure 3.**
HHV-8 Oropharyngeal Shedding by Day of Study. Mean log copy of HHV-8 detected in saliva is summarized among all participants providing data for each study day. Zero copies were assigned a log value of zero.

Panel A. All participants receiving valganciclovir followed by placebo (n=13).

Panel B. All participants receiving placebo followed by valganciclovir (n=13).

Panel C. Only HIV positive participants receiving valganciclovir followed by placebo (n=8).

Panel D. Only HIV positive participants receiving placebo followed by valganciclovir (n=8).

**Table 1**

Baseline Characteristics of Participants

| Characteristic | HIV-negative (n=10) | HIV-positive (n=16) | Total (n=26) |
|---|---|---|---|
| Age, median (range) | 45 (37-66) | 40 (24-54) | 42 (24-66) |
| Race / Ethnicity, n (%) | | | |
| White | 8 (80%) | 9 (56%) | 17 (65%) |
| Non-white | 2 (20%) | 7 (44%) | 9 (35%) |
| CMV Status, n (%) | | | |
| Positive | 9 (90%) | 16 (100%) | 25 (96%) |
| Negative | 1 (10%) | 0 (0%) | 1 (4%) |
| CD4+ T-Lymphocyte Coount (cells / mm$^3$), median (range) | N/A | 434 (49-936) | N/A |
| Plasma HIV RNA Level, mean log$^{10}$ copies / mL (range) | N/A | 3.8 (2.2 -5.3) | N/A |
| Antiretroviral use, n (%) | | | |
| None | N/A | 8 (50%) | N/A |
| non-HAART | N/A | 1 (6%) | N/A |
| HAART | N/A | 7 (44%) | N/A |

Abbreviations: HIV - Human Immunodeficiency Virus; RNA - Ribonucleic Acid; HAART - Highly Active Antiretroviral Therapy; CMV - Cytomegalovirus

**Table 2**

Effect of Valganciclovir on Human Herpesvirus 8 Oropharyngeal Shedding Frequency and Quantity

| Outcome Measure | Placebo Arm | Valganciclovir Arm | Estimated Measure of Effect[*] (95% Confidence Interval) | P-value |
|---|---|---|---|---|
| Proportion of Days with HHV-8 Detected in Saliva | 583/1333 (44%) | 318/1360 (23%) | RR = 0.54 (0.33, 0.90) | 0.02 |
| Mean $Log_{10}$ HHV-8 Quantity (Copies/mL) in Saliva During Study Arm[+] (range) | 5.0 (2.7-7.9) | 4.7 (2.7-7.6) | Coefficient = -0.44 (-0.76, -0.12) | 0.007 |

Abbreviations: HHV-8 - Human Herpesvirus 8, RR - Relative Risk

[*]Adjusted for treatment arm, human immunodeficiency virus infections status and highly active antiretroviral therapy use

[+]Calculated only for days during which HHV-8 was detected in saliva