

# Valganciclovir Reduces T Cell Activation in HIV-infected Individuals With Incomplete CD4<sup>+</sup> T Cell Recovery on Antiretroviral Therapy

Peter W. Hunt,<sup>1</sup> Jeffrey N. Martin,<sup>1</sup> Elizabeth Sinclair,<sup>1</sup> Lorrie Epling,<sup>1</sup> Juli Teague,<sup>1</sup> Mark A. Jacobson,<sup>1</sup> Russell P. Tracy,<sup>2</sup> Lawrence Corey,<sup>3</sup> and Steven G. Deeks<sup>1</sup>

<sup>1</sup>Departments of Medicine, Epidemiology, and Biostatistics, University of California, San Francisco; <sup>2</sup>Departments of Pathology and Biochemistry, University of Vermont College of Medicine, Burlington; <sup>3</sup>Departments of Laboratory Medicine, Medicine and Microbiology, University of Washington, Fred Hutchison Cancer Research Center, Seattle

**Background.** Elevated immune activation persists during treated human immunodeficiency virus (HIV) infection and is associated with blunted CD4 recovery and premature mortality, but its causes remain incompletely characterized. We hypothesized that asymptomatic cytomegalovirus (CMV) replication might contribute to immune activation in this setting.

**Methods.** Thirty antiretroviral therapy–treated HIV-infected CMV-seropositive participants with CD4 counts <350 cells/mm<sup>3</sup> were randomized to receive valganciclovir 900 mg daily or placebo for 8 weeks, followed by an additional 4-week observation period. The primary outcome was the week 8 change in percentage of activated (CD38<sup>+</sup> HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells.

**Results.** Fourteen participants were randomized to valganciclovir and 16 to placebo. Most participants (21 [70%] of 30) had plasma HIV RNA levels <75 copies/mL. The median CD4 count was 190 (IQR: 134–232) cells/mm<sup>3</sup>, and 12 (40%) of 30 had detectable CMV DNA in saliva, plasma, or semen at baseline. CMV DNA continued to be detectable at weeks 4–12 in 7 (44%) of 16 placebo-treated participants, but in none of the valganciclovir-treated participants ( $P = .007$ ). Valganciclovir-treated participants had significantly greater reductions in CD8 activation at weeks 8 ( $P = .03$ ) and 12 ( $P = .02$ ) than did placebo-treated participants. These trends were significant even among those with undetectable plasma HIV RNA levels.

**Conclusions.** CMV (and/or other herpesvirus) replication is a significant cause of immune activation in HIV-infected individuals with incomplete antiretroviral therapy–mediated CD4<sup>+</sup> T cell recovery.

**Clinical Trials Registration.** NCT00264290.

Despite clear improvements in morbidity and mortality in the modern antiretroviral therapy era, treated human immunodeficiency virus (HIV)–infected individuals continue to have at least a 10-year shorter life

expectancy than the general population and remain at higher risk for many non–AIDS-associated morbidities associated with aging [1–3]. This risk is greatest in those with persistently low CD4<sup>+</sup> T cell counts [4–6]. This is of major clinical relevance, since up to one-third of HIV-infected individuals initiating antiretroviral therapy at CD4<sup>+</sup> T cell counts <200 cells/mm<sup>3</sup> fail to restore normal CD4<sup>+</sup> T cell counts, even after 10 years of viral suppression [7–9]. We and others have observed that most HIV-infected individuals continue to have abnormal T cell activation despite years of treatment-mediated viral suppression [10–13], which is associated with blunted CD4<sup>+</sup> T cell recovery [11, 14]. Higher plasma levels of the inflammatory cytokine interleukin-6 (IL-6) are also associated with increased all-cause mortality and cardiovascular disease events among treated

Received 2 September 2010; accepted 30 December 2010.

Potential conflicts of interest: none reported.

Presented in part: 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, California, 27 February through 2 March 2010. Abstract 380.

Correspondence: Peter W. Hunt, MD, UCSF Positive Health Program, SFGH Building 80, Ward 84, 995 Potrero Ave, San Francisco, CA 94110 (phunt@php.ucsf.edu).

**The Journal of Infectious Diseases** 2011;203:1474–83

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0022-1899 (print)/1537-6613 (online)/2011/20310-0001\$14.00

DOI: 10.1093/infdis/jir060

HIV-infected individuals, which further suggests a negative effect of inflammation and immune activation on clinical outcomes among these patients [15].

Although the causes of persistent immune activation during suppressive antiretroviral therapy remain incompletely characterized, several observations suggest that asymptomatic cytomegalovirus (CMV) replication may be an important contributor. First, the prevalence of CMV coinfection approaches 90% among HIV-infected individuals and perhaps even higher among homosexual men [16–18]. Second, among healthy HIV-uninfected individuals, asymptomatic CMV coinfection is associated with higher T cell activation and is responsible for nearly 10% of the entire circulating memory T cell repertoire [19, 20]. CMV-specific T cell responses are even higher in HIV-infected individuals, particularly those receiving antiretroviral therapy [21]. Third, CMV infection is associated with more rapid progression to non-CMV AIDS events and mortality in both untreated and treated HIV infection [22–26]. Last, higher CMV-specific immune responses have been strongly associated with greater atherosclerosis in both HIV-infected and -uninfected individuals [27, 28], and treatment of CMV reduces the risk of atherosclerosis in solid-organ transplant recipients [29]. Chronic immune activation from asymptomatic CMV infection may also have long-term effects on the immune system. For example, asymptomatic CMV coinfection is associated with accelerated immunosenescence and mortality among elderly HIV-uninfected individuals, which suggests a major role of this virus in aging [30–36]. Although the long-term effects of CMV in HIV-infected individuals have not been well studied, there is a growing appreciation for accelerated immunosenescence and aging in HIV-infected individuals [37]. It remains unclear, however, whether CMV replication increases HIV disease progression and risks of age-associated diseases by increasing immune activation or whether it is simply a marker for immunodeficiency.

