

Valganciclovir for the Suppression of Epstein-Barr Virus Replication

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A thesis

submitted in partial fulfillment of the
requirements for the degree of

Master of Public Health

University of Washington

2015

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Program Authorized to Offer Degree:

Epidemiology

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Abstract

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Epstein-Barr virus (EBV), a common herpesvirus, is the main causative agent of infectious mononucleosis. Following primary infection, EBV persists in host cells and can cause B cell transformation, leading to the development of EBV-associated malignancies. The role for antiviral therapy in treating and preventing EBV-associated disease remains unclear. Using oral swabs collected from a randomized, double-blind, placebo-controlled crossover study that our group had previously conducted, we sought to determine the impact of valganciclovir on both the rate and quantity of oral EBV shedding. Twenty-six men were randomly assigned to receive either oral valganciclovir, 900mg daily, or oral placebo once daily for eight weeks. Participants then received no study drug for a two week “washout period,” followed by the alternate treatment (valganciclovir or placebo) for a second eight-week period. Valganciclovir reduced the proportion of days on which EBV was detected by 72% (relative risk 0.28, 95% confidence interval 0.08-0.55; $p=0.003$), and reduced the quantity of virus detected by 0.77 logs (95% confidence interval 0.62-0.91 logs; $p<0.001$). These results were consistent regardless of participants’ HIV status and use of antiretroviral therapy. These findings could have significant clinical applications in both the treatment and prevention of EBV-associated disease.

Background and Significance:

Epstein-Barr virus (EBV) is a common double-stranded gamma-herpesvirus. Most acute EBV infections are subclinical; however, when EBV does cause clinical disease, it most commonly causes infectious mononucleosis, and is the main etiologic agent to do so. Following acute infection, the virus persists in host B and T lymphocytes, monocytes, and epithelial cells. Studies conducted in a wide variety of populations have found that EBV is frequently present in the saliva of immunocompetent and immunosuppressed individuals with no associated clinical symptoms, a state known as “asymptomatic shedding” [1, 2]. Unlike other herpesviruses, EBV can cause B cell transformation and is associated with malignancies including Hodgkin, non-Hodgkin and primary central nervous system lymphomas, post-transplant lymphoproliferative disease (PTLD), nasopharyngeal carcinoma, and gastric cancer [3].

Acyclovir has been demonstrated to reduce EBV replication by inhibiting viral DNA polymerase [4]; however, the role of antiviral medications in the management of EBV-related disease remains unclear. Multiple small studies have evaluated the use of acyclovir (both oral and intravenous) or its pro-drug formulation, valacyclovir, for the treatment of infectious mononucleosis. While these studies have consistently found that acyclovir and valacyclovir reduced oral shedding of EBV [5-7], only one study suggested that valacyclovir (one gram every eight hours for 14 days) expedited the resolution of clinical symptoms associated with infectious mononucleosis [7].

The data evaluating the use of antivirals in the treatment and prevention of EBV-associated malignancies also remains controversial. Because EBV-associated malignancies typically develop in the setting of latent, rather than lytic, infection, the role of antiviral treatments for these late complications of EBV infection is unclear. Though multiple case reports and small studies have suggested a possible role for antiviral treatment of post-transplant lymphoproliferative disorder (PTLD) and hemophagocytic syndrome [8-12], the lack of larger trials has hindered a better understanding of the impact of antiviral therapy in disease treatment. Further data elaborating the effect of antiviral prophylaxis and treatment for immunosuppressed patients at risk for complications of EBV infection could significantly reduce the burden of EBV-associated disease and are sorely needed.

Valganciclovir is an oral valine ester prodrug of the antiviral medication ganciclovir. It can be taken once daily, and achieves adequate serum drug levels required for treatment of infection with cytomegalovirus (CMV), another herpesvirus, for which the drug is indicated. Few data exist examining the impact of valganciclovir on EBV replication *in vivo*. However, increasing observational and anecdotal data support the role of valganciclovir in suppressing replication and reducing associated clinical disease associated with EBV [13-15] and the related gamma-herpesvirus, human herpesvirus-8 (HHV-8) [16]. Further data are required to better elucidate the role of valganciclovir in suppressing EBV replication and in reducing EBV's impact in chronic infection.

