

## Kinetics of Cytomegalovirus Load Decrease in Solid-Organ Transplant Recipients after Preemptive Therapy with Valganciclovir

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**The availability of valganciclovir (VGCV) has significantly simplified the treatment of human cytomegalovirus (HCMV) infection after solid-organ transplantation. We show that there was no difference in the kinetics of the decrease in HCMV load after preemptive therapy with VGCV in 22 solid-organ transplant recipients ( $T_{1/2} = 2.16$  days), compared with that in 23 patients treated with intravenous ganciclovir (GCV) ( $T_{1/2} = 1.73$  days;  $P = .63$ ). Preemptive therapy with VGCV provides control of HCMV replication that is comparable to that achieved with preemptive intravenous therapy with GCV.**

Human cytomegalovirus (HCMV) remains an important cause of morbidity after solid-organ transplantation. Infection has been associated with a number of direct and indirect effects in immunocompromised hosts, including hepatitis, prolonged pyrexia, and acute and chronic graft rejection [1]. HCMV replicates rapidly in the human host, and the viral load is directly related to the probability of disease development [2–4]. Ganciclovir (GCV) has become one of the most commonly used antivirals to control HCMV replication in solid-organ transplant recipients. Therapeutic approaches include prophylaxis, which is usually administered to patients at high risk of developing HCMV disease, and preemptive therapy, in which the drug is administered on the basis of the detection of active

HCMV replication by sensitive laboratory methods such as the antigenemia assay or polymerase chain reaction (PCR). Recently, controlled clinical trials of the valine ester prodrug of GCV, valganciclovir (VGCV), have shown that it is effective in the treatment of HCMV in HIV-infected patients [5, 6] and in the prophylaxis of high-risk solid-organ transplant recipients [7]. VGCV offers many advantages over existing formulations of GCV—high plasma levels of drug can be achieved through oral dosing, and patients no longer require extensive hospitalization for intravenous (iv) infusions. A dose of 900 mg of VGCV provides plasma GCV exposure that is approximately comparable to a 5-mg/kg dose of iv GCV [8, 9]. At present, there have been no trials that have compared preemptive therapy with VGCV with that of iv GCV in solid-organ transplant recipients. However, increasingly, VGCV is being used in such a treatment modality. We therefore investigated whether the kinetics of the control of HCMV replication in patients receiving VGCV were comparable to those that we have previously observed in patients receiving iv GCV [10].

**Subjects, materials, and methods.** In a retrospective non-randomized study, renal- or liver-transplant recipients receiving either oral VGCV (900 mg twice/day) or iv GCV (5 mg/kg twice/day) for the treatment of HCMV infection were identified between October 2001 and September 2003. Patients were monitored for HCMV DNAemia at least twice weekly, when they were hospitalized, or when they attended the outpatient clinic. HCMV infection was defined as 2 consecutive positive HCMV PCR results (cutoff, 200 genomes/mL). HCMV PCR was done on a routine basis with an in-house TaqMan (ABI)-based method adapted from our previously published method [11]. Briefly, DNA was extracted from 200  $\mu$ L of whole blood by use of a Qiagen extraction kit (Minden), according to the manufacturer's instructions. Real time-amplification of HCMV DNA used glycoprotein B-specific primers, as described elsewhere [12] (5'-GAGGACAACGAAATCCTGTTGGGCA-3' [gB1] and 5'-TCGACGGTGGAGATACTGCTGAGG-3' [gB2]). The 150-bp product was detected in real time by use of a 29-mer TaqMan probe (5'-CAATCATGCGTTTGAAGAGGTTAGTCCACG-3' [gb-P3]), which was labeled at the 5' end with 6-FAM and at the 3' end with TAMRA. The conditions for the PCR were as follows: 2.5  $\mu$ L of 1 $\times$  PCR buffer (that contained 1.5 mmol/L MgCl<sub>2</sub>; Qiagen), 5  $\mu$ L of MgCl<sub>2</sub> (25 mmol/L), 7.5  $\mu$ L of dNTPs (6.25 mmol/L each nucleotide), 1  $\mu$ L of gB1 and gB2 (15 pmol/ $\mu$ L), 1  $\mu$ L of gb-P3 (5 pmol/ $\mu$ L), and 0.25  $\mu$ L of HotStarTaq polymerase (0.25 IU/ $\mu$ L; ABI) made up to a final volume of 20  $\mu$ L with sterile water. Then, 5  $\mu$ L of extracted or control