To determine whether asymptomatic CMV (and/or other herpesvirus) replication contributes to immune activation in patients with treated HIV infection, we conducted a randomized placebo-controlled trial of valganciclovir in HIV-infected individuals with incomplete CD4<sup>+</sup> T cell recovery during antiretroviral therapy. Valganciclovir has potent activity against CMV and most other herpesviruses and is safe in patients with HIV infection [38]. Furthermore, a prophylactic regimen of 900 mg per day taken for 8 weeks decreases salivary CMV DNA levels by 80% in asymptomatic HIV-infected and -uninfected individuals [39].

## METHODS

### Study Participants

CMV-seropositive adults with chronic HIV infection who had been receiving a stable antiretroviral therapy regimen for at least

6 months and with CD4<sup>+</sup> T cell counts <350 cells/mm<sup>3</sup> for ≥1 year were eligible to participate in the study. We chose to enroll individuals with persistently low CD4<sup>+</sup> T cell counts to enrich for higher T cell activation levels [11, 14]. Patients were ineligible if they reported <90% adherence to their antiretroviral regimen; had any serious illness requiring hospitalization or intravenous antibiotics during the preceding 3 months; had active CMV end-organ disease necessitating treatment; had received ganciclovir or valganciclovir during the previous month; were receiving concurrent nephrotoxic, immunosuppressive, or immunomodulatory drugs; were pregnant or breastfeeding; or had any of the following abnormal laboratory values: absolute neutrophil count, <1000 cells/mm<sup>3</sup>; platelet count, <100,000 cells/mm<sup>3</sup>; hemoglobin, <8 mg/dL; or creatinine clearance, <50 mL/min. To enrich for participants with high T cell activation, the percentage of activated (CD38<sup>+</sup> HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells was measured at screening on fresh whole blood and potential participants with <10% activated CD8<sup>+</sup> T cells were excluded. All participants provided written informed consent for the study, and the study was approved by the Committee on Human Research at the University of California San Francisco.

### Study Procedures

Enrolled participants were randomized to receive oral valganciclovir or placebo, 900 mg daily for 8 weeks, followed by a 4-week observation period. Randomization was stratified by plasma HIV RNA level (<75 vs ≥75 copies/mL). Participants, clinicians, and laboratory personnel were blinded to treatment assignment. Participants were monitored biweekly during the treatment period for CD4<sup>+</sup> T cell counts and plasma HIV RNA levels and screened for evidence of toxic effects (complete blood count and renal function tests). Saliva, peripheral blood mononuclear cells (PBMCs), and plasma were cryopreserved at baseline and every 4 weeks for immunological and virological testing at the end of the study. Semen was also cryopreserved at baseline and at week 8 from consenting male participants.

### Laboratory Measurements

**Screening for T Cell Activation.** The percentage of activated (CD38<sup>+</sup> HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells was measured on fresh whole blood at screening to exclude participants with <10% activated CD8<sup>+</sup> T cells using methods described elsewhere [14].

**T Cell Activation on Trial Samples.** T cell activation was measured on PBMCs in batch at the end of the study using methods that have been optimized and validated for PBMCs. Cryopreserved PBMCs were rapidly thawed in warm media, washed, stained with Viacount (Millipore), and run on a Guava PCA (Millipore) for cell count and viability. One sample was excluded for low cell viability (23%), but the remaining samples ranged from 70% to 97% viability (median, 92%). PBMCs were stained with AquaAmine Reactive Dye (Invitrogen) to exclude nonviable cells and for CD3, CD4, CD38, HLA-DR (BD

Biosciences), and CD8 (Invitrogen). Stained cells were run on a BD LSR II and data analyzed using FlowJo (Tree Star). The frequencies of CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD8<sup>+</sup> T cells expressing both CD38 and HLA-DR were determined using fluorescent minus 1 gates to define double-positive cells.

**Herpesvirus DNA Levels.** DNA was extracted from cryopreserved saliva, plasma, and seminal plasma for the quantification of CMV with a double-primer set to the UL55 and UL123-exon 4 as reported elsewhere [39]. Assays for *Epstein-Barr virus* (EBV), human herpesvirus type 8 (HHV-8), and HHV-6 were also performed on the same samples with the same extraction methods as previously described [39–41]. Samples with  $\geq 3$  copies/reaction and  $> 150$  copies/mL of sample were considered to have a positive result. Several negative and positive controls were run with each reaction, including 2 reaction mixtures without DNA (negative controls) and  $\geq 1$  sample with a known quantity of CMV DNA. An internal control was amplified with each specimen to ensure that negative results were not attributable to PCR inhibition.

