

Contribution of Population Pharmacokinetics to Dose Optimization of Ganciclovir-Valganciclovir in Solid-Organ Transplant Patients

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Treatment of solid-organ transplant (SOT) patients with ganciclovir (GCV)-valganciclovir (VGCV) according to the manufacturer's recommendations may result in over- or underexposure. Bayesian prediction based on a population pharmacokinetics model may optimize GCV-VGCV dosing, achieving the area under the curve (AUC) therapeutic target. We conducted a two-arm, randomized, open-label, 40% superiority trial in adult SOT patients receiving GCV-VGCV as prophylaxis or treatment of cytomegalovirus infection. Group A was treated according to the manufacturer's recommendations. For group B, the dosing was adjusted based on target exposures using a Bayesian prediction model (NONMEM). Fifty-three patients were recruited (27 in group A and 26 in group B). About 88.6% of patients in group B and 22.2% in group A reached target AUC, achieving the 40% superiority margin ($P < 0.001$; 95% confidence interval [CI] difference, 47 to 86%). The time to reach target AUC was significantly longer in group A than in group B (55.9 ± 8.2 versus 15.8 ± 2.3 days, $P < 0.001$). A shorter time to viral clearance was observed in group B than in group A (12.5 versus 17.6 days; $P = 0.125$). The incidences of relapse (group A, 66.67%, and group B, 9.01%) and late-onset infection (group A, 36.7%, and group B, 7.7%) were higher in group A. Neutropenia and anemia were related to GCV overexposure. GCV-VGCV dose adjustment based on a population pharmacokinetics Bayesian prediction model optimizes GCV-VGCV exposure. (This study has been registered at ClinicalTrials.gov under registration no. NCT01446445.)

Human cytomegalovirus (CMV) is a common viral pathogen affecting solid-organ transplant (SOT) recipients, in whom it causes significant morbidity. CMV infection may be clinically manifested as either an acute viral syndrome or a tissue-invasive disease (1–5). Without antiviral drug prophylaxis, most CMV infections occur during the first 3 months posttransplant, when patients are receiving intensive immunosuppressive regimens for the prevention of graft rejection. SOT patients at the highest risk of developing CMV infection are seronegative recipients of organs from seropositive donors (CMV D⁺/R⁻) and those receiving T-cell-depleting agents (6).

The “gold standard” therapy for the prevention and treatment of CMV disease in SOT patients is intravenous ganciclovir (i.v. GCV) and/or oral valganciclovir (VGCV) at doses that should be adjusted according to renal function. Insufficient antiviral dosing may result in a lack of clinical efficacy and the development and selection of resistant viral strains, whereas overdosing may increase toxicity (7). According to Wiltshire et al. (8), exposure values of 40 to 50 $\mu\text{g} \cdot \text{h}/\text{ml}$, as measured by the area under the concentration-time curve (AUC) at steady state, result in a low incidence of breakthrough viremia during prophylactic treatment, whereas lower AUCs are associated with up to 8-fold-higher viral replication rates.

However, it is well known that both GCV and VGCV display high pharmacokinetic (PK) interindividual variability (7, 8). Our group developed a population pharmacokinetics (PPK) modeling approach to GCV-VGCV dosing in SOT patients with CMV infection. Interpatient variability was explained by differences in serum creatinine clearance (CL_{CR}) (52.03%), whereas body weight explained only 4% of it and thus was not included in our

current PPK model (9). Also, we examined the adequacy of the manufacturer's recommended dosing schedule to achieve target AUC values and found that potential dosing refinements were clearly possible. In this pharmacokinetic study, we hypothesized that by applying our previously developed PPK model as a tool for Bayesian prediction, we could optimize GCV-VGCV treatment and more rapidly achieve target AUC values as well as maintain them throughout the treatment period. Thus, the primary endpoint of this study was a pharmacological parameter assessed as the percentage of patients achieving the target therapeutic exposure.

MATERIALS AND METHODS

Study design. This was a two-arm, randomized, open-label, single-center trial with adult SOT recipients treated with GCV-VGCV as either prophylaxis or treatment for CMV infection (viremia or disease) (EudraCT no. 2010-021433-32; ClinicalTrials.gov registration no. NCT01446445). The trial was designed as a superiority study based on the percentage of defined

Received 3 September 2015 Returned for modification 7 October 2015

Accepted 1 January 2016

Accepted manuscript posted online 11 January 2016

Citation Padullés A, Colom H, Bestard O, Melilli E, Sabé N, Rigo R, Niubó J, Torras J, Lladó L, Manito N, Caldés A, Cruzado JM, Grinyó JM, Lloberas N. 2016.

Contribution of population pharmacokinetics to dose optimization of ganciclovir-valganciclovir in solid-organ transplant patients. *Antimicrob Agents Chemother* 60:1992–2002. doi:10.1128/AAC.02130-15.

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TABLE 1 Dosing adjustments based on CL_{CR} in the solid-organ transplant population of oral valganciclovir and i.v. ganciclovir

Oral valganciclovir			i.v. ganciclovir	
CL_{CR} (ml/min)	Treatment dose	Prophylaxis dose	CL_{CR} (ml/min)	Treatment dose
≥ 60	900 mg/12 h	900 mg/24 h	≥ 70	5 mg/kg/12 h
40–60	450 mg/12 h	450 mg/24 h	50–70	2.5 mg/kg/12 h
25–40	450 mg/24 h	450 mg/48 h	25–50	2.5 mg/kg/24 h
10–25	450 mg/48 h	450 mg twice per week	10–25	1.25 mg/kg/24 h

target AUC values (40 to 50 $\mu\text{g} \cdot \text{h/ml}$) achieved in each group with a superiority margin of 40%.

Patients were randomly allocated into two groups depending on the dosing adjustment strategy to be applied (1:1). Group A received GCV-VGCV according to the manufacturer's dosing recommendations based on Cockcroft-Gault-calculated CL_{CR} and body weight according to the criteria showed in Table 1. Doses were adjusted based on the individual CL_{CR} at each time point. Group B received initial doses of GCV-VGCV as calculated using our previously developed PPK model (Table 2) (9). Subsequent doses were then adjusted applying a Bayesian prediction model to ongoing measurements of drug concentrations and renal function.

