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Standard ganciclovir dosing results in slow decline of cytomegalovirus viral loads

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Background: Cytomegalovirus (CMV) can cause severe disease, including rejection in transplant recipients. Ganciclovir and its oral prodrug valganciclovir have been used as first-line therapy for CMV disease in transplant recipients. The exposure targets of ganciclovir are not exactly known, and toxicity and resistance have interfered with ganciclovir therapy.

Objectives: To evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of ganciclovir in transplant recipients.

Methods: We used patient data from a previous observational study on ganciclovir therapeutic drug monitoring (TDM) in prophylaxis and therapy. The ganciclovir concentrations and CMV viral loads were determined during routine clinical care. The PK/PD population modelling and simulations were done with non-parametric methodology using the Pmetrics program.

Results: Eighty-five patients were included in the PK modelling. The final PK model was a two-compartment model with first-order absorption and elimination. A subset of 17 patients on CMV therapy were included in the PD modelling. A median of 4 (range 2–8) viral loads were obtained per patient. A simulation of 10 000 patients showed that an approximately 1 $\log_{10}$ reduction of CMV viral load will be observed after 12.5 days at the current recommended dose.

Conclusions: The developed linked PK/PD population model and subsequent PD simulations showed slow decline of CMV viral load and it appears that dosing of (val)ganciclovir in this study might have been inadequate to achieve fast reduction of viral load. It is clear that further studies are needed to specify the PD effects of ganciclovir by performing systematic measurements of both ganciclovir concentrations and CMV viral loads.

Introduction

Cytomegalovirus (CMV) is a double-stranded DNA virus within the family Herpesviridae. CMV infection is defined by detection of CMV virus in a bodily fluid.[1] CMV disease refers to the detection of the virus with associated end-organ damage and/or clinical symptoms.[1] CMV disease can cause severe complications in transplant recipients. The clinical manifestations are varied and include viraemia and end-organ disease, such as retinitis, colitis, hepatitis, pneumonitis and uveitis.[2,3] CMV disease can cause prolonged hospitalization, graft rejection and death.[2,4,5]

Different therapeutic strategies are used for the management of CMV infection and disease. Solid organ transplant (SOT)

recipients generally receive routine prophylaxis for 3–12 months.[6,7] In contrast, a pre-emptive approach is generally used for HSCT recipients, which indicates serial monitoring of CMV before antiviral therapy is commenced in the case of a primary infection or reactivation. Ganciclovir, either intravenously administered or orally administered as the pro-drug valganciclovir, is commonly used for prophylaxis and treatment of CMV infection.[8,9] A pre-emptive approach rather than daily prophylactic therapy minimizes exposure to ganciclovir, which often causes significant adverse events, such as myelosuppression (e.g. thrombocytopenia, neutropenia), to which HSCT recipients are most sensitive.[10,11]

Ganciclovir is a synthetic analogue of 2′-deoxyguanosine, which inhibits replication of herpes viruses.[12] The pharmacokinetic (PK) parameters of ganciclovir are generally known; it has low protein binding (1%–2%), is mostly eliminated through the kidneys and thus the half-life is prolonged during renal failure.[13,14] The pharmacodynamics (PD) of ganciclovir to control CMV—both in the context of prevention and established infection—are poorly understood. Furthermore, drug exposure targets that are associated with a high and low probability of therapeutic success and toxicity, respectively, are not well understood. A total drug PD target of a 24 h area under the concentration–time curve (AUC_{24h}) of >50 mg·h/L has been associated with suppression of viral replication during prophylaxis and 80–120 mg·h/L has been suggested during treatment.[15–18] However, this is based on one study investigating prophylaxis with ganciclovir and expert opinions. A strong relationship between ganciclovir trough and peak concentrations and treatment outcomes has not been observed.[19–23] Due to the lack of PK/PD data, the benefit of ganciclovir therapeutic drug monitoring (TDM) for CMV disease has been long debated. However, absence of evidence does not automatically mean evidence of absence.[19–24] Therefore, the aim of this study was to assess the PK and PD of ganciclovir in transplant recipients using a linked PK/PD population model.