**Herpes Simplex Virus Type 2 Serological Characteristics.** Baseline serum samples were tested using a gG-2-specific serology kit (HerpeSelect Herpes Simplex Virus Type 2 [HSV-2] enzyme-linked immunosorbent assay; Focus Diagnostics).

**Inflammatory Biomarkers.** Plasma samples from weeks 0, 4, 8, and 12 were assessed for biomarkers, including levels of high-sensitivity C reactive protein (hs-CRP), IL-6, D-dimer, soluble CD14, and cystatin C, by means of immunoassay at the Laboratory for Clinical Biochemistry Research at the University of Vermont [15, 42, 43]. Coefficients of variation were 5.1%, 12.2%, 15.0%, 6.7%, and 2.5%, respectively.

### Statistical Analysis

The primary analysis compared the change in percentage of activated CD8<sup>+</sup> T cells from baseline to week 8 between valganciclovir- and placebo-treated participants. Changes in the percentage of activated T cells across all time points were assessed with generalized estimating equations, and differences in the change from baseline between groups at each time point were assessed with interaction terms. The percentage of activated T cells was log-transformed to satisfy model assumptions. Linear predictions from the models were back-transformed for graphical representation of the data. Changes in the proportion of participants with positive herpesvirus DNA levels over time were assessed with the Cochran Q test. Between-group comparisons of proportions were assessed with the Fisher exact test.

The power calculations were as follows. We assumed a standard deviation of 5% in the week 8 change in percentage of activated CD8<sup>+</sup> T cells from baseline, a likely rate of study noncompletion of 2 enrolled participants per study arm, and a Type I error rate of 5%, yielding 80% power to detect a difference between groups as small as 5.5% with 15 planned participants in each arm.

## RESULTS

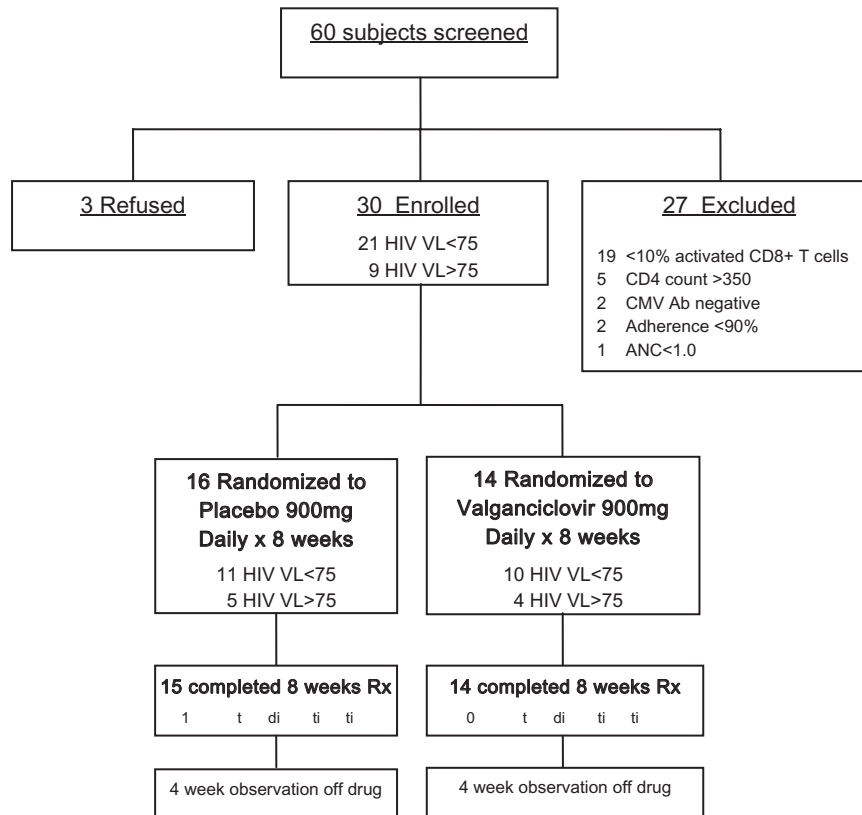
### Characteristics of Participants

Of 60 screened subjects, 3 refused participation, 27 were excluded, and 30 met inclusion criteria and were enrolled (Figure 1). The most common reason for exclusion was  $< 10\%$  activated CD8<sup>+</sup> T cells ( $n = 19$ ; median, 5.9%, range: 3.7% to 7.6%). Most enrolled participants (93%) were men, the median age was 49 (IQR: 44 to 56), and the median duration of antiretroviral therapy was 27 (IQR: 18 to 38) months (Table 1). Most had current CD4<sup>+</sup> T cell counts of  $< 200$  cells/mm<sup>3</sup> and self-reported pretreatment nadir CD4 counts of  $< 50$  cells/mm<sup>3</sup>, and there was no evidence for a difference between treatment arms ( $P > .18$  for both comparisons). Among the 9 participants with detectable viremia at baseline, the median plasma HIV RNA level was 4.1 log<sub>10</sub> copies/mL (range, 3.4–5.7 log copies/mL) and there was no evidence for a difference between arms. The majority of participants randomized to placebo and valganciclovir were HSV-2 seropositive (63% and 86%, respectively), and approximately one-third in each group were receiving daily acyclovir prophylaxis at enrollment, which continued throughout the study. All 14 participants randomized to valganciclovir completed the trial, but 1 of the 16 placebo-treated participants discontinued the study medication prematurely (congestive heart failure exacerbation).

