ORIGINAL RESEARCH ARTICLE

Thoroughly Validated Bayesian Estimator and Limited Sampling Strategy for Dose Individualization of Ganciclovir and Valganciclovir in Pediatric Transplant Recipients

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Abstract

Background and Objective Given a high pharmacokinetic inter-individual variability and a low exposure target achievement, ganciclovir (GCV) therapeutic drug monitoring is sometimes used in children. We aimed to develop and validate Bayesian estimators based on limited sampling strategies for the estimation of GCV area under the concentration–time curve from 0 to 24 h in pediatric transplant recipients treated with valganciclovir (VGCV) or GCV.

Methods Solid organ transplant or stem-cell transplant recipients who received GCV or VGCV and had available GCV concentrations per standard of care were retrospectively included in this study for pharmacokinetic modeling and development of Bayesian estimators using the iterative two-stage Bayesian method. Validation datasets included additional child recipients of a solid organ transplant or stem-cell transplant, and child recipients of a kidney or liver transplant enrolled in a previous study. Various combinations of three or two sampling times, applicable in clinical practice, were assessed based on the relative mean bias, standard deviation, and the root mean square error in a development dataset and three independent validation datasets.

Results In the development dataset, the mean bias/standard deviation/root mean square error for the 1 h/2 h/3 h and 1 h/3 h limited sampling strategies were − 1.4%/9.3%/9.1% and − 3.5%/12.2%/12.3%, respectively for GCV, while for VGCV, the mean bias/standard deviation/root mean square error for the 1 h/2 h/6 h and 1 h/6 h limited sampling strategies were 0.7%/13.5%/13.3% and − 0.1%/12.1%/11.8%, respectively. In the independent validation datasets, seven (13%) and five (14%) children would have had misclassifications of their exposure using these Bayesian estimators and limited sampling strategies for VGCV and GCV, respectively.

Conclusions Three plasma samples collected at 1 h/2 h/3 h and 1 h/2 h/6 h post-dose for GCV and VGCV respectively, are sufficient to accurately determine GCV area under the concentration–time curve from 0 to 24 h for pharmacokinetic-enhanced therapeutic drug monitoring.

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Key Points

Bayesian estimators and limited sampling strategies including samples at 1 h/2 h/3 h and 1 h/2 h/6 h were developed for intravenous GCV and enteral VGCV, respectively, in pediatric transplant recipients.

The Bayesian estimators developed have been validated in three external datasets.

These Bayesian estimators are available to the medical community at https://pharmaco.chu-limoges.fr/.

1 Introduction

Ganciclovir (GCV) and its prodrug valganciclovir (VGCV) are the first-line drugs for the prophylaxis and treatment of cytomegalovirus (CMV) disease in solid organ transplant (SOT) and stem cell transplant (SCT) recipients [1–3]. No significant relationship has been observed between GCV trough concentration (C_0) and GCV efficacy [4–6]. However, a GCV $AUC_{0–24 h}$ between 40 and 60 mg h/L is often referred to as a surrogate efficacy and safety target in adult transplant recipients. Indeed, a GCV area under the concentration–time curve from 0 to 24 h ($AUC_{0–24 h}$) of 50 mg h/L was associated with an average incidence of CMV viremia of 1.3% during prophylaxis, whereas a GCV $AUC_{0–24 h}$ of 25 mg h/L was associated with an eight-fold higher incidence [7]. Moreover, the predicted incidence of neutropenia increased above 20% when GCV $AUC_{0–24 h}$ was > 60 mg h/L in adults with SOT [7, 8] and the predicted incidence of anemia increased from 26.6 to 51.9% when GCV $AUC_{0–24 h}$ exceeded 50 mg h/L [9]. These systemic exposure targets of GCV have been extrapolated to pediatric patients because of the lack of pharmacodynamic or exposure-effect studies in this population.