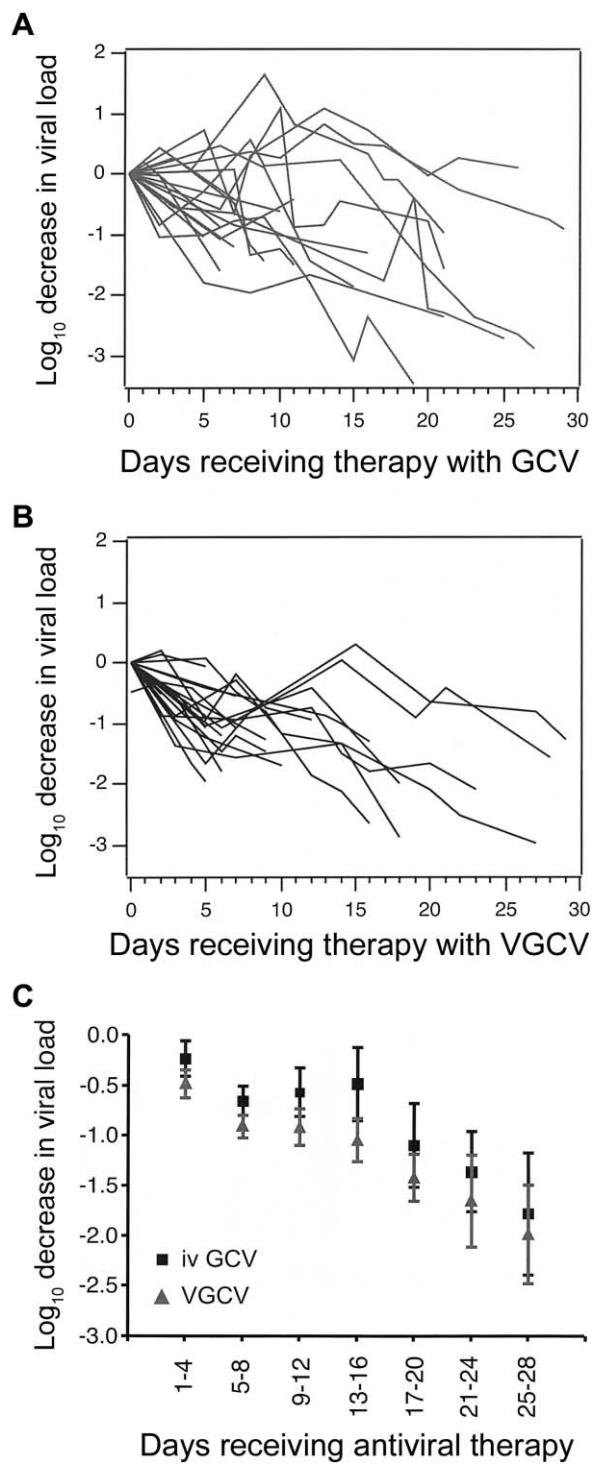
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**Figure 1.** A, Decrease in human cytomegalovirus (HCMV) load from baseline, after the initiation of antiviral therapy for individual patients receiving intravenous (iv) ganciclovir (GCV; A) or oral valganciclovir (VGCV; B). The change in HCMV load from baseline is plotted against days receiving antiviral therapy (data shown for the first 28 days of antiviral therapy). C, Log<sub>10</sub> decrease in HCMV load after preemptive therapy with VGCV (900 mg twice daily) or iv GCV (5 mg/kg twice daily). The mean reduction in HCMV load ( $\pm 1$  SD) is shown for each 3-day window after therapy, for the 28 days of therapy. Squares, iv GCV; triangles, VGCV.