### Changes in Herpesvirus DNA Levels

At baseline, 7 (44%) of 16 placebo-treated participants and 5 (36%) of 14 valganciclovir-treated participants had detectable CMV DNA levels in saliva, seminal plasma, or peripheral blood plasma. Median (range) titers in those with detectable levels were 3737 (218 to 28,255), 15,541 (153 to 862,223), and 159 (158 to 160) copies/mL, respectively. CMV DNA levels were most commonly detectable in seminal plasma and saliva (Figure 2), with only 2 participants having detectable peripheral blood plasma CMV DNA levels at baseline (both placebo-treated). Whereas there was no evidence for a change in CMV DNA levels in the placebo arm (titers or proportion positive), valganciclovir significantly reduced CMV DNA levels such that no valganciclovir-treated participant had a detectable CMV DNA level at any site after baseline, even 4 weeks after stopping therapy ( $P = .007$  for between-group comparison of proportions with a detectable level at any postbaseline time point).

We also assessed the effect of valganciclovir on other herpesvirus DNA levels. Salivary EBV DNA was detectable in 11 (69%) of 16 placebo-treated participants and 11 (79%) of 14 valganciclovir-treated participants at baseline. Whereas there was no evidence for a change in the proportion of placebo-treated participants with detectable EBV DNA levels, the proportion of valganciclovir-treated participants with detectable salivary EBV DNA levels declined significantly through week 8, then rebounded to near pretreatment levels by week 12 ( $P = .003$  for changes within valganciclovir-treated participants). A much lower proportion of



**Figure 1.** Screening and enrollment status. The disposition of all screened subjects is outlined. Of 60 screened subjects, 27 were excluded for the reasons noted, 3 refused participation, and 30 were enrolled. Randomization was stratified by plasma human immunodeficiency virus (HIV) RNA level (<75 or ≥75 copies/mL) in block sizes of 2. All 14 participants randomized to valganciclovir completed the trial, whereas 1 of the 16 placebo-treated participants discontinued study medication prematurely (congestive heart failure exacerbation). ANC, absolute neutrophil count; CMV, cytomegalovirus; VL, virus level.

participants in each group had detectable HHV-8 or HHV-6 DNA levels in either saliva or semen, and there was no evidence for a change in this proportion in either treatment group over time. There was also no evidence for a change in salivary HHV-8 or HHV-6 DNA levels over time in valganciclovir-treated participants with detectable levels at baseline.

### Changes in T Cell Activation

At baseline, the median percentage of activated (CD38<sup>+</sup> HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells was 20.0% in the placebo arm (interquartile range [IQR], 16.4%–35.5%) and 20.2% (IQR, 15.9%–35.8%) in the valganciclovir arm. Whereas there was no evidence for a change in the percentage of activated CD8<sup>+</sup> T cells at any time point in placebo-treated participants, the percentage of activated CD8<sup>+</sup> T cells declined by a mean 4.0% at week 8 (95% CI, –0.9% to –5.7%) and 4.1% at week 12 (95% CI, –1.1% to –6.8%) in valganciclovir-treated participants, representing an approximately 20% relative decrease from baseline levels (Figures 3A and 3B). Compared with placebo-treated participants, valganciclovir-treated participants experienced a greater reduction in CD8<sup>+</sup> T cell activation at weeks 8 ( $P = .03$ ) and 12 ( $P = .02$ ) (Figure 3C), even

when analysis was restricted to those with plasma HIV RNA levels <75 copies/mL ( $P = .04$  and  $P = .006$ , respectively) or those with <20.2% activated CD8<sup>+</sup> T cells at baseline ( $P = .01$  and  $P = .01$ , respectively). This valganciclovir-mediated reduction in T cell activation is comparable to roughly 40% of the observed increase in CD8<sup>+</sup> T cell activation in treated HIV-infected individuals relative to HIV-uninfected control participants studied in the same laboratory (median, 10% [IQR: 6% to 14%] activated CD8<sup>+</sup> T cells; data not shown). When the analysis was restricted to those with negative HSV-2 serology or those continuing to receive acyclovir prophylaxis ( $n = 16$ ), valganciclovir-treated participants continued to have a greater median reduction in the percentage of activated CD8<sup>+</sup> T cells at week 8 (–3.2%, IQR: –4.8% to –1.0%) than did placebo-treated participants (+0.8%, IQR: –0.7 to +3.3%) but this difference was not statistically significant ( $P = .07$ ). Whereas there was no evidence for a change in CD4<sup>+</sup> T cell activation levels in the placebo arm ( $P > .36$  for all time points), there were trends toward a decrease in CD4<sup>+</sup> T cell activation levels at weeks 4 ( $P = .12$ ) and 8 ( $P = .08$ ) in the valganciclovir arm, but the differences between arms were not significant ( $P > .24$  for all time points) (Figure 3D–F). There was no evidence for a