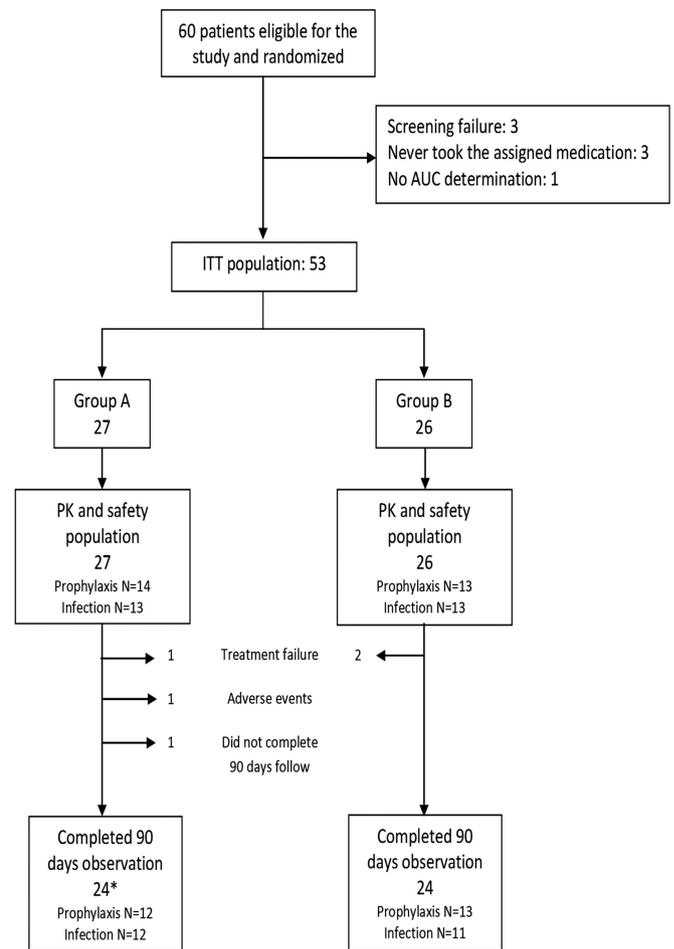
Kidney, liver, or heart transplant recipients were eligible to participate in the study if they were ≥ 18 years of age and were treated with GCV or VGCV as either prophylaxis or treatment of CMV infection, according to standard clinical practice. Patients were excluded if they had a calculated CL_{CR} below 10 ml/min using the Cockcroft-Gault equation, had a history of hypersensitivity to GCV-VGCV, or were receiving concomitant treatment with other anti-CMV agents. Patients under prophylaxis received oral VGCV for 90 days, whereas those with CMV infection were treated with GCV-VGCV until two consecutive negative CMV viral load tests were obtained, performed at least 1 week apart.

Patients with CMV infection included patients with CMV viremia (evidence of CMV replication regardless of symptoms) and those with CMV disease. CMV disease included both viral syndrome and tissue-in-

TABLE 2 Initial doses based on the PPK model in the solid organ transplant population of oral valganciclovir and iv ganciclovir

Oral valganciclovir			i.v. ganciclovir	
CL_{CR} (ml/min)	Treatment dose	Prophylaxis dose	CL_{CR} (ml/min)	Treatment dose
100	1,000 mg/12 h	1,000 mg/24 h	100	10 mg/kg/12 h
95	950 mg/12 h	950 mg/24 h	95	9.5 mg/kg/12 h
90	900 mg/12 h	900 mg/24 h	90	9 mg/kg/12 h
85	850 mg/12 h	850 mg/24 h	85	8.5 mg/kg/12 h
80	800 mg/12 h	800 mg/24 h	80	8 mg/kg/12 h
75	750 mg/12 h	750 mg/24 h	75	7.5 mg/kg/12 h
70	700 mg/12 h	700 mg/24 h	70	7 mg/kg/12 h
65	650 mg/12 h	650 mg/24 h	65	6.5 mg/kg/12 h
60	600 mg/12 h	600 mg/24 h	60	6.0 mg/kg/12 h
55	550 mg/12 h	550 mg/24 h	55	5.5 mg/kg/12 h
50	500 mg/12 h	500 mg/24 h	50	5.0 mg/kg/12 h
45	450 mg/12 h ^a	450 mg/24 h ^a	45	4.5 mg/kg/12 h
40	400 mg/12 h	400 mg/24 h	40	4.0 mg/kg/12 h
35	350 mg/24 h	350 mg/48 h	35	3.5 mg/kg/12 h
30	300 mg/24 h	300 mg/48 h	30	3.0 mg/kg/12 h
25	250 mg/24 h	250 mg/48 h	25	2.5 mg/kg/24 h
20	200 mg/48 h	200 mg/72 h	20	2.0 mg/kg/24 h

^a Solution was used when doses did not fit the tablet presentation.



*1 patient exitus at Day 110 (did not complete 6 months follow)

FIG 1 Study patient allocation and disposition.

vative disease. Identification of the viral syndrome caused by CMV required the following: (i) viral load of $> 1,000$ copies/ml, (ii) temperature of $> 38^\circ\text{C}$ with no other source to account for it, and (iii) one of the following findings: leukocyte count of $< 4,000/\text{mm}^3$, atypical lymphocytes of $> 3\%$, elevation of transaminases, and platelet count of $< 100,000/\text{mm}^3$. Tissue-invasive disease required histopathological evidence of CMV, with or without virus culture of the tissue.

Oral VGCV was administered as tablets (Valcyte, 450 mg; F. Hoffmann-La Roche Ltd., Basel, Switzerland) and/or solution (Valcyte, 50-mg/ml suspension; F. Hoffmann-La Roche Ltd.). Solution was used when treatment dose did not fit the tablet presentation. Patients with CMV infection could receive either oral VGCV or i.v. GCV according to standard clinical practice. For those under i.v. treatment, sequential treatment with i.v. GCV (Cymevene; F. Hoffmann-La Roche Ltd.) for a maximum of 5 days, followed by oral VGCV, was administered.

The study was conducted in accordance with the Declaration of Helsinki and good clinical practice (GCP) guidelines. The study protocol was approved by the Bellvitge Hospital's Ethics Research Committee. Written informed consent was obtained from each patient prior to randomization.