Our group has previously conducted a randomized controlled trial (RCT) to determine the impact of valganciclovir on oral shedding rates of HHV-8 and CMV, and found the drug reduced shedding of both viruses significantly [17]. Using oral swabs already collected from this study, we sought to determine the impact of valganciclovir on *in vivo* replication of EBV.

Methods:

Study subjects:

Twenty-six men (10 HIV-negative and 16 HIV-positive) who had previously demonstrated oropharyngeal shedding of HHV-8 on $\geq 40\%$ of days evaluated enrolled [17]. Individuals were excluded if they were taking medications with known activity against human herpesviruses, had known bone marrow suppression, hypersensitivity to ganciclovir, renal or hepatic dysfunction, or a history of CMV disease. Persons with HIV on antiretroviral therapy (ART) were required to remain on a stable regimen throughout the study period.

Study setting:

All data were collected between February 2003 and February 2005 at the University of Washington Virology Research Clinic in Seattle, Washington.

Study design and procedures:

The study was a randomized, double-blind, placebo-controlled crossover study. Randomization was completed through a computer-generated random number assignment, and stratified by HIV status. Study participants were randomly assigned to receive either oral valganciclovir, 900mg daily, or oral placebo once daily for eight weeks. Following the initial eight-week administration of either drug or placebo, participants received no study drug for a two-week “washout period.” Subsequently, participants received the alternate treatment (valganciclovir or placebo) for a second eight-week period.

Data collection:

All participants collected daily oropharyngeal swabs, and maintained a diary of symptoms including constitutional and gastrointestinal symptoms, as well as missed school or work days and visits to a healthcare provider.

Serum samples were tested for HIV and CMV antibodies using commercial enzyme immunoassays. DNA was extracted from patient-collected oropharyngeal swabs for real-time quantitative polymerase chain reaction (PCR) amplification of EBV using primers to amplify the BALF5 gene [18]. Participants were considered positive for oral EBV shedding if PCR analysis detected >150 copies of EBV DNA/mL [19].

Statistical Analysis:

The primary endpoint evaluated for this analysis was the reduction of oral EBV shedding during valganciclovir administration. This endpoint was assessed by determining the frequency of EBV shedding, as defined by the ratio of days on which EBV was detected out of all days on which oral samples were obtained, on both valganciclovir and on placebo. Additionally, the reduction in EBV shedding was determined by evaluating the quantity of EBV shed by each individual while receiving valganciclovir versus placebo. The crossover study design allowed each individual to serve as his own control.

Data were collected and managed at the University of Washington; data managers were blinded to participants' study arm. To evaluate the efficacy of valganciclovir in reducing the frequency of EBV

shedding, we created generalized linear mixed models using the Poisson distribution and log link. To evaluate the efficacy of valganciclovir in reducing the quantity of EBV shed by each individual, we used linear mixed effects models. The mean \log_{10} copies per milliliter of EBV detected was calculated using all days for which EBV was detected. Samples for which no EBV DNA were detected were excluded from this analysis. We tested the efficacy of the washout period by creating covariates for time period and for the interaction between time period and treatment.

Results:

Participant characteristics:

Participants' ages ranged from 24 to 66 years old, with a median age of 42 years. Seventeen of 26 participants (65%) reported their race as white. All participants self-identified as men who have sex with men (MSM), and 16 participants (62%) were HIV-positive. Mean HIV RNA plasma level was \log_{10} 3.8 copies per mL (range 2.2-5.3 copies/mL), and median CD4 T cell count was 434 cells/mm³ (range, 49-936 cells/mL). Half (8 of 16) of the HIV-positive individuals were receiving treatment with at least three antiretroviral medications, including at least one non-nucleoside reverse transcription inhibitor or protease inhibitor (**Table 1**).

Adherence to protocol and safety of valganciclovir:

Of 3,276 anticipated swabs, 2931 (89.5%) were available for analysis for EBV. Because swabs were collected daily for eight weeks during both placebo and drug arms, each participant was expected to submit 56 swabs per study arm. For all participants on both study arms, the mean number of swabs collected was 50 (range, 3-61; standard deviation 10.24). While on valganciclovir, participants submitted a mean number of 49 swabs each (range, 29-62; standard deviation 8.64). While on placebo, participants submitted a mean number of 50 swabs per person (range, 3-61; standard deviation 11.78). HIV-positive participants submitted a mean number of 51 swabs (range 3-61; standard deviation 10.6), while HIV-negative participants submitted a mean number of 48 swabs (range, 28-61; standard deviation 9.7).