**Table 1. Baseline Characteristics of Participants**

Characteristic	Placebo group (n = 16)	Valganciclovir group (n = 14)
Age, years	50 (44–59)	48 (43–55)
Male sex, no. (%)	16 (100)	12 (86)
ART regimen, no. (%)		
PI-based	11 (69)	11 (79)
NNRTI-based	3 (19)	2 (14)
NRTIs only	2 (13)	1 (7)
Duration of ART regimen, months	27 (17–35)	28 (16–40)
Self-reported ART adherence past 30 days, %	100 (96–100)	100 (96–100)
CD4 <sup>+</sup> T cell count, cells/mm <sup>3</sup>	187 (108–218)	207 (176–249)
Self-reported CD4 count nadir, cells/mm <sup>3</sup>	64 (20–114)	18 (8–54)
Plasma HIV RNA level, copies/mL, no. (%)		
<75	11 (69)	10 (71)
≥75	5 (31)	4 (29)
HSV-2 seropositive, no. (%)	10 (63)	12 (86)
Currently taking acyclovir, no. (%)	6 (38)	5 (36)
Estimated creatinine clearance, mL/min	85 (73–101)	94 (82–108)
Absolute neutrophil count, ×10 <sup>3</sup> cells/mm <sup>3</sup>	2.2 (1.9–3.3)	2.9 (1.9–3.5)
Hemoglobin, mg/dL	14.6 (13.3–15.2)	14.4 (13.4–15.4)
Platelet count, ×10 <sup>3</sup> /mm <sup>3</sup>	215 (178–270)	255 (223–304)

relationship between the presence of detectable CMV DNA at baseline and either the baseline frequency of activated T cells or the extent of reduction in T cell activation during valganciclovir therapy.

#### Changes in Soluble Biomarkers

Whereas we observed no evidence for a change in plasma hs-CRP levels at any time point in placebo-treated participants ( $P > .44$  for all time points), hs-CRP levels declined by week 8 in valganciclovir-treated participants ( $P = .02$ ), but the difference in week 8 changes between treatment groups was not statistically significant ( $P = .21$ ) (Figure 3G–I). There was no evidence for a change in plasma IL-6, d-Dimer, soluble CD14, or cystatin C levels at any time point within either treatment group.

#### Changes in CD4<sup>+</sup> T Cell Counts and Plasma HIV RNA Levels

There was no evidence for a change from baseline in either CD4<sup>+</sup> T cell counts or plasma HIV RNA levels in either valganciclovir- or placebo-treated participants at any time point, nor was there evidence for changes over time in either measure as assessed by linear mixed models (Figures 4A and 4B).

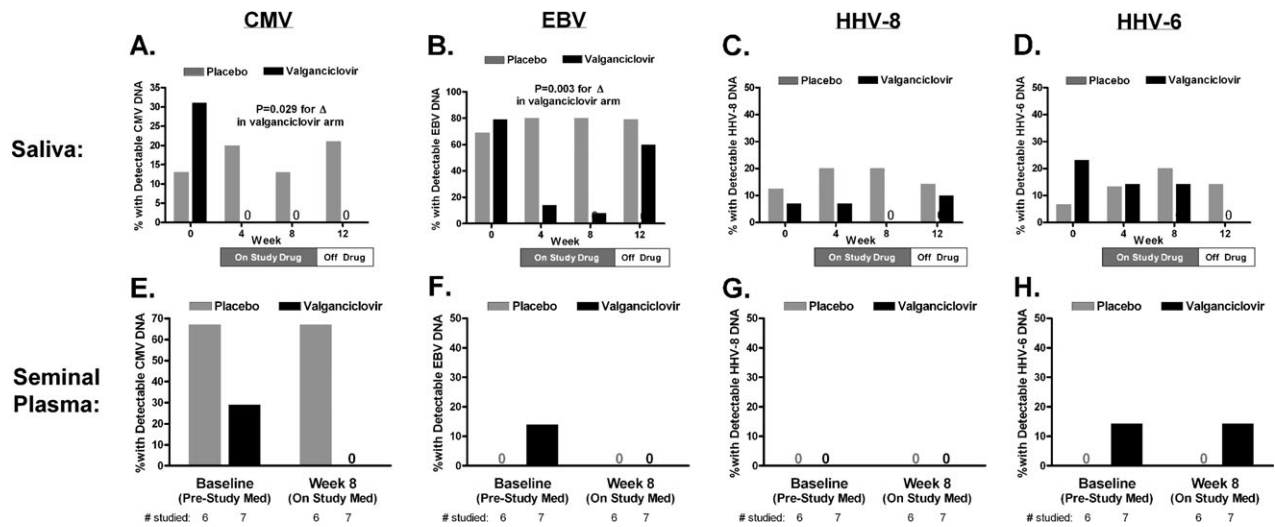
#### Adverse Events

There was only 1 grade 3 adverse event during the trial: a placebo-treated patient was hospitalized for congestive heart failure prior to the scheduled week 4 visit. There were no other

serious adverse events. Specifically among valganciclovir-treated participants, there was no evidence for a change in absolute neutrophil counts ( $P = .61$ ), hemoglobin levels ( $P = .37$ ), platelet counts ( $P = .67$ ), or serum creatinine concentrations (mean,  $-0.006$  [95% CI:  $-0.012$  to  $+0.001$ ] mg/dL per week through week 8;  $P = .12$ ) during the 8-week treatment interval.