Study endpoints. The primary study endpoint was defined as the percentage of patients achieving target AUC values between 40 and 50 $\mu\text{g} \cdot \text{h/ml}$. In all patients included in the study, AUC values were estimated at steady state in one or more scenarios: (i) 3 days after treatment onset, (ii) 3 days after any dose adjustment, (iii) after any change in CL_{CR} of > 10

TABLE 3 Demographic and baseline characteristics for all ITT patients

Characteristic ^f	Value for:		P value
	Group A (n = 27)	Group B (n = 26)	
Type of treatment (no.)			1.000 ^c
Prophylaxis	14	13	
Infection	13	13	
Sex			0.218 ^c
No. (%) female/no. (%) male	9 (33.3)/18 (66.7)	13 (50)/13 (50)	
Age (yrs)			0.586 ^d
Mean ± SD	52.6 ± 19.6	55.1 ± 14.7	
Wt (kg)			0.939 ^d
Mean ± SD	70.1 ± 13.6	70.4 ± 16.4	
Transplanted organ, no.			0.852 ^c
Kidney	23	25	
Liver	2	0	
Heart	2	1	
HLA-A mismatches, no.			0.601 ^c
No mismatch	1	3	
1 or 2 mismatches	22	22	
Missing	4	1	
HLA-B mismatches, no.			0.573 ^c
No mismatch	0	0	
1 or 2 mismatches	23	25	
Missing	4	1	
HLA-DR mismatches, no.			0.341 ^c
No mismatch	3	3	
1 or 2 mismatches	20	22	
Missing	4	1	
Donor/recipient CMV IgG serostatus at time of transplantation, no. (%) ^e			0.241 ^c
D ⁺ /R ⁻	10 (37.0)	4 (16)	
D ⁺ /R ⁺	15 (55.6)	16 (64) ^a	
D ⁻ /R ⁺	2 (7.4)	4 (16)	
D ⁻ /R ⁻	0	1 (3.8)	
Induction immunosuppression, no. (%) ^e			0.779 ^c
ATG	9 (33.3)	10 (38.5)	
Basiliximab	17 (63)	16 (61.5)	
None	1 (3.7)	0	
CMV risk, no. (%) ^{b,e}			0.570 ^c
High/low	18 (66.7)/9 (33.3)	14 (56)/11 (44) ^a	
Rejection episode, no. (%) ^e			1.000 ^c
Yes/no	3 (12)/22 (88)	2 (8.7)/21 (91.3)	
Maintenance immunosuppression ^e			0.795 ^c
CsA + MMF + corticoids	2 (7.5)	4 (15.4)	
TAC + MMF + corticoids	26 (92.5)	19 (73.1)	
CsA + MMF	0	1 (3.8)	
TAC + MMF	0	1 (3.8)	
CsA + corticoids	0	1 (3.8)	
Serum creatinine (μmol/liter)	157.8	144.8	0.560 ^d
CL _{CR} (ml/min)	55.8	54.3	0.823 ^d

(Continued on following page)

TABLE 3 (Continued)

Characteristic ^f	Value for:		P value
	Group A (n = 27)	Group B (n = 26)	
Hemoglobin (g/liter)	108.5	102	0.070 ^d
Leukocytes ($\times 10^9$ cells/liter)	6.84	7.15	0.741 ^d
Lymphocytes ($\times 10^9$ cells/liter)	1.23	1.33	0.749 ^d
Neutrophils ($\times 10^9$ cells/liter)	4.9	5.1	0.818 ^d
Platelets ($\times 10^9$ cells/liter)	207	239	0.126 ^d

^a For one of the CMV IgG-seropositive recipients, the donor's IgG serostatus was missing.

^b Patients classified according to donor and recipient IgG serostatus and induction therapy: high risk (D^+/R^- with any induction therapy or D^-/R^+ and D^+/R^+ treated with antithymocyte globulin) and low risk (D^-/R^- or D^-/R^+ and D^+/R^+ treated with basiliximab or no induction therapy).

^c Chi-square.

^d *t* test.

^e Risk factor associated with therapeutic efficacy.

^f ATG, antithymocyte globulin; TAC, tacrolimus; MMF, mycophenolate mofetil; CsA, cyclosporine.

ml/min, or (iv) after any change in drug administration route. Exposures on days 30, 60, and 90, if the treatment was ongoing, and the time needed to achieve target AUC values were also assessed.

The secondary study endpoints were measurements of time to viral clearance (time to first negative viral load result), recurrence of CMV infection (6 months follow-up), and incidence of late-onset CMV infection (infection occurring after the discontinuation of prophylaxis). Determinations of viral loads in patients with CMV infection were performed until day 45. Hematological and biochemistry laboratory parameters were measured at baseline and every time PK samples were collected, as well as on days 30, 60 and 90, if the treatment was ongoing. Assessments of safety and tolerability were also performed.

Plasma ganciclovir concentration measurements. Blood samples were collected 0.5 to 1.5, 4 to 5, and 6 to 8 h after dose according to a limited-sampling strategy previously developed by our group (10). Plasma GCV concentrations were determined by a validated ultrahigh-performance liquid chromatography assay coupled with UV detection (11).

GCV dose, plasma GCV concentrations, and CL_{CR} in every patient were used for a maximum *a posteriori* (MAP) Bayesian probability estimate of GCV systemic exposure with a PPK model previously developed and implemented in the nonlinear mixed-effects modeling (NONMEM) computer program, version 7.2 (Icon Development, Ellicott City, MD).

Briefly, in the PPK model the PK disposition of GCV was best described by a two-compartment open linear model with first-order absorption process and elimination from the central compartment. Renal function given by CL_{CR} was the most influential covariate in clearance (CL). The final pharmacokinetic parameters were as follows: GCV CL was $7.49 \times (CL_{CR}/57)$ liters/h (57 was the mean population value of CL_{CR}); the central and peripheral distribution volumes were 31.9 liters and 32.0

liters, respectively; intercompartmental clearance was 10.2 liter/h; the first-order absorption rate constant was 0.895 h^{-1} ; bioavailability was 0.825; and lag time was 0.382 h (9).

Viral load monitoring. CMV DNA replication analysis was conducted with the Abbott real-time CMV amplification reagent kit (Abbott Molecular, Des Plaines, IL) using 500 μl of plasma-EDTA (quantification limit, 20 to 10^7 copies/ml). DNA was obtained 24 h after sample collection using magnetic DNA extraction on an m24sp instrument. Patients who tested positive (viral load higher than 1,000 copies/ml) received i.v. GCV or oral VGCV until two consecutive negative CMV viral load tests were obtained, performed at least 1 week apart.

Safety monitoring. Hematological adverse events were assessed at every sampling time point as present or absent. Anemia was defined as a hemoglobin concentration of <100 g/liter, neutropenia as a neutrophil cell count of $<1 \times 10^9$ cells/liter, and thrombocytopenia as a platelet count of $<50 \times 10^9$ cells/liter (12).