DNA was added to each reaction before the PCR. In addition each TaqMan PCR run contained a dilution series of cloned HCMV gB DNA in triplicate of  $1-10^4$  genomes [13]. PCR cycling conditions were 2 min at 50°C, 10 min at 95°C, and 60 cycles of 15 s at 95°C and 15 s at 60°C. All clinical samples were analyzed in duplicate, and the average HCMV load was calculated by use of the sequence detection system software available on the ABI 7700 platform.

Anti-HCMV therapy was recommended after 2 consecutive positive HCMV PCR results were obtained and was continued until 2 consecutive negative HCMV PCR results were obtained. The choice of anti-HCMV drug was made by the treating physician. The dose of VGCV and GCV was adjusted according to the renal function of the patient, as recommended by the manufacturer.

The length of a viremic episode was defined as the interval between the last negative HCMV PCR result, followed by at least 2 consecutive positive results until the first negative PCR result. Treatment delay was defined as the time from the first positive PCR result until the initiation of antiviral therapy. Baseline HCMV load was defined as the HCMV load measured at the time of starting antiviral therapy. A linear curve was fitted through all available HCMV load data before and after antiviral therapy. The doubling time or half-life was calculated by use of standard exponential growth or decay functions [4]. Groups with continuous variables were compared by use of the Mann-Whitney *U* test. Difference in proportions between groups were calculated by use of Fisher's exact test. All statistical analysis was performed as intent-to-treat analysis with the software R [14].

**Results.** During the period from September 2001 to October 2003, 22 patients (15 liver- and 7 renal-transplant recipients) received preemptive therapy with VGCV (900 mg twice daily). During the same time period, a further 23 patients (12 liver- and 11 renal-transplant recipients) received preemptive therapy with iv GCV (5 mg/kg twice daily). In all patients studied, HCMV loads were determined twice per week before and during therapy. With the exception of 2 patients (both of whom received iv GCV), patients received antiviral therapy until they became negative for HCMV by PCR. A total of 107 samples were quantified before the initiation of antiviral therapy (median, 3 samples/patient), and a further 177 HCMV PCR-positive samples were quantified after the start of antiviral therapy (median, 4 samples/patient).

The demographic characteristics of the 2 treatment groups were well matched in terms of treatment allocation (23 GCV vs. 22 VGCV recipients), age (mean, 43.8 years for GCV recipients vs. 49.3 years for VGCV recipients), and sex (GCV recipients, 14 men/9 women; VGCV recipients, 9 men/13 women). Immunosuppressive regimens were equally distributed within each treatment group ( $P = .57$ ), with patients administered a reg-

**Table 1. Virological response after the initiation of antiviral therapy, according to treatment.**

Characteristic	Treatment		<i>P</i> <sup>a</sup>
	iv GCV	VGCV	
Time to negative PCR, days	14 (2–230)	15 (2–110)	.86
Treatment length, days	16 (1–33)	20 (6–49)	.19
Half-life, days	1.73 (0.87–11.55)	2.16 (0.75–6.93)	.63
Log <sub>10</sub> decrease, genomes/mL			
Day 7	−0.64 (−1.79 to 0.57)	−1.07 (−1.96 to −0.07)	.14
Day 14	−1.17 (−3.07 to 1.07)	−0.98 (−2.12 to 0.29)	.92

**NOTE.** Data are expressed as median (range). GCV, ganciclovir; PCR, polymerase chain reaction; VGCV, valganciclovir.

<sup>a</sup> Mann-Whitney *U* test; significance was set at *P* ≤ .05.