## DISCUSSION

Although antiretroviral therapy has dramatically improved the health of HIV-infected individuals, abnormal immune activation persists in most of these individuals and predicts subsequent morbidity and earlier mortality. To assess whether low-level asymptomatic CMV replication contributes to persistent immune activation in these patients, we conducted a randomized placebo-controlled trial of valganciclovir among HIV-infected individuals with persistently low CD4<sup>+</sup> T cell counts despite antiretroviral therapy. Eight weeks of valganciclovir therapy resulted in potent suppression of CMV DNA levels, which persisted for at least 4 weeks after therapy was discontinued. Valganciclovir also resulted in significantly greater declines in the frequency of activated CD8<sup>+</sup> T cells than placebo, which persisted for at least 4 weeks after treatment cessation. Reductions in hs-CRP levels were also observed with valganciclovir. Because we observed no change in plasma HIV RNA levels among viremic participants and the reduction in T cell



**Figure 2.** Changes in herpesvirus DNA levels in saliva and semen with valganciclovir therapy. Herpesvirus DNA levels were assessed by polymerase chain reaction (lower limit of detection, 150 copies/mL) on saliva (at weeks 0, 4, 8, and 12) and seminal plasma (at weeks 0 and 8 only). The proportion of participants with a positive herpesvirus DNA level result is plotted for both valganciclovir and placebo groups in saliva and seminal plasma for cytomegalovirus (CMV) (A and E), Epstein-Barr virus (EBV) (B and F), human herpesvirus (HHV) type 8 (C and G), and HHV type 6 (D and H). Plotted *P* values test whether the proportion of participants with a positive DNA level result changes over time within each treatment group (Cochran *Q* test). The proportion of participants with positive CMV and EBV DNA levels in saliva declined significantly with valganciclovir therapy but not with placebo. The proportion of participants with detectable salivary CMV DNA levels remained suppressed 4 weeks after study drug discontinuation, whereas the proportion with detectable EBV levels rebounded to pretreatment levels. Whereas 2 (29%) of 7 valganciclovir-treated participants had detectable CMV DNA plasma at baseline, none had it at week 8.

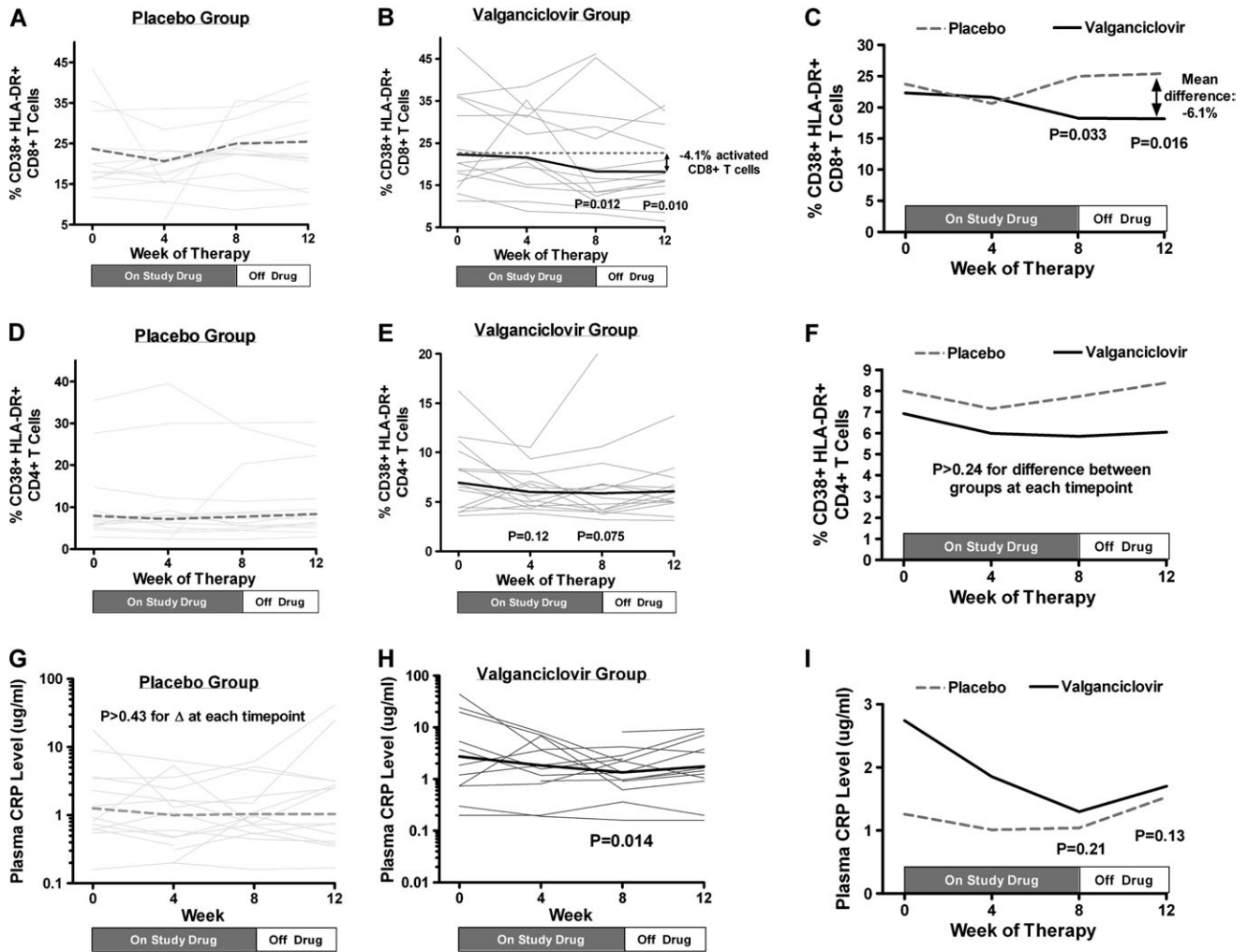
activation remained significant when analysis was restricted to those with undetectable plasma HIV RNA levels, the reduction in T cell activation with valganciclovir therapy does not seem to be explained by a direct effect on HIV replication. These results suggest that CMV and/or other herpesvirus coinfections are a substantial cause of in vivo T cell activation among treated HIV- and CMV-coinfected individuals.