Methods

Patients

Patients aged 18 years or older that were included in the PK study received ganciclovir or valganciclovir for prophylaxis, pre-emptive therapy or therapy against CMV and/or human herpes virus type 6 (HHV-6) as standard-of-care at the University Medical Center Groningen (UMCG), Groningen, The Netherlands.[25] TDM of ganciclovir and CMV viral load determination was performed as part of routine patient care.[25] The Medical Ethics Review Board of the UMCG confirmed that medical research involving human subject act (WMO) approval was not needed for this study (METc 2018/020). Before transplantation, included patients signed an informed consent for approval of data collection for observational clinical studies. Ganciclovir measurements that were obtained during renal replacement therapy were excluded from the population PK modelling, due to missing data on the replacement therapy (e.g. filtration rates, filtrate). For the PD modelling, only data for patients on CMV treatment were included. The patients had to have at least two CMV plasma viral loads in order to assess the decline of viral loads and population PK data available at the time of viral load obtainment.

Drug, dose and schedule

Valganciclovir [oral—Valganciclovir Accord (Accord Pharmaceuticals, Ahmedabad, India) 450 mg film-coated tablets; Valcyte (Roche, Basel, Switzerland) powder for oral solution 50 mg/mL] and ganciclovir [intravenous—Ganciclovir (Sandoz, Basel, Switzerland) 500 mg powder for solution for infusion] were administered according to the estimated glomerular filtration rate (eGFR; calculated with CKD-EPI). Regardless of eGFR, every patient received a first day full dose 900 mg twice daily (orally) or 5 mg/kg twice daily (intravenously). Therapeutic dosing in different eGFR ranges was as follows for oral dosing: 40–59 mL/min/1.73 m^2, 450 mg twice daily; 25–39 mL/min/1.73 m^2, 450 mg once daily; and 10–24 mL/min/1.73 m^2, 450 mg every other day. Therapeutic dosing in different eGFR ranges was as follows for intravenous dosing: 50–69 mL/min/1.73 m^2, 2.5 mg/kg twice daily; 25–49 mL/min/1.73 m^2, 2.5 mg/kg once daily; and 10–24 mL/min/1.73 m^2, 1.25 mg/kg once daily. If eGFR was <10 mL/min/1.73 m^2 the dose was determined by the attending pharmacist and virologist, based on early TDM.

PK sampling and bioanalysis

Plasma drug concentration measurements were obtained 24 h after the start of therapy. For oral therapy, trough concentrations (C_{min}) were obtained immediately prior to dosing. For intravenous therapy, a trough concentration was obtained followed by a peak concentration (C_{max}). The intravenous drug was administered as a 1 h infusion; the peak concentration was obtained 1 h after the infusion was finished.

Ganciclovir concentrations were measured in the Clinical Pharmacy and Pharmacology Laboratory of the UMCG three times per week. Ganciclovir was measured using LC-MS/MS. The assay was based on a previously described method with some modification to utilize a more sensitive LC-MS/MS platform.[26] Briefly, 500 μL of precipitation reagent {internal standard of 0.05 mg/L ganciclovir[$^2H_5$] (Alsachim, Illkirch, France) in methanol} was added to 100 μL of serum. Samples were vortexed for 1 min. After centrifugation (5 min at 9500 **g**), 0.2 μL of supernatant was injected into the LC-MS/MS system (Thermo Fisher Scientific triple quadrupole Quantiva MS/MS system with a Thermo Fisher Scientific Vanquish UPLC system, Waltham, MA, USA).

Chromatographic separation was performed on a Waters T3 HSS 1.8 μL 50 × 2.1 mm analytical column (Milford, MA, USA) and by means of a gradient with a flow of 0.8 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The highest coefficient of variation of the linear range was 12.6% at the lower limit of quantification (LLOQ) level with a bias of 1%. Accuracy and precision were validated at LLOQ (0.1 mg/L), low (0.2 mg/L), medium (8 mg/L) and high (16 mg/L) levels and their largest deviations were: LLOQ—4.7 CV%, 9.5% bias, within run precision 3.6% and between run precision 3.0%; low—4.0% CV%, 13.0% bias, within run precision 2.9% and between run precision 6.7%; medium—2.4 CV%, −2.0% bias, within run precision 1.7% and between run precision 0.5%; and high—1.8 CV%, 2.1% bias, within run precision 1.3% and between run precision 0.9%. Analysis time was 1.4 min.