Sample size calculation and statistical methods. The study was designed as a superiority trial. The primary endpoint was defined as a 40% or higher superiority margin in the number of patients reaching the AUC target. Thus, the percentages of patients with AUC achieved assessed by median AUC values for each patient were compared between group B (Bayesian prediction dose adjustment group) and group A (treated per the manufacturer's dosing recommendations). A sample size of 27 patients per group provided 80% statistical power to claim superiority of one group over the other ($P < 0.05$). The percentages of patients with median AUC values, within each patient, that reached the therapeutic target were compared between both by a chi-square test. Superiority was declared when statistically significant differences between proportions were found and the lower bound of the one-sided 95% confidence interval (CI) for the difference between

TABLE 4 Baseline viral data characteristics of per-protocol-treated patients for CMV infection (viremia or disease)

Characteristic	Value for:		P value
	Group A (n = 12) ^a	Group B (n = 11) ^b	
Previous anti-CMV strategy, no. (%)			
Prophylactic	5 (58.3)	1 (9.1)	0.378 ^c
Preemptive	7 (41.7)	10 (90.9)	
Median baseline viral load (copies/ml)	3,542	3,392	0.611 ^d
Patients with previous prophylactic treatment	33,774	4,809	0.739 ^d
Patients under preemptive treatment	3,406	3,379	0.696 ^d

^a In group A (13 ITT patients), 1 patient was excluded because the 90-day protocol follow-up was not reached due to the treatment failure (dosing increase).

^b In group B (13 ITT patients), 2 patients were excluded. One patient was excluded from the PK analysis because foscarnet was initiated as an add-on treatment, and the other patient did not reach the 90-day follow-up (dosing increase).

^c Chi-square.

^d Mann-Whitney test.

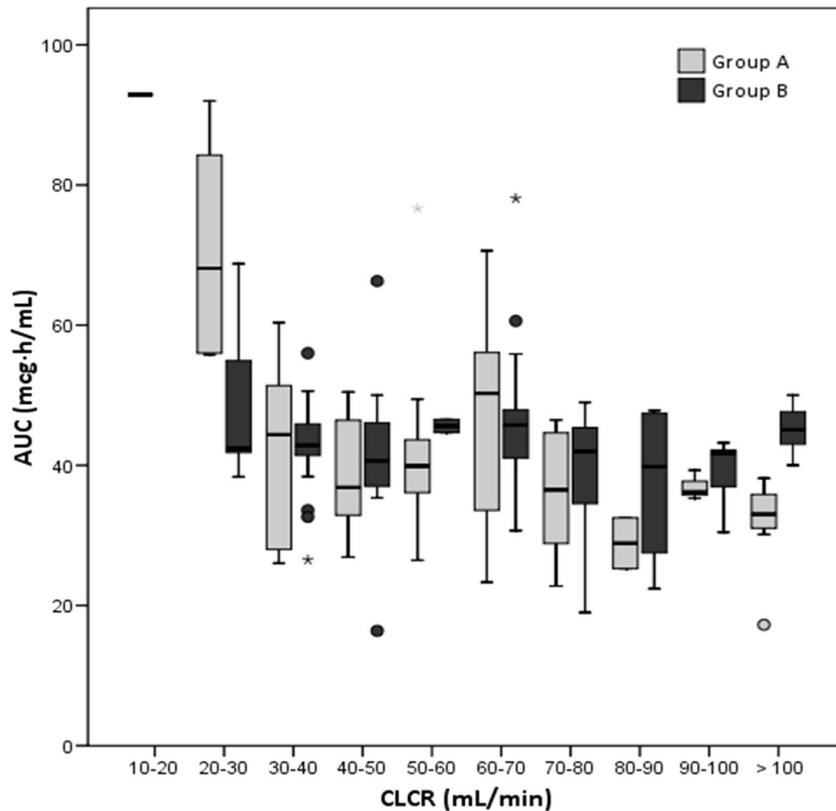


FIG 2 Average estimated systemic exposure of GCV following administration of oral VGC by CL_{CR} cutoff values.

groups was above the superiority margin of 40% (group B versus group A), assuming a 20% incidence of the primary endpoint in group A. Although our previous study showed that AUC values obtained with i.v. GCV and oral VGCV were comparable (13), the analysis of the primary endpoint was performed separately.

The primary endpoint was also evaluated using isolated GCV determinations for every patient and by different CL_{CR} cutoffs. Differences between groups in the time to achieve target AUC values were assessed using a Kaplan-Meier survival analysis.

Statistical analysis of secondary endpoints included a *t* test for normally distributed data, a chi-square test for categorical variables, and a Kaplan-Meier survival analysis for time to viral clearance comparisons. The ability to predict relapse and late-onset infection based on dosing strategy after discontinuation of prophylactic treatment was assessed by an analysis of area under the ROC (receiver-operator curve). All statistical analyses were performed using SPSS v19.0, with a significance level of 0.05.

Statistical analyses were performed using the intention-to-treat (ITT) population for the primary endpoint. Analysis of secondary safety endpoints included all randomized patients who had received at least one dose of study medication and had at least one AUC determination and one safety assessment. The per-protocol population analysis for the efficacy endpoint included only those patients who completed treatment and the 90-day follow-up period.

RESULTS

Baseline characteristics. A total of 60 SOT patients eligible to receive GCV or VGC for either prophylaxis or CMV infection were enrolled. The ITT population included 53 patients: 27 were assigned to group A and 26 to group B. Study patient allocation and disposition are shown in Fig. 1. There were no significant differences in main demographic or baseline characteristics be-

tween groups (Table 3). The baseline viral data characteristics of patients with CMV infection are shown in Table 4. Only three patients received sequential treatment with i.v. GCV followed by oral VGCV.

Evaluation of systemic exposure. A total of 155 AUC determinations from 53 patients were analyzed. We excluded data after intravenous administration from the statistical analysis (5 AUC determinations not shown). Median AUC values by CL_{CR} cutoff interval are shown in Fig. 2. Analysis of median systemic exposure during the study period within each patient showed that 88.64% (23/26) of patients treated following the Bayesian prediction based on the PPK model (group B) achieved the target AUC values, compared to only 22.2% (6/27) of patients treated by following the manufacturer's dosing recommendations (group A), thus fulfilling the 40% superiority margin in target AUC values established as a primary endpoint ($P < 0.001$; 95% CI for the difference, 47 to 86%). Median AUC values were 38.2 $\mu\text{g} \cdot \text{h}/\text{ml}$ in group A and 42.7 $\mu\text{g} \cdot \text{h}/\text{ml}$ in group B.

The time required to reach target AUC values was longer in group A than in group B (log rank statistics = 15.48, $P < 0.001$, 56.7 days [95% CI = 40.24 to 73.07], versus 16.8 days [95% CI = 11.67 to 21.87]) (Fig. 3A). Differences remained statistically significant ($P < 0.001$) when patients on prophylaxis or treatment of CMV infection were considered separately, even though the time to target AUC was longer in prophylaxis-treated individuals, as patients were visited weekly. The times between samples and AUC determinations were longer in patients receiving prophylaxis than in those receiving treatment for CMV infection (Fig. 3B and C).