Treating herpesvirus coinfections has long been pursued as a potential strategy to delay HIV disease progression. Prior to the introduction of protease inhibitors in the mid-1990s, several small trials established a mortality benefit to high-dose acyclovir when used in combination with 1 or 2 antiretroviral drugs [44]. More recently, standard prophylactic doses of acyclovir to prevent genital ulcer disease have been shown to decrease the rate of CD4<sup>+</sup> T cell decline in untreated HIV- and HSV-2-coinfected individuals in a large international trial [45]. Although some research groups have suggested that acyclovir might exhibit a direct antiviral effect on HIV replication [46, 47], these in vitro studies have used much higher concentrations of acyclovir than used in current dosing regimens and evidence of acyclovir-associated resistance mutations in HIV-1 reverse transcriptase has not been demonstrated in vivo. This has led many to hypothesize that the beneficial effect of acyclovir on HIV progression is mediated by a reduction in HSV-2-induced immune activation [45].

Because asymptomatic chronic CMV infection elicits massive immune responses in HIV-uninfected individuals [20] and even

higher responses in HIV-infected individuals receiving anti-retroviral therapy [21], we suspect that the valganciclovir-mediated reductions in T cell activation that we observed were largely mediated by reductions in CMV replication. While we have not seen a relationship between detectable salivary CMV DNA levels and T cell activation in this trial or in a recent observational study [48], the relationship between CMV replication and immune activation is likely to be complex. For example, individuals with the strongest immune responses to very low amounts of CMV replication are likely to have the strongest antiviral responses, limiting the detection of CMV in bodily fluids.

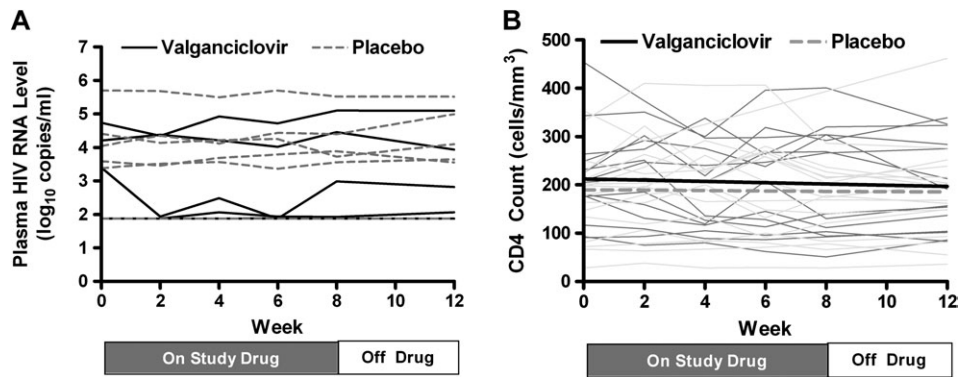
However, we cannot definitively exclude the possibility that valganciclovir's effect on T cell activation is mediated by suppression of other herpesviruses. EBV DNA levels also declined significantly during valganciclovir therapy but, unlike those of CMV DNA, rebounded to pretreatment levels 4 weeks after treatment cessation. Because CD8<sup>+</sup> T cell activation remained suppressed at this time, it seems unlikely that valganciclovir's effect on CD8<sup>+</sup> T cell activation is mediated by a reduction in EBV replication. Although we observed no evidence for a decline in HHV-8 or HHV-6 levels during valganciclovir therapy, a recent trial using the exact same dose and duration of valganciclovir—but more frequent sampling of the oropharynx and enrichment for participants with high salivary HHV-8 DNA levels—found significant reductions in HHV-8 DNA [39].