Several population pharmacokinetic (POPPK) analyses of GCV have been published in children, mostly in recipients of a SOT. These studies highlighted frequent insufficient GCV exposure for both intravenous (IV) GCV and enteral VGCV, a large inter-individual variability in pharmacokinetic (PK) parameters and a low probability of target attainment [10–12]. The actual US Food and Drug Administration-recommended dosing regimen based on body surface area using the Mosteller formula [13] and the creatinine clearance (CrCL) using the modified Schwartz formula [14] leads to overexposure in younger children and underexposure in older children [10–12, 15]. As a result, many other dosing regimens have been proposed in children,

mostly weight based [11, 12, 15]. However, the probability of patients achieving the exposure target range ($AUC_{0–24 h}$ = 40–60 mg h/L) is still low, with a range of 23–65% in simulation studies [10–12, 15].

Given the high PK interindividual variability and the low probability of target achievement in children, therapeutic drug monitoring is highly recommended to ensure systemic exposure that optimizes the benefit-risk balance [16]. Considering that no significant correlation has been observed between GCV C_0 and $AUC_{0–24 h}$, therapeutic drug monitoring is generally performed based on the $AUC_{0–24 h}$ [17, 18].

Determining the $AUC_{0–24 h}$ using the reference trapezoidal method is even more challenging in pediatrics than in adults because it requires many blood samples. Few studies have developed maximum a posteriori Bayesian estimators (MAP-BEs) based on limited sampling strategies (LSSs). In pediatric transplant recipients, only two papers reported LSSs for GCV after VGCV administration [12, 18]. The first study developed and validated in an independent dataset a MAP-BE for kidney transplant recipients based on the three-point LSS 0 h/2 h/4 h [18]. The second only showed a correlation between the two-point trapezoidal AUC from 2 to 5 hours ($AUC_{2–5 h}$) and the $AUC_{0–24 h}$ without any external validation [12]. Additionally, no BE has been reported so far for pediatric SCT, whether receiving enteral VGCV or IV GCV. The aims of the present study were to develop, validate, and make available POPPK models and BEs based on LSSs for the estimation of GCV $AUC_{0–24 h}$ after VGCV or IV GCV administration in pediatric SOT or SCT recipients.

2 Materials and Methods

2.1 Patient Population

For the development of the POPPK model and BEs (development dataset), we retrospectively included children from a tertiary pediatric hospital center (CHU Sainte-Justine, Montreal, QC, Canada). Children were included if: they had undergone a transplantation (SOT or SCT) between January 2007 and December 2015; they received VGCV or IV GCV for the prevention of CMV infection; and had a complete GCV PK profile (four or more PK samples) performed per standard of care. At CHU Sainte-Justine, the strategy to prevent CMV disease is a pre-emptive approach: CMV DNA in peripheral blood (CMV DNAemia) is monitored weekly during the first 100 days and then monthly until 6 months after transplantation. Antiviral therapy with IV GCV (5 mg/kg/every 12 h) or enteral VGCV (10 mg/kg/every 12 h) is started whenever CMV DNAemia is detected above a significant threshold that is not standardized and depends on risk factors (CMV DNAemia value, time since transplant, CMV status). Treatment duration is based on CMV DNA

clearance and risk factors. The Institutional Review Board of the CHU Sainte-Justine approved the protocol and since 2015, a clinical pharmacology database has been approved and implemented that prospectively collects data from children with available PK concentrations, as per standard of care.

2.2 Sample Collection and Analytical Method

As per the local standard of care, GCV therapeutic drug monitoring was performed after a minimum of six doses (steady state) with a AUC_{0-24h} target of 40–60 mg h/L. Blood samples were routinely collected in EDTA microtainers (0.25 mL) at pre-dose; 0.5; 1; 1.5; 2; 3; 6; and 12 h for IV GCV, and pre-dose; 0.5; 0.75; 1; 1.5; 2; 4; 6; and 12 h for enteral VGCV. Samples were centrifuged at 3500 rpm for 10 min (2634 g) immediately after sampling, and plasma stored (-30 °C) at the hospital clinical laboratory. Plasma concentrations were determined within 72 h of sampling, using high-performance liquid chromatography with diode array detection (Electronic Supplementary Material [ESM S1]) [19].