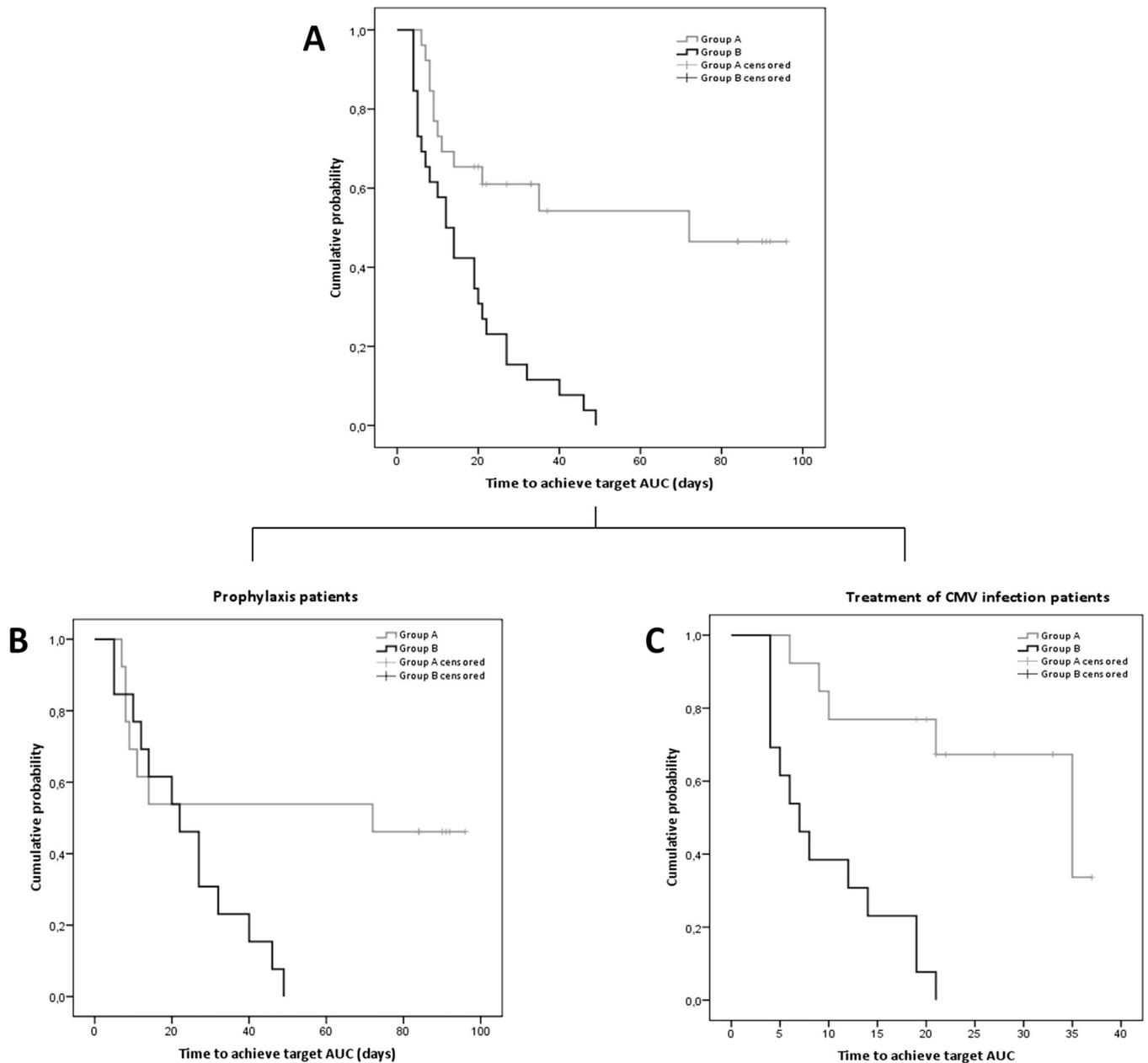


FIG 3 Rate of first achievement of AUC target according to randomization group over the follow-up period (Kaplan-Meier analysis using log rank statistics) for all patients included (A), for patients on prophylaxis (B), and for patients on treatment of CMV infection (C). Time scale is based on days under GCV-VGCV treatment.

Considering all AUC determinations, the Bayesian prediction approach resulted in a higher proportion of AUC values falling within the therapeutic range (19.2% for group A, versus 65.9% for group B; $P < 0.001$; 95% CI for the difference, 33 to 60%) and fulfilling the 40% superiority margin. The proportions of on-target AUC values by CL_{CR} and prophylactic versus treatment of CMV infection in each group are shown in Table 5. Differences in AUC values between groups were statistically significant for patients with CL_{CR} s of <30 ml/min ($P = 0.008$; 95% CI for the difference, 38 to 105%) or >60 ml/min ($P = <0.001$; 95% CI for the difference, 43 to 77%). No significant differences were observed in patients with CL_{CR} s between 30 and 50 ml/min (Table 5).

In some patients in group B, the dose of GCV or VGCV was adjusted due to subtherapeutic (24.7%) or supratherapeutic (9.41%) AUC values. Subsequent AUC values fell within target exposure limits in all cases.

Clinical outcome analysis. (i) Patients on treatment for CMV infection. The time to viral clearance was shorter in patients in group B than in patients assigned to group A (group A, 17.6 days, and group B, 12.5 days), reflecting a 40.8% reduction (log rank statistics = 2.34; $P =$ not significant [NS]). A Kaplan-Meier curve for time to viral clearance is shown in Fig. 4. The median two-exponential decay of viral load for each group until day 45 is displayed in Fig. 5.