As previously reported, adherence to both placebo and valganciclovir was very high. Two-hundred and eighty-six pills were returned, of 5560 pills dispensed. Based on the number of pills returned to research staff, the estimated median adherence rate to medication (valganciclovir and placebo) was 97.1% (range, 73-100%). Adherence rates were similar in the placebo and valganciclovir study arms ($p=0.68$) [17]. Medication was well tolerated: no serious adverse events occurred throughout the study. No participant experienced renal insufficiency, anemia, or thrombocytopenia on either placebo or valganciclovir [17].

Impact of valganciclovir on oropharyngeal shedding of EBV:

EBV was detected at least once in 25 of 26 participants. Administration of valganciclovir reduced both the proportion of days on which EBV was detected and the quantity of virus detected. Among all participants receiving valganciclovir, oral EBV was detected on 229 of 1286 days (17.81%), compared with 803 of 1309 days (61.34%) of days on placebo. Therefore, valganciclovir was associated with a 72% reduction in the frequency of EBV shedding in the oropharynx compared with placebo, with a relative risk (RR) of 0.28 (95% confidence interval 0.08-0.55; $p=0.003$). When evaluating the quantity of EBV shed in the oropharynx on days for which virus was detected, we found a mean \log_{10} copies of EBV per milliliter of 4.32 (range, 2.18-7.23 copies/mL) among those participants using placebo, compared with a mean \log_{10} copies of EBV per milliliter of 3.61 (range, 2.22-7.24 copies/mL) among those participants using valganciclovir. Valganciclovir therefore significantly reduced the quantity of EBV by 0.77 logs (95% CI, 0.62-0.91 logs; $p<0.001$).

While on placebo, HIV-negative participants had EBV detected on 27.6% of days evaluated, compared with 84.3% for HIV-positive individuals receiving HAART and 81.1% for HIV-positive individuals not receiving HAART. While on valganciclovir, HIV-negative participants had EBV detected on 7.6% of days evaluated, compared with 26.6% for HIV-positive individuals receiving HAART and 20.5% for HIV-positive individuals not receiving HAART (**Table 2**).

When we adjusted for HIV status and receipt of antiretrovirals, the impact of valganciclovir on EBV shedding remained pronounced. In our adjusted analysis, valganciclovir again reduced the number of

days on which EBV was detected by 72% (RR, 0.28 [95% confidence interval 0.21-0.41]; $p < 0.001$), and reduced the quantity of EBV by a mean of 0.77 logs (95% CI, 0.62-0.91 logs; $p < 0.001$) (**Table 3**). When we included in our model covariates to evaluate the interactions of both HIV status and receipt of HAART on valganciclovir use, we did not find a significant difference in the impact of valganciclovir use based on HIV status ($p = 0.99$). No significant differences were found in our results when we included in the model covariates accounting for either the time period or for the interactions between the time period and valganciclovir use, indicating that there was no sequence effect and that the 14-day washout period between the receipt of placebo and drug was effective.

Discussion:

Our results indicate that oral valganciclovir administered once daily was highly successful in reducing both the rate of oral shedding of EBV and the quantity of virus shed. These findings could have significant clinical applications in both the treatment and prevention of EBV-associated disease. In immunocompetent hosts, the role for antiviral treatment of primary EBV infection remains poorly defined. Acute EBV infection is usually self-limited, and generally presents as infectious mononucleosis, a clinical syndrome defined by fever, cervical lymphadenopathy, and pharyngitis. Multiple studies examining the effect of antiviral therapy (either combined with oral steroids or used in isolation) on acute EBV infection found a reduction in oral EBV shedding; however, this reduction was not associated with significant changes in the duration of clinical symptoms or in overall clinical outcomes [6, 20, 21]. One randomized control trial similarly found no difference in duration of clinical symptoms between patients receiving acyclovir versus placebo for EBV-associated infectious mononucleosis, though did find a statistically-significant difference between composite clinical endpoints [5]. A more recent meta-analysis evaluating the use of anti-viral medications in severe complications of EBV-associated infectious mononucleosis—including, though not limited to, central and peripheral nervous system manifestations, hepatitis, acute kidney injury, and myocarditis—similarly found no consistent clinical benefit with the administration of anti-viral medications. Of note, however, the study was limited by the small number of cases available, generally presented as case reports or case series, and the highly varied treatment regimens received by individual patients [22]. One small randomized trial evaluating the use of valacyclovir in infectious