Ganciclovir concentrations were used to calculate AUC_{24h} values using Bayesian simulation (MW/Pharm++ version 1.87, Mediware, Prague, Czech Republic) and dosage changes were performed after. The simulation software used the following PK parameters: volume of distribution of 0.74 ± 0.15 L/kg (lean body mass), elimination rate constant of 0.023 ± 0.1 h^{-1}, renal elimination rate constant of 0.0021 ± 0.001 h^{-1}/(mL/min/1.73 m^2) and, for oral valganciclovir, bioavailability of 0.6 ± 0.15, absorption rate constant of 0.895 ± 4.64 h^{-1} and lag time of 0.825 ± 1.54 h.[27]

CMV viral load quantification

CMV viral loads were determined twice weekly at the Department of Microbiology and Infection Prevention of the UMCG. The CMV viral load was measured in plasma, using a previously described method.[28] Briefly, 190 μL of clinical sample was collected and nucleic acids were extracted using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics,

Mannheim, Germany). An in-house CMV PCR (LLOQ 536 IU/mL) was prepared using the 2X TaqMan Universal Mastermix (Life Technologies, Carlsbad, CA, USA) and performed on an Applied Biosystems 7500 (Life Technologies). Cycling conditions were 2 min at 50°C and 10 min at 95°C followed by 42 repeats of 15 s at 95°C and 1 min at 60°C. For the population PD modelling the viral loads were converted to $\log_{10}$ scale. The lower limit of detection was 2.0 $\log_{10}$ copies/mL.

Population PK and PD modelling

The PK modelling was performed using a non-parametric population methodology with the program Pmetrics (version 1.5.2) for R (version 3.6.1) (Laboratory of Applied Pharmacokinetics and Bioinformatics, Los Angeles, CA, USA).[29] In order to stabilize the modelling process and avoid biases in modelling a sparse dataset, the PK and PD were modelled in a two-step process. First, a population PK model was fitted to the data. The initial PK parameters were not fixed to a single value. The non-parametric software estimated population mean, median and individual PK parameters and a range of support points. The individual PK parameters (developed in the population PK model) for each patient were then supplied as a covariate in the new dataset, where the measured CMV $\log_{10}$ viral loads were in the measurement (output) column. Thereafter the PD model was fitted to the individual PD data (CMV viral loads).

One-, two- and three-compartment structural PK models were fitted to the data. The fitting and different PK models were assessed and compared using a visual inspection of the observed-versus-predicted ganciclovir concentration goodness-of-fit plots, Akaike information criterion (AIC), Bayesian information criterion (BIC) and assessment of log likelihood values. Models were distinguished by comparing twice the difference in log likelihood values against a χ^2 distribution with the degrees of freedom determined by the difference in parameter number between the respective models.

The base structural PK/PD model was as follows:

$$\text{Equation 1}: \frac{dX_1}{dt} = B(1) \times F - (K_a \times X_1)$$

$$\text{Equation 2}: \frac{dX_2}{dt} = K_a \times X_1 + RateIV - \left(\frac{CL}{V_c} + KCP\right) \times X_2 + KPC \times X_3$$

$$\text{Equation 3}: \frac{dX_3}{dt} = KCP \times X_2 - KPC \times X_3$$

Equations 1–3 describe the PK of ganciclovir and its oral prodrug. The amounts of drug (in mg) in the gut, central compartment and peripheral compartment are described by X_1, X_2 and X_3, respectively, dX_n/dt describes the rate of change of mass of drug in the respective compartments, B(1) and RateIV describe the bolus input of valganciclovir and the intravenous infusion of ganciclovir (in mg), respectively, F is the bioavailability of valganciclovir, K_a is the absorption rate constant (h^{-1}) describing the rate of transfer of drug from the gut to the central compartment, CL is the first-order clearance of ganciclovir from the central compartment (L/h), V_c is the volume of the central compartment (L), and KCP and KPC are the first-order intercompartmental rate constants (h^{-1}).

The impact of covariates was then assessed. Weight, age, sex, WBC count, platelets, alkaline phosphatase, ALT, AST, GGT and eGFR were investigated if these have any relationship with the PK parameters estimated from each patient.

The final model included eGFR as a covariate that affected CL in the following way:

$$\text{Equation 4}: CL = CL_0 + CL_1 \times eGFR$$

CL_0 (L/h) is the intercept and CL_1 [L/h/(mL/min/1.73 m^2)] is the slope in the linear relationship between CL and eGFR.