The proportion of patients with CMV recurrence was higher in

TABLE 5 Proportions of on-target AUC values by group, CL_{CR} cutoff value, and type of treatment

Classification variable for statistical analysis	% measurement achieving the target AUC (40–50 µg · h/ml)		% difference (95% CI)	P value
	Group A (= 73)	Group B (= 82)		
All patients included	19.2	65.9	47.0 (33–60)	<0.001
Patients with indicated CL _{CR} cutoff value				
<30 ml/min	0	71.4	71.4 (38–105)	0.008
30–40 ml/min	37.5	64.7	27.2 (–13–68)	0.467
40–50 ml/min	36.4	57.1	20.8 (–21–63)	0.582
50–60 ml/min	36.7	100	64.6 (35–92)	0.252
>60 ml/min	8.6	68.3	59.7 (43–77)	<0.001
Type of treatment				
Prophylaxis	21.3	62.9	41.6 (27–56)	<0.001
Treatment of CMV disease	15.4	71.4	56.0 (34–78)	<0.001

group A than in group B (66.67% [8/12] versus 9.01% [1/11]), and 85% of patients who relapsed had received GCV-VGCV according to the manufacturer's dosing recommendations (group A). No differences were found in time to recurrence between groups. The ability to predict relapse based on dosing strategy, recipient and donor CMV serostatus, and immunological risk assessed by a sensitivity/specificity ROC analysis revealed the dosing strategy as the most accurate variable predicting the viral relapse ($P = 0.007$; AUC, 0.835; 95% CI, 0.66 to 1.02; sensitivity, 100%; specificity, 91.7%) (Fig. 6A).

(ii) **Patients on CMV prophylaxis.** There were no incident cases of CMV viremia during the prophylactic period. The inci-

dence of late-onset infection during the 3 months following discontinuation of prophylaxis was lower in group B than in group A (7.7% [1/13] versus 36.7% [4/11]). However, the accuracy of our dose adjustment strategy predicting late-onset CMV infection was not statistically significant ($P = 0.168$; AUC, 0.706; 95% CI, 0.45 to 0.96; sensitivity, 100%; specificity, 40%) (Fig. 6B).

Adverse events. About 32.7% of patients reported at least one hematological adverse event during treatment. Neutropenia occurred in 7.5% of patients (7.4% in group A [2 patients] and 7.7% in group B [2 patients]). Two episodes of neutropenia, out of 27 cases in which the AUC values were supratherapeutic (7.4%), one in each group, were related to GCV overexposure. In the other two patients, toxicity was associated with concomitant treatment with co-trimoxazole. The difference in the occurrence of anemia was associated with GCV overexposure. In those patients with AUC values higher than 50 µg · h/ml, the incidence of anemia was 51.9% (14/27 determinations), versus 26.6% (31/128 determinations) in those patients whose values were under or within the defined target AUC ranges ($P = 0.010$).

Dosage. Patients in group B received higher doses of either i.v. GCV or oral VGCV than patients in group A. The median daily dose of oral VGCV for patients under prophylaxis was lower in group B than in group A. Dosage distributions are shown in Fig. 7.

DISCUSSION

This is the first prospective, randomized, controlled superiority trial with SOT patients comparing conventional GCV-VGCV dosing for either CMV prophylaxis or infection treatment to a Bayesian prediction model approach in an attempt to optimize GCV exposure. Herein, we show that the proportion of patients achieving therapeutic target AUC values was higher after GCV-VGCV dose adjustment using a Bayesian prediction model approach than simply following the manufacturer's dosing recommendations. A preestablished superiority margin of 40% in the number of patients reaching a therapeutic target AUC value, the study primary endpoint, was fulfilled. The time to target AUC values was also shorter in patients dosed according to the Bayesian prediction model approach. These results are important since therapeutic exposure to GCV-VGCV has been previously correlated with treatment efficacy (8); thus, the achievement of therapeutic drug exposure is especially advisable in the treatment of

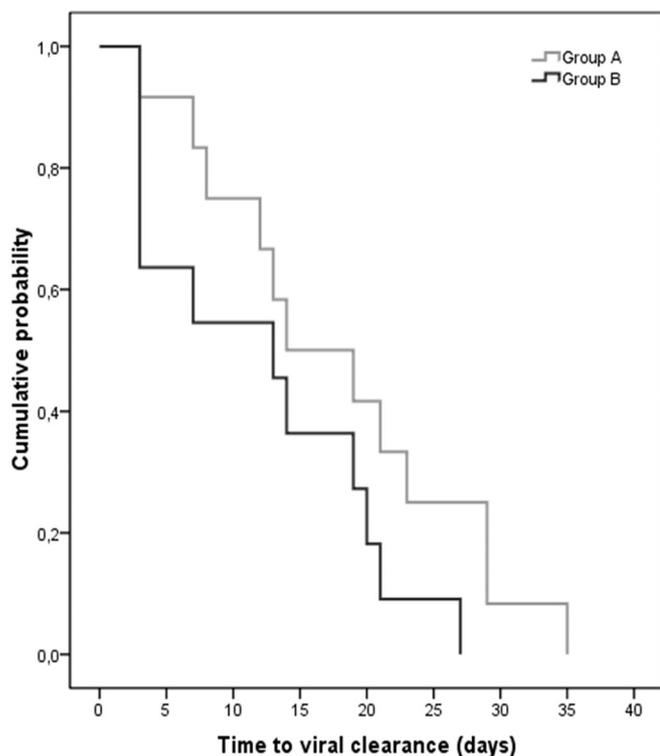


FIG 4 Kaplan-Meier curves showing cumulative probability of viral clearance in patients treated with either oral VGCV or i.v. GCV.