**Figure 3.** Changes in T cell activation and C reactive protein (CRP) levels with valganciclovir therapy. The percentage of activated (CD38<sup>+</sup> HLA-DR<sup>+</sup>) CD8<sup>+</sup> T cells, percentage of activated CD4<sup>+</sup> T cells, and plasma CRP levels were assessed over time in both placebo-treated (A, D, G) and valganciclovir-treated participants (B, E, H) with generalized estimating equations. The thin gray lines (A, B, D, E, G, H) indicate individual participant changes, and the thick lines represent the estimated mean changes at each time point within each treatment arm. Mean changes from baseline at each time point were also plotted and compared between placebo- and valganciclovir-treated participants with generalized estimating equations (C, F, I), with *P* values referring to differences in the change from baseline between treatment arms at each time point. Whereas there was no evidence for a change from baseline in the percentage of activated CD8<sup>+</sup> T cells at any time point for placebo-treated participants (A), valganciclovir-treated participants experienced a mean decline of 4% activated CD8<sup>+</sup> T cells by week 8 (*P* = .01) and continued to have a mean 4.1% fewer activated CD8<sup>+</sup> T cells than baseline at week 12 (*P* = .01, B). Compared with placebo-treated participants, those receiving valganciclovir experienced a greater decline in CD8<sup>+</sup> T cell activation from baseline at week 8 (*P* = .03) and from baseline to week 12 (*P* = .02, C). Whereas there was no evidence for a change in CD4<sup>+</sup> T cell activation levels in the placebo arm (*P* > .36 for all time points, D), there were trends toward a decrease in CD4<sup>+</sup> T cell activation levels at weeks 4 (*P* = .12) and 8 (*P* = .08) in the valganciclovir arm (E), but the differences between arms were not significant (*P* > .24 for all time points, F). Whereas there was no evidence for a change in the placebo arm (*P* > .43 for all time points, G), CRP levels declined significantly by week 8 in the valganciclovir arm (*P* = .01, H), but the difference in CRP changes between arms was not significant (*P* ≥ .13 for all time points, I).

Valganciclovir also has known antiviral activity against HHV-6 [49]. Last, although there seemed to be reductions in CD8<sup>+</sup> T cell activation even in valganciclovir-treated participants who were either HSV-2 seronegative or receiving concurrent acyclovir prophylaxis, we cannot rule out the possibility that reductions in HSV-2 replication may have partially contributed to the effects observed. A similar trial of acyclovir in HIV-infected

individuals receiving antiretroviral therapy might help address this possibility, because acyclovir has excellent activity against HSV-2 but very little activity against CMV. Last, although acyclovir clearly reduces plasma HIV RNA levels in untreated HIV-infected individuals [45], we observed no evidence for a reduction in plasma HIV RNA levels in viremic valganciclovir-treated participants, and the effect of valganciclovir on CD8<sup>+</sup>



**Figure 4.** Changes in plasma human immunodeficiency virus (HIV) RNA levels and CD4<sup>+</sup> T cell counts during valganciclovir therapy. Changes in plasma HIV RNA levels (A) and CD4<sup>+</sup> T cell counts (B) were assessed over time in valganciclovir- and placebo-treated participants. Each line represents an individual participant's trajectory. Dark thick lines in panel B represent the estimated mean changes over time within each treatment group from a linear mixed model. There was no evidence for changes over time in either plasma HIV RNA levels or CD4<sup>+</sup> T cell counts in either treatment group.

T cell activation remained significant even when analysis was restricted to participants with undetectable plasma HIV RNA levels.

While we did not observe any evidence for a change in CD4<sup>+</sup> T cell counts during the 12-week observation period, our prior cross-sectional study suggests that each absolute 4% increase in the frequency of activated CD8<sup>+</sup> T cells would be associated with just 28 fewer CD4<sup>+</sup> T cells gained over a median of 2 years of suppressive antiretroviral therapy, so our trial was far too small and short to rule out an effect on CD4<sup>+</sup> T cell counts [14]. However, it remains possible that T cell activation caused by CMV replication may have a lesser effect on CD4<sup>+</sup> T cell recovery than T cell activation caused by other factors (ie, HIV release from latently infected cells, microbial translocation, and so forth). Similarly, although we observed some evidence for a valganciclovir-mediated decrease in hs-CRP levels, a larger trial would be necessary for adequate power to detect changes in other surrogate markers of interest, including the inflammatory biomarkers IL-6, D-dimer, and endothelial function, given the high within-subject variability of these measurements. Subsequent studies will also need to assess whether these findings are generalizable to individuals with higher CD4<sup>+</sup> T cell counts and lower CD8<sup>+</sup> T cell activation levels.

The clinical relevance of the observed valganciclovir-mediated reduction in immune activation is also unclear, and still larger studies would be necessary to establish a benefit of valganciclovir (or other anti-CMV therapy) in preventing AIDS- and non-AIDS-associated morbidity and mortality. Although we did not observe any toxic effects of valganciclovir in this study, the known marrow-suppressive and teratogenic effects of valganciclovir must be balanced against the potential for clinical benefit if such trials are to be conducted.

In summary, this randomized controlled trial of valganciclovir revealed that CMV and/or other herpesvirus replication is a significant cause of persistent T cell activation in patients with

treated HIV infection. Although the clinical relevance of this finding remains unclear, persistent immune activation and inflammation is a major predictor of premature morbidity and mortality among these patients [15]. Furthermore, given persistent functional T cell defects and immunosenescence even in treated HIV-infected individuals with optimal CD4<sup>+</sup> T cell recovery [50], and the clear associations between CMV and immunosenescence in HIV-uninfected individuals [30–36], a larger trial of anti-CMV therapy for treated HIV-infected patients is clearly warranted.

## Funding

This work was supported in part by the University of California San Francisco (UCSF)/Gladstone Center for AIDS Research (P30 AI27763, P30 MH59037), UCSF-San Francisco General Hospital General Clinical Research Center (Clinical Research Feasibility Funds), NIAID (AI065244 and AI055273), the Center for AIDS Prevention Studies (P30 MH62246), the UCSF Clinical and Translational Science Institute (UL1 RR024131-01), and an investigator-initiated research grant, as well as donated drug and placebo from Roche Laboratories, Inc.

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