mononucleosis did find an impact on clinical resolution [7]. None of the randomized trials, however, evaluated valganciclovir specifically. *In vitro* experiments find that the EBV tyrosine kinase has variable affinity for the antiherpetic antivirals [23]. Indeed, we observed valganciclovir to be associated with more profound reductions in shedding of another human gamma-herpesvirus, HHV-8, compared with acyclovir or valaciclovir [24]. Thus, valganciclovir could have a role to play in the treatment of complicated initial EBV infection.

Given the difficulty, toxicity, and expense of treating late-stage complications of EBV infection, clinicians have long sought to create preventive strategies for those populations at risk for late-stage disease. Recipients of both solid organ transplants (SOT) and of hematopoietic cell transplants (HCT) are heavily immunosuppressed and at risk for PTLD. Among those who do develop PTLD, mortality is high: 87.5% in one retrospective cohort in Australia [25]. Patients with increased rates of EBV shedding and higher quantities of EBV shed have been found to be at greater risk for the development of PTLD [26]. Those patients who newly acquire EBV infection following their transplant—such as those who were themselves EBV-seronegative with a seropositive donor—are also at particular risk for PTLD. Various studies have therefore examined the use of prophylactic antiviral therapy in transplant recipients to reduce the risk of PTLD. Results from studies have been varied, with multiple early studies of anti-viral prophylaxis detecting no impact on PTLD incidence [27, 28]. However, one early study was able to detect a reduction in PTLD incidence from 3.9% to 0.5% of SOT recipients who received either acyclovir (administered orally four times daily) or ganciclovir (administered intravenously) while they received highly immunosuppressive antilymphocyte antibody therapy [29]. A subsequent multi-center case-control study evaluating renal-only transplants found up to an 83% reduction in the risk of PTLD, with variation between anti-viral agents. Notably, ganciclovir was associated with a 38% risk reduction of early PTLD for each 30 days during an individual's first year post-transplant [30]. In a study of lung transplant patients, researchers also found a reduction of PTLD from 4.2% among a historical comparison group to 0.76% in recipients of prophylaxis with either acyclovir, valaciclovir, or ganciclovir [25].

Valganciclovir has better oral availability than ganciclovir [31] and could therefore play an important role in anti-viral prophylaxis against PTLD. In one study of children post-liver transplantation, those with

detectable EBV DNA were initiated on prophylactic valganciclovir (520mg/sqm) twice daily with no alteration in their immunosuppressive regimen. One child of 47 (2.1%) developed suspected new PTLD, and the valganciclovir was found to be well tolerated [32]. However, further data are needed to better elucidate the role of valganciclovir in PTLD prophylaxis.

EBV also causes severe clinical manifestations in persons with HIV disease, including oral hairy leukoplakia, end-organ inflammation, and lymphoma [33, 34]. Previous studies by our group [35, 36] have shown a reduction in EBV replication associated with ART use, which was also observed in this study. Valganciclovir continued to be associated with a reduction in EBV shedding among HIV-positive men on ART in our study, though the effect was attenuated modestly in comparison to ART-naïve participants. The role valganciclovir could play in the clinical management of HIV-infected patients in the ART era remains to be defined.

Our study has several limitations. First, all study participants were selected for study participation based on their known history of HHV-8 shedding. It is possible that individuals with high rates of oral HHV-8 shedding may also have higher rates of EBV shedding than the general population. Additionally, all study participants were male. These limitations may narrow the generalizability of our findings. Further studies with a less selected population may be required to ensure more generalizable results. Second, the optimal dose of valganciclovir needed to prevent EBV-related disease is unclear. The induction phase of treatment for cytomegalovirus (CMV) generally requires 900mg of valganciclovir given twice daily; subsequent maintenance therapy requires only 900mg daily. Because of the potential toxicities of the drug, we used the lower dose. However, further studies are needed to compare different dosing strategies in order to determine differential clinical benefit and toxicity profile.