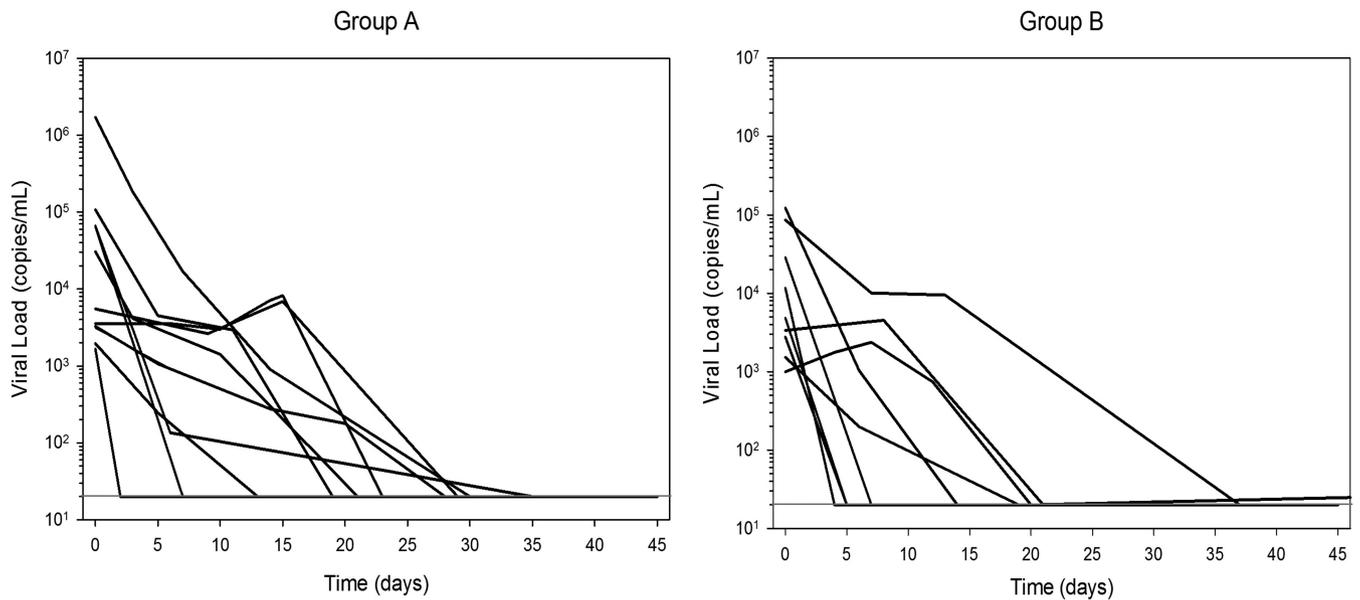


FIG 5 Plots of individual viral load decays observed until day 45 for patients treated according to the manufacturer's recommendation (left) and for patients with dose adjusted by Bayesian approach (right). The gray line represents the limit of quantification of the analytical method. (Relapse data are not shown.)

CMV infection in order to rapidly control and inhibit viral replication.

A previously reported study by our group showed both under- and overexposure to GCV in patients following manufacturer's dosing recommendations (13). Based on our own PPK model simulations, and using the manufacturer's initial dose recommendations, GCV-VGCV dosing results in overexposure in patients

with CL_{CR} values below 30 ml/min and underexposure in patients with CL_{CR} values over 60 ml/min. The current study confirms that the manufacturer's label dosing algorithm (14, 15) is appropriate for only approximately one-fifth of patients, most of whom fall within CL_{CR} values between 30 and 50 ml/min. To date, there are limited data concerning GCV dose optimization (16), and some authors suggested testing the adequacy of drug level in

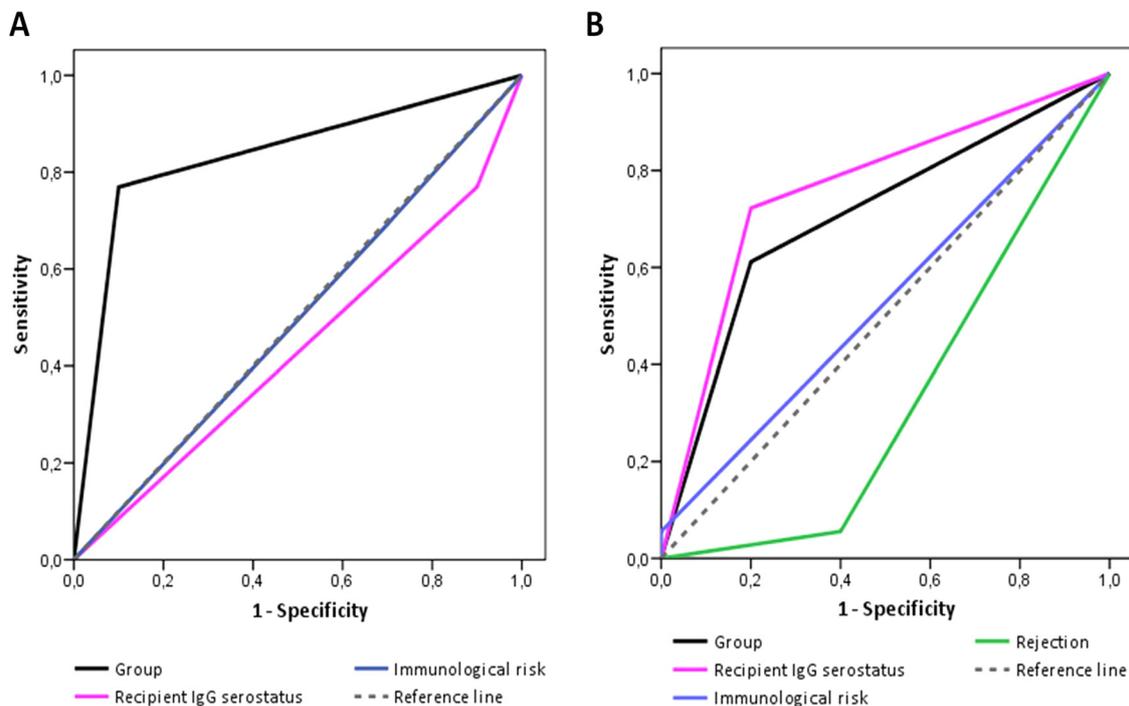


FIG 6 Receiver-operator curve (ROC) of dose adjustment based on target AUC, recipient IgG serostatus, and immunological risk for predicting relapse (A) and of dose adjustment based on AUC target, recipient IgG serostatus, episodes of rejection, and immunological risk for predicting late CMV disease (B).