The PD were modelled in the following way:

$$\text{Equation 5}: \frac{dX_4}{dt} = Initial\ condition - K_{kmax} \times \left(\frac{\left(\frac{X_2}{V_c}\right)^{Hk}}{\left(\frac{X_2}{V_c}\right)^{Hk} + EC_{50}^{Hk}}\right) \times X_4$$

The rate of change of viral copies in the plasma was determined by plasma concentrations of ganciclovir (i.e. X_2/V_c). Since no PD data were acquired in the absence of therapy, the rate of spontaneous viral replication could not be directly estimated. X_4 is the viral load in plasma. The initial condition (i.e. initial CMV viral load at time 0, viral set point) was estimated as a parameter (viral copies/mL). K_{kmax} is the maximal rate of drug-induced viral kill ($\log_{10}$ viral copies/mL/h), EC_{50} is the ganciclovir concentration inducing half-maximal rate of kill (mg/L) and H_k is the slope (Hill) function. Using this equation, we assumed that each patient was at viral steady state, where viral load production was equal to viral load clearance and the viral load was constant.

Monte Carlo simulations

The Monte Carlo simulations were performed using a two-step approach. In the first step the developed PK model was used to simulate 100 patients with the licensed regimen of 5 mg/kg ganciclovir twice daily intravenously with a median eGFR of 71.1 mL/min (range 51.9–90.3). For each of the simulated patients the individual PK parameters (i.e. F, V_c, K_a, CL_0, CL_1, KCP, KPC) were added as covariates to use in the second simulation. In the second step the PD model was used to perform 100 additional simulations from each of the initial simulated 100 individuals resulting in 10 000 simulated patients with estimated CMV viral loads. The PMfinal object is used as input for the simulations, where the final PK or PD model supports points and the distribution around these points is used as input. The last timepoint for the simulation was at 335 h.

Results

Patients

The PK model was fitted to data from 85 patients (both on CMV prophylaxis and treatment) with 306 ganciclovir concentrations. A median of 3 (range 1–11) ganciclovir concentrations were measured per patient; the median time until the next dose was 1.7 h (IQR 0.4–8.2) and 11.3 h (IQR 5.5–19.8) since the last dose. The median age was 57 years (IQR 46–64), 48 patients (56%) were male, the median weight was 72.6 kg (IQR 61.5–83.5) and the median eGFR was 69 mL/min/1.73 m^2 (IQR 55–100). Over one-quarter (28%) of the patients had received an HSCT and 14% of the patients had received a lung transplant. The demographics of the patients used for the population PK model are presented in Table 1. The population PK model was used to calculate the average total drug AUC_{24h} values, where the median was estimated to be 94 mg·h/L (IQR 87–139). Seventeen patients (only on CMV treatment) with 69 CMV $\log_{10}$ viral loads were included in the PD model. The patient characteristics used for PD modelling are presented in Table 2. A median of 4 (range 2–8) viral loads were available for each patient. We observed significant interpatient variability in PD, with the lowest CMV viral load being close to the lower limit of detection and the highest starting viral load being

Table 1. Demographics of patients included for the PK modelling ($N = 85$)

	Median (IQR)/*n* (%)
Age (years)	57 (46–64)
Male	48 (56.5)
Weight (kg)	72.6 (61.5–83.5)
Height (cm)	175 (168–181)
Transplant	
HSCT	31 (28)
lung	16 (14)
kidney	11 (10)
liver	13 (12)
heart	7 (6)
small intestine	3 (3)
combined SOT	4 (4)
CMV treatment	48 (56.5)
CMV prophylaxis	37 (43.5)
eGFR (mL/min/1.73 m^2)	69 (55–100)
Ganciclovir concentration (mg/L)	2 (0.9–3.3)

6.0 $\log_{10}$ copies/mL (Figure 1). The estimated median viral load at the initiation of therapy was 3 $\log_{10}$ copies/mL. The median time to undetectable viral load was 13 days and two patients did not reach an undetectable viral load during the study period. For five patients, persistent viraemia was observed, with a time to undetectable viral load exceeding 25 days.

Population PK and PD modelling

The final PK model was a two-compartment model with first-order absorption and elimination. There was a relationship between CL estimated from the base model and eGFR. eGFR was included as a covariate on clearance, which resulted in a better fit of the model to the data. The individual observed-predicted ganciclovir coefficient of determination was $r^2 = 0.76$ (Figure 2a). The final population PK model parameters are reported in Table 3. The individual PK parameters from posterior predictions were used as covariates in the PD model.