Our findings can contribute to the current science in multiple ways. First, our results indicate that valganciclovir was highly efficacious in reducing both the rate and quantity of EBV shedding in asymptomatic individuals. Because active EBV shedding is associated with increased risk of PTLD development in at-risk individuals [26], valganciclovir could be a useful agent for PTLD prophylaxis. Second, we were able to achieve these results with a daily dose of valganciclovir that was safer, better

tolerated and more affordable than the typical twice-daily dosing. In doing so, we increased the feasibility of employing this dosing schedule as a prophylactic strategy for management of transplant recipients, though it remains unknown if the drug at this dose would have a similar effect on EBV replication in this patient population. This dose could also be a more attractive option for individuals without known immunosuppression who have severe sequelae of primary EBV infection. Though many clinical questions remain, the potential utility of valganciclovir as an important prophylactic or therapeutic agent will hopefully guide further research to answer these remaining questions.

Table 1: Demographic and clinical baseline characteristics of participants

Characteristic	HIV infection status		Total (n=26)
	Negative (n=10)	Positive (n=16)	
Age, median (range), years	45 (37-66)	40 (24-54)	42 (24-66)
Race or ethnicity			
White	8 (80)	9 (56)	17 (65)
Non-white	2 (20)	7 (44)	9 (35)
CD4 T lymphocyte count, median (range), cells/mm	NA	434 (49-93)	NA
Plasma HIV RNA level, mean (range), log ₁₀ copies/mL	NA	3.8 (2.2-53)	NA
Receipt of antiretrovirals			
None	NA	8 (50)	NA
HAART	NA	8 (50)	NA

Table 2: Rate and quantity of viral shedding among study participants by HIV status, ARV use, and study arm

	Valganciclovir			Placebo		
	HIV positive (N=16)		HIV negative (N=10)	HIV positive (N=16)		HIV negative (N=10)
	HAART (N=8)	No HAART (N=8)		HAART (N=8)	No HAART (N=8)	
EBV shedding rate ¹	26.6 (110/414)	20.5 (84/409)	7.6 (35/463)	81.1 (304/375)	84.3 (359/426)	27.6 (140/508)
Mean quantity EBV detected ^{2,3}	3.9 (1.3)	3.4 (1.0)	3.4 (1.0)	4.2 (1.2)	4.7 (1.2)	3.6 (1.1)
CMV shedding rate ¹	4.6 (19/414)	0.5 (2/407)	2.0 (8/394)	12.9 (48/373)	12.2 (52/425)	11.8 (54/457)
Mean quantity CMV detected ^{2,3}	2.5 (0.3)	2.4 (0.2)	2.5 (0.3)	2.8 (0.4)	4.5 (1.0)	3.3 (0.4)
HHV8 shedding rate ¹	18.1 (78/430)	40.6 (172/424)	14.4 (73/506)	45.2 (180/398)	65.9 (280/425)	28.6 (146/511)
Mean quantity HHV8 detected ^{2,3}	4.0 (0.9)	4.7 (1.0)	5.1 (1.2)	4.7 (1.0)	4.9 (1.2)	4.9 (1.0)

¹%(EBV positive swabs / total swabs collected)

²log₁₀ copies/mL

³mean (standard deviation)

Table 3: Impact of valganciclovir on the frequency and quantity of oropharyngeal shedding of Epstein-Barr virus (EBV)

	Placebo	Valganciclovir	Measure of Effect (95% CI)	P value
Proportion of days with EBV detected (%)	803/1309 (61.34)	229/1286 (17.81)	RR 0.292 (0.252-0.338)	<0.001
Quantity of EBV detected (range), mean log ₁₀ copies/mL	2.647 (0-7.324)	0.643 (0-7.242)	Coefficient, -2.066 (-2.192 to -1.941)	<0.001

Adjusted for HIV infection status and use of antiretroviral therapy.

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