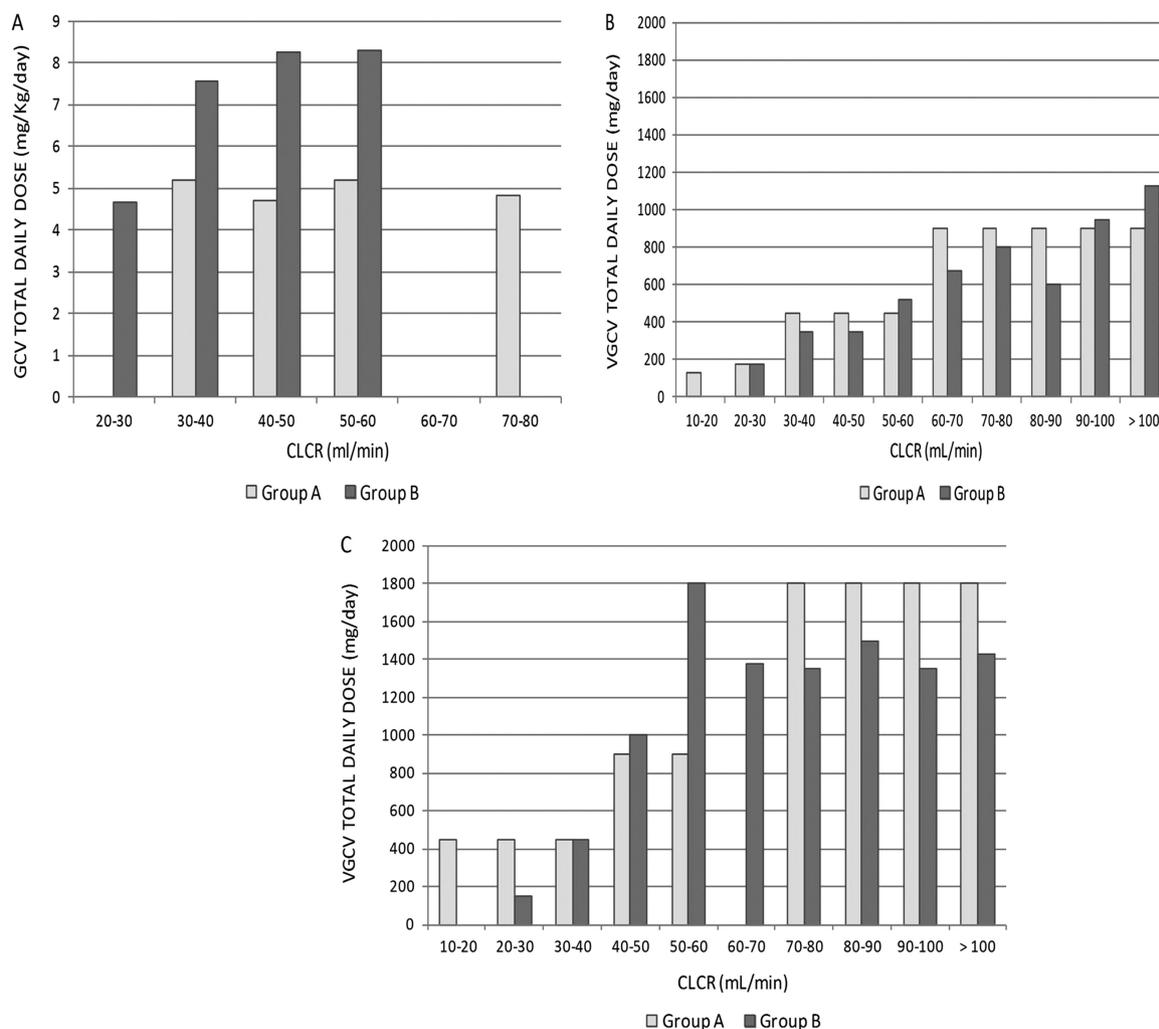


FIG 7 Dosage distributions considering the randomization group, route of administrations, type of treatment, and CL_{CR} cutoffs. (A) i.v. GCV dosage; (B) oral VGCV dosage in patients on prophylaxis; (C) oral VGCV dosage in patients on treatment for CMV infection.

patients with critical CMV infection in which GCV resistance is suspected (6).

Clinical outcome analysis of patients treated for CMV infection following such an approach also showed a trend toward reduced time to viral clearance. However, since our study had a primarily pharmacokinetic focus, it was therefore not powered to assess clinical efficacy.

Besides dosing adequacy, other factors that may result in longer treatment duration are a high baseline viral load and a high net state of immunosuppression (6). According to Asberg et al. (17), the baseline viral load can be a significant predictor of viral clearance. In our study, the baseline viral load was lower than 200,000 copies/ml in all patients, as the majority had received preemptive therapy, and no differences between groups were observed.

With regard to CMV recurrence, our study showed a rate of 28%, which is in line with earlier reports showing rates of 25% to 30% (18–20). However, a trend toward higher recurrence rates was observed in patients treated according to the manufacturer's dosing recommendations (group A). Even though response to antiviral therapy is likely dependent on a complex interaction be-

tween the virus and the host immunity (6, 17, 21), our results suggest that therapeutic drug exposure may help improve the control of viral replication.

In our study, the incidence of hematological adverse events was similar to that previously reported (32.7) (8). There were no differences between groups in the incidence of neutropenia, and 14.8% of patients that presented AUC values of $>50 \mu\text{g} \cdot \text{h/ml}$ developed neutropenia. The small sample size did not allow achieving statistical significance, but Wiltshire et al. (8) reported a weak tendency to increase the incidence of neutropenia with higher GCV exposure. A significantly superior incidence of anemia was observed in those patients with AUC values higher than $50 \mu\text{g} \cdot \text{h/ml}$ who presented CL_{CR} values below 30 ml/min. In fact, patients with worse renal function ($CL_{CR} < 30 \text{ ml/min}$) do not fit the target AUC when dosed using the manufacturer recommendations.

In conclusion, this PK study achieved the primary endpoint, with 88.4% of patients reaching target exposure when dosed according to a PPK model using a Bayesian prediction approach, with a superiority margin of 40%. GCV-VGCV treatment following the manufacturer's dosing recommendations resulted in only

18.5% of patients falling within the therapeutic range. Applying our PPK model, using a Bayesian prediction model approach, constitutes a significant step forward in optimizing GCV dosing in the management of CMV infection and may allow for individualized anti-CMV therapy. It was mainly for those patients with CL_{CR} values below 30 ml/min and over 60 ml/min that CGV-VGCV dosage did not achieve the optimal drug exposure. Our study was an application of therapeutic drug monitoring, but further dose tailoring studies, focused on clinical efficacy as a primary endpoint, may show improved clinical outcomes. Further powered randomized dose tailoring clinical trials are warranted to assess the clinical benefits of PPK-based dosage of GCV-VGCV in the prevention and management of CMV infections in SOT recipients.

ACKNOWLEDGMENTS

We gratefully acknowledge Gema Cerezo for technical assistance with this study.

N. Lloberas is a researcher from ISCIII Miguel Servet (CP06/00067) and REDinREN RD12/0021/003. This study was supported by grants from Instituto de Salud Carlos III and Ministerio de Sanidad y Consumo (PI12/01564), MSPSI (EC10-144), and Fondo Europeo de Desarrollo Regional (FEDER).

We have no financial or personal conflicts of interest related to this article.

A. Padullés performed research, analyzed data and wrote the manuscript; H. Colom, J. M. Grinyó, and N. Lloberas designed the research, performed research, analyzed data, wrote the manuscript, and contributed new reagents and analytical tools; O. Bestard, J. Torras, and J. M. Cruzado performed research and wrote the manuscript; E. Melilli, N. Sabé, L. Lladó, and N. Manito performed research; R. Rigo and J. Niubó performed research and contributed new reagents and analytical tools, and A. Caldés analyzed data.

FUNDING INFORMATION

This work was supported by Spanish Public Funding sources: Instituto de Salud Carlos III (ISCIII) provided funding to Nuria Lloberas under grant number PI12/01564 (FIS) and grant number EC10-144 (MSP clinical trial); and REDin REN.

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