The population PD model showed a good fit of the model to the data with an $r^2 = 0.94$ after the Bayesian step (Figure 2b). The estimated mean K_{kmax} was 0.01 $\log_{10}$ copies/mL/h (SD 0.01), Hk was 2.56 (SD 3.24) and EC_{50} was 13.86 mg/L (SD 8.03). The final PD model parameters are shown in Table 4. The

Table 2. Demographics of 17 patients included for PD modelling

Number	Age (years)	Sex	Transplant	CMV	D/R CMV status	Starting CMV ($\log_{10}$ copies/mL)	Time to undetectable viral load (days)	Average daily AUC_{24h} (mg·h/L)
1	39	F	liver	reactivation	D + R+	3.43	13	41.3
2	53	F	HSCT—allogeneic	pre-emptive	D + R−	2.56	–	91.9
3	19	F	heart	primary CMV	D + R−	2.85	12	158.35
4	72	M	HSCT—allogeneic	pre-emptive	D + R+	2.20	7[a]	237.7
5	71	F	HSCT—allogeneic	pre-emptive	D + R+	2.12	10	309.55
6	58	M	HSCT—allogeneic	pre-emptive	D − R+	2.79	3	94.1
7	65	M	HSCT—allogeneic	pre-emptive	D + R−	2.57	27	142.9
8	66	M	liver	primary CMV	D − R−	4.24	25	77.1
9	73	M	kidney	primary CMV	D − R−	5.87	114[b]	92.25
10	49	M	kidney	primary CMV	D − R−	5.78	140	85.3
11	64	F	liver	reactivation	D + R−	3.10	29	59.2
12	61	F	HSCT—allogeneic	pre-emptive	D + R+	3.15	8	87.0
13	46	F	HSCT—allogeneic	pre-emptive	D + R+	2.69	24	97.2
14	38	F	HSCT—allogeneic	pre-emptive	D + R+	3.01	3[a]	87.1
15	67	M	HSCT—allogeneic	pre-emptive	D + R+	2.15	–	135.1
16	58	M	HSCT—allogeneic	pre-emptive	D + R+	3.08	13	103.8
17	60	M	HSCT—allogeneic	pre-emptive	D − R+	3.33	28[a]	370.35
Summary median count	60	8 F and 9 M	11 HSCT, 3 liver, 2 kidney and 1 heart	11 pre-emptive, 2 reactivation and 4 primary CMV	8 D + R+, 4 D + R−, 2 D − R+ and 3 D − R−	3.01	13	94.1

F, female; M, male; D/R, donor/recipient.
[a]One of multiple treatment durations.
[b]To 2.23 $\log_{10}$ CMV copies/mL.

estimated CMV viral load decline was slow or non-evident. The time to undetectable viral load for most patients was more than 500 h of therapy (approximately 21 days). Individual CMV viral load profiles with the model estimates for all included patients are presented in Figure S1 (available as Supplementary data at *JAC* Online).

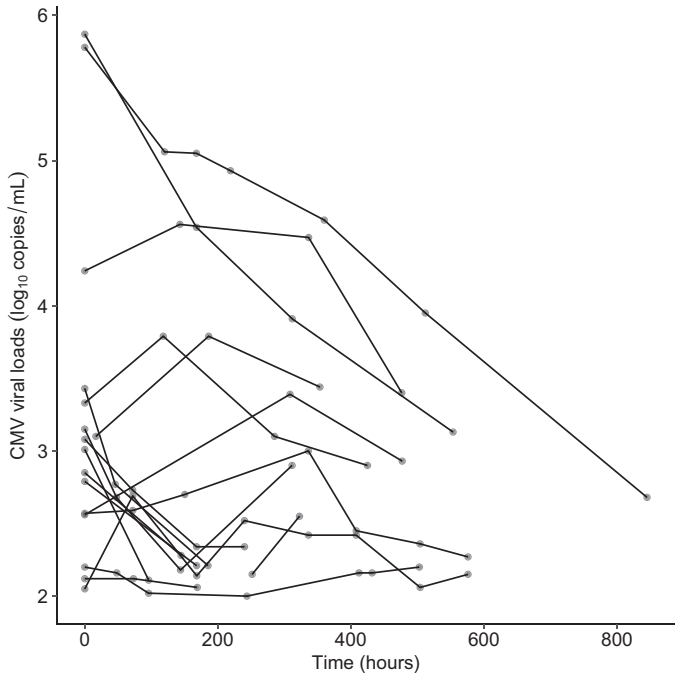

Figure 1. Viral load over time for 17 patients included in the PD study.