imen that contained either cyclosporin (GCV recipients, *n* = 5; VGCV recipients, *n* = 3) or tacrolimus (GCV recipients, *n* = 16; VGCV recipients, *n* = 17). More patients with primary HCMV infections (donor HCMV positive [D<sup>+</sup>], recipient HCMV negative [R<sup>-</sup>]) received GCV (*n* = 7) than VGCV (*n* = 2); however, this difference did not reach statistical significance (*P* = .27, Fisher's exact test). Treatment groups were well matched for D<sup>-</sup>R<sup>+</sup> (4 GCV recipients and 4 VGCV recipients) and D<sup>+</sup>R<sup>+</sup> (12 GCV recipients and 14 VGCV recipients) combinations. Virological parameters before starting antiviral therapy were comparable for HCMV load at treatment initiation (GCV, 3.55 log<sub>10</sub> genomes/mL vs. VGCV, 3.81 log<sub>10</sub> genomes/mL; *P* = .67), peak HCMV load (GCV, 3.87 log<sub>10</sub> genomes/mL vs. VGCV, 4.15 log<sub>10</sub> genomes/mL; *P* = .68), and doubling time (GCV, 2.03 days vs. VGCV, 1.82 days; *P* = .47). The treatment delay was 8 days in the GCV group and 9 days in the VGCV group (*P* = .98).

After therapy, patients who had received VGCV had a median half-life of decrease in HCMV load of 2.16 days, compared with a median half-life of decrease in HCMV load of 1.73 days in patients who received iv GCV (*P* = .63). The median decrease in HCMV load at day 7 was −1.07 log<sub>10</sub> genomes/mL in patients who received VGCV, compared with −0.65 log<sub>10</sub> genomes/mL in patients who received iv GCV, although this difference did not reach statistical significance (*P* = .14). At day 14, the decrease in HCMV load was similar in both treatment groups (GCV, −1.17 log<sub>10</sub> genomes/mL vs. VGCV, −0.98 log<sub>10</sub> genomes/mL; *P* = .92). Overall, the time to become PCR negative for HCMV was comparable between the 2 groups (median, 14 days for GCV vs. 15.5 days for VGCV; *P* = .86).

Individual decrease profiles for patients receiving preemptive therapy with iv GCV or VGCV are shown in figure 1A, and a composite figure summarizing all patients is shown in figure 1B. As noted in table 1, the decrease rates of HCMV load between the 2 treatment groups were almost identical.

**Discussion.** In the absence of data from randomized, controlled, clinical trials of VGCV, we used a single center's ex-

perience of preemptive therapy to compare the posttherapy kinetics of HCMV replication in solid-organ transplant recipients receiving either VGCV or iv GCV. We reasoned that, because VGCV use was becoming more widespread in the preemptive-therapy setting, it was important to show that the ability to control HCMV replication rapidly and effectively with VGCV was comparable to that achieved with iv GCV. The results clearly show that VGCV (900 mg twice daily) and iv GCV (5 mg/kg twice daily) have similar efficacy levels for the control of HCMV replication in this therapeutic setting. These results concur with the pharmacokinetics of VGCV observed in liver-transplant recipients and in HIV-infected patients—that is, a 900-mg dose of VGCV produces plasma GCV levels comparable to those produced by a 5-mg/kg iv dose of GCV. We have previously shown that replication events occurring before the initiation of preemptive therapy can have a substantial effect on the observed response to therapy [15]. Hence, rapid viral replication (a fast doubling time) and high HCMV load at the start of therapy are both associated with a slower rate of decrease after the initiation of therapy. Such events are consistent with the non-steady-state viral dynamics that are apparent during the period when HCMV loads are 200–10,000 genomes/mL and are reflected in the overshoot in HCMV load frequently observed after the initiation of therapy in the D<sup>+</sup>R<sup>-</sup> setting. In the present analyses, HCMV load at the initiation of therapy and the average doubling time of virus before therapy were well matched between patients who received VGCV and those who received iv GCV. The present study therefore provides the first detailed assessment of HCMV kinetics after preemptive therapy with VGCV in solid-organ transplant recipients and emphasizes the rapid control of HCMV replication that is achievable. However, for completeness, more examples of the control of replication by VGCV in the D<sup>+</sup>R<sup>-</sup> setting is warranted. However, there was no difference in the recurrence of viremia between patients who received GCV and those who received VGCV (7/23 vs. 5/22). The inclusion of measures of viral dynamics in future clinical trials of VGCV in other clinical

settings will facilitate our understanding of the factors required for the successful control of HCMV in transplant recipients.

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