Simulations

Based on the median weight of 73 kg, an absolute dosage of 365 mg twice daily intravenously was used. The output of the 10 000 simulated patients showed that an approximately 1 $\log_{10}$ reduction of CMV viral load was achieved after 12.5 days (300 h). For 5% of the simulated population the decline was estimated to be faster and after 100 h (approximately 4 days) a 1 $\log_{10}$ reduction was achieved. Within this group, 1481 simulated patients reached 2 $\log_{10}$ copies (the detection limit); the median time to reach 2 $\log_{10}$ copies for these patients was 196 h (IQR 191–218). The viral load decline for the 10 000 simulated patients with time is presented in Figure 3.

Table 3. Final population PK parameters of 85 patients

Parameter	Mean	Median	SD	Shrinkage (%)
F	0.419	0.356	0.255	43.17
K_a (h^{-1})	1.169	0.201	2.214	34.91
V (L)	24.272	23.722	16.026	48.85
CL_0 (L/h)	1.349	0.786	1.906	65.27
CL_1 [L/h/(mL/min/1.73 m^2)]	0.060	0.066	0.029	55.91
KPC (h^{-1})	7.003	5.701	6.235	46.30
KCP (h^{-1})	6.461	7.003	4.811	51.77

F is the bioavailability, K_a is the absorption rate constant, V is the central volume of distribution, CL_0 is the intercept and CL_1 is the slope in the linear relationship between CL and eGFR, and KCP and KPC are the first-order intercompartmental rate constants.
Clearance was calculated as shown in Equation 4.

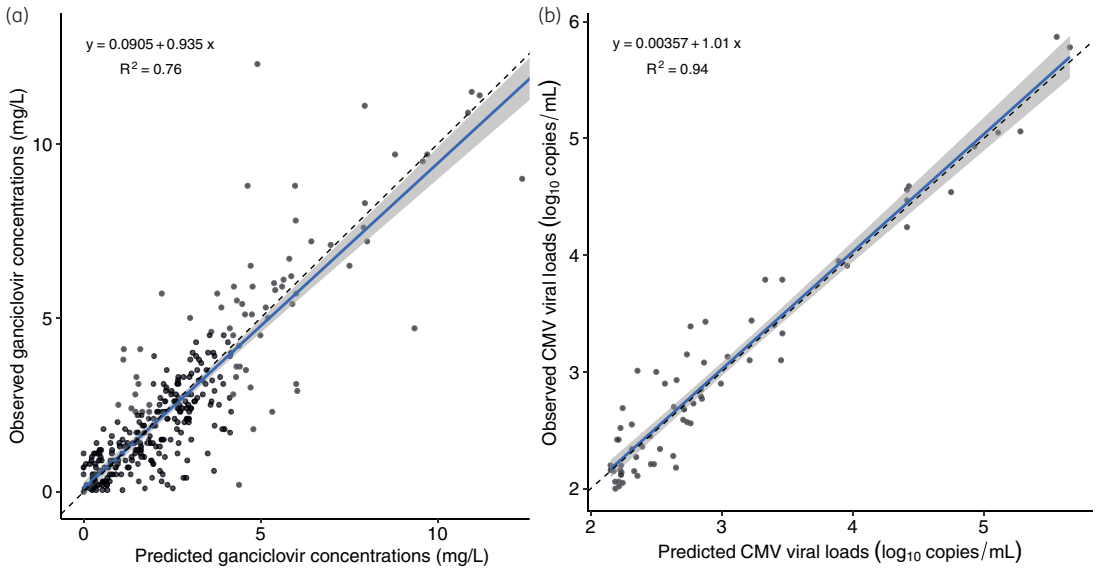

Figure 2. Observed versus predicted individual ganciclovir concentrations (PK model; a) and individual CMV viral loads (PD model; b) after the Bayesian step. The solid lines represent the linear regressions of the observed and predicted values. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 4. PD parameters of 17 patients

Parameter	Mean	Median	SD	Shrinkage (%)
K_{kmax} (log_{10} copies/mL/h)	0.01	0.01	0.01	1.72
H_k	2.56	1.74	3.24	0.54
IC (copies/mL)	50 086.37	756.05	131 481.69	0.004
EC_{50} (mg/L)	13.86	16.76	8.03	13.18

K_{kmax}, maximal rate of drug-induced viral kill; H_k, slope (Hill) function; IC, initial condition; EC_{50}, ganciclovir concentration inducing half-maximal rate of kill.

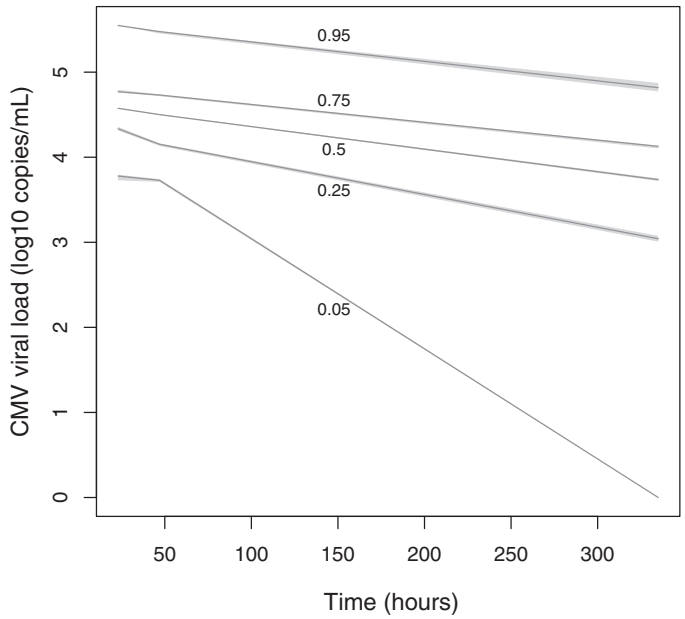

Figure 3. CMV viral load decline over time for 10 000 simulated patients. The black lines represent the 5th, 25th, 50th, 75th and 95th percentiles of the simulated CMV viral loads. The grey shaded areas represent the 95% CI.

Discussion

Our study describes the PK and PD of ganciclovir in transplant recipients. The developed linked PK/PD population model and subsequent simulations showed slow viral load reduction of CMV. In clinical practice we would expect an approximate 1 log_{10} reduction of viral loads within a week of therapy.[30–32] We used internationally accepted dosages of ganciclovir and valganciclovir, but observed a slow decline, so either the current dosing and exposure was inadequate or the immune status of the patient was that unfavourable that the effect of antiviral therapy was not enough for an adequate viral response. Currently, drug exposure targets used for ganciclovir therapy are based on either *in vitro* studies or simulations or extrapolated from prophylaxis studies and the PD are not well described.[15,18] For foscarnet and newer medications like letermovir and maribavir the PK/PD relationships have not yet been confirmed. Investigating the exposure–effect relationship is critical for effective CMV treatment.

There are various population PK models published on valganciclovir and ganciclovir with different populations. The median K_a of 0.2 h^{-1} is close to what Wiltshire *et al.*[33] reported in SOT recipients (K_a of 0.13 h^{-1}) and Chen *et al.*[34] reported in adult kidney transplant recipients (K_a of 0.23 h^{-1}). However, in other publications, higher K_a values in SOT recipients have been reported—Perrotet *et al.*[35] and Caldés *et al.*[36] reported K_a values of 0.56 and 0.895, respectively. The bioavailability has not often been reported, but in a wide range of 0.4 to 0.83, which aligns with our median of 0.4.[35–37] The median volume of distribution of 23.7 L is similar to what was observed by Caldés *et al.*,[36] Billat *et al.*[38] and Wiltshire *et al.*[33] Our reported clearance value cannot be directly compared with the reported models as these have different equations for estimating the clearance as well as different covariates included; however, our values are similar to the Pmetrics model by Billat *et al.*,[38] who reported a CL/F of 0.58 L/h. Our KPC and KCP were different from the Billat *et al.*[38] model (KPC 0.02 h^{-1} and KCP 72.3 h^{-1}); however, only Billat *et al.*[38] reported the KPC and KCP and in other published PK models for SOT recipients different structural models were used.

We observed an extremely slow reduction of viral load following ganciclovir antiviral therapy for CMV in our linked PK/PD model and in our simulated patients. This may be caused by a number of factors. Firstly, patients may have been infected with wild-type virus and have experienced concentration-dependent therapeutic failure. Secondly, the severity of CMV disease and clearance of the virus may be affected by the degree of impairment in cellular immunity.[39] In a systematic review, the *in vitro* EC_{50} values for ganciclovir in different CMV strains ranged from 0.04 to 37.2 μM (approximately 0.01–9.5 mg/L); however, the majority of EC_{50} values were <10 μM (2.5 mg/L).[40] Thus, ganciclovir is expected to be effective at lower concentrations than other antiviral drugs. Here, we estimated a high mean EC_{50} of 13.86 mg/L (54 μM) with our model, which is higher than reported in the *in vitro* results. This could mean that higher doses of ganciclovir are needed to achieve sufficient viral load decline. However, dose escalation is difficult as a still high incidence of myelotoxicity occurs, which often leads to therapy cessation.[11,41–43] Thirdly, some patients might have had resistance to ganciclovir due to mutations in genes UL97 and UL54.[44] When viral response to treatment is less than expected, while drug levels are within the target range, viral resistance is considered and tested for in our centre. This was not the case in the samples included in this study. A variable response to ganciclovir has been observed in an earlier study, but no association with the response to ganciclovir exposure could be established, due to the small sample size ($n = 7$).[31]

Previous studies suggested that ganciclovir therapy may be optimized using TDM.[22,45–47] TDM has been used to guide dosing during renal replacement therapy,[47] in deep-seated and sanctuary infection sites (e.g. brain extracellular fluid),[45] in children, in transplant recipients and in individuals living with HIV.[19,22,46] Stockmann *et al.*[18] suggested the use of AUC_{24h} for therapy optimization; however, this is not based on a robust study investigating viral load suppression in patients with high CMV viral loads. While the available data have shown potential benefits of higher ganciclovir exposure, using AUC_{24h} for therapy optimization, no consensus has been reached on PD targets associated with a high probability of efficacy and an acceptable safety. An in-depth analysis on the PK of this observational study was presented in

a separate publication.[25] Briefly, the results of the study suggest monitoring ganciclovir AUC_{24h}, as trough concentrations seemed to have worse target attainment when compared with AUC_{24h}. Besides, there is less evidence available for using trough concentration over AUC_{24h}.[18,48] In addition, during augmented renal clearance, extensive underexposure and inter- and intra-individual variability during dialysis and kidney failure was observed.

In order to design a study for resolving the PK/PD relationship between ganciclovir exposure and the antiviral response, *in vitro* and *in vivo* studies should be used to define an optimal PK/PD target in preclinical models that thereafter should be confirmed in a prospective clinical trial as the PD of ganciclovir remain unclear. However, such models have not been developed yet, including for the drugs under investigation to be used for therapy of CMV (e.g. letermovir). Creating these models for antivirals is complex as the exposure targets are not as well-known as for antibiotics (e.g. MIC).[49] Using EC_{50} or IC_{50} (half-maximal inhibitory concentration) is recommended; however, other parameters (e.g. viral load, CD4 cell count) and host-specific factors should also be considered.[49] Limiting a prospective study to either SOT or HSCT may be beneficial, as the therapeutic approach is different and the CMV burden varies between the groups. The exposure–effect and exposure–toxicity relationships of drugs being developed for CMV should be incorporated into the clinical trials.

There are several limitations of our study. In clinical practice, samples were collected less systematically than the protocol prescribed, which resulted in a low number of patients that were used to develop the PD model ($n = 17$). This may lead to suboptimal information to resolve PK/PD relationships as we observed >30% shrinkage in the parameters of the PK model. Thus, the parameter estimates for individuals are biased towards the population estimates. In addition, we have not fixed bioavailability and absorption in our PK modelling step as we were not certain what exact values to fix to due to the variability in the available literature. Thus, the values were estimated and we acknowledge that a degree of uncertainty remains for bioavailability and absorption. Another drawback was the relatively low viral load in most patients at the start of therapy, due to our strict monitoring policy. As a result, dynamics were subtle. However, as limited data are available on PD of ganciclovir in CMV, this study was necessary to describe PD in transplant recipients, to help in designing an intervention study.

It appears that the dosing of (val)ganciclovir in this study might have been inadequate to achieve fast reduction of viral load. It is clear that further studies are needed to specify the PD effects of ganciclovir by performing systematic measurements of both ganciclovir concentrations and CMV viral loads. As ganciclovir TDM is not routinely performed, this study was an important first step to specify the PK and PD of ganciclovir and guide future clinical studies in this area of clinical research.

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

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