2.3 PK Modeling and Model Evaluation

Pharmacokinetic modeling was performed using the iterative two-stage Bayesian method with home-made software (ITSIM) [20]. Intravenous GCV and enteral VGCV were modeled separately. For both administration routes, one-compartment and two-compartment structural models with first-order elimination were investigated. For enteral VGCV, different models of absorption were investigated, including a lag time and one or two gamma distributions (because some PK profiles showed two absorption peaks). All PK profiles were considered as independents because it was not part of our objectives to explore the inter-occasion variability. A combined error model was used to describe the residual variability.

Associations between CrCL or bodyweight (WT) and PK parameters (central volume of distribution and clearance [CL]) were investigated using visual examination (scatterplots or boxplots). The covariates were included in the model using exponent allometric functions (Equations 1–3). Scaling was centralized to median WT or CrCL. Covariates were tested in the model using stepwise forward addition and backward elimination.

$$CL = CL_{std} \times \left(\frac{CrCL}{MedCrCL} \right)^x \tag{1}$$

$$CL = CL_{std} \times \left(\frac{WT}{MedWT} \right)^y \tag{2}$$

$$V = V_{std} \times \left(\frac{WT}{MedWT} \right)^z \tag{3}$$

where CL_{std} and V_{std} represent population estimates for a typical patient, CrCL is the creatinine clearance for the ith individual, WT is the bodyweight for the ith individual, MedCrCL and MedWT represent median CrCL and weight for our population, and x, y, and z are the estimated exponents.

The final model was selected based on a combination of a decrease of the Akaike information criterion and Bayesian information criterion and the visual examination of the individual and the goodness-of-fit plots: individual-predicted and population-predicted vs observed concentrations, weighted residuals vs time and weighted residuals vs individual-predicted concentrations. Internal evaluation of the model was performed using prediction-corrected visual predictive checks [21]. A total of 1000 replicates were simulated using the final model to generate expected concentrations and the 90% prediction intervals. The observed data and its 90% intervals were overlaid onto the prediction intervals and compared visually.

2.4 Correlation Between $AUC_{0-12/24\,h}$ and C_0

The relationship between $AUC_{0-12/24\,h}$ estimates and observed C_0 (within 30 min before the administration) was investigated for IV GCV and enteral VGCV using linear regression and the coefficients of determination were calculated.

2.5 BE and LSS

Based on the POPPK models developed, the best combinations of three or two sampling times applicable in clinical practice were selected by calculating the mean relative prediction error (relative bias), standard deviation (SD) and range of bias, imprecision (relative root mean square error [RMSE]), and the number of $AUC_{0-24\,h}$ estimates out of $\pm$ 10%, 15%, and 20% intervals with respect to the trapezoidal $AUC_{0-24\,h}$. The selection of the sampling times was made with a maximum deviation of $\pm$ 0.25 h (i.e., 15 min) from the selected time. Using the surrogate efficacy target range $AUC_{0-24\,h}$ = 40–60 mg h/L, the MAP-BE $AUC_{0-24\,h}$ estimates ($AUC_{BE,0-24\,h}$) were graphically compared to the trapezoidal $AUC_{0-24\,h}$ ($AUC_{trap,0-24\,h}$) and the number of exposure misclassifications potentially leading to inaccurate dose adjustment was calculated. For this purpose, GCV $AUC_{0-24\,h}$ < 40 mg h/L was classified as underexposure and GCV $AUC_{0-24\,h}$ > 60 mg h/L as overexposure.

2.6 Validation

The performances of the selected LSSs (2 and 3 points) were evaluated in three independent datasets and the relative mean bias, SD and range, RMSE, and number of $AUC_{0-24\,h}$ out of ± 10, 15, and 20% the trapezoidal $AUC_{0-24\,h}$ were calculated. The $AUC_{BE,0-24\,h}$ were also graphically compared to the reference $AUC_{trap,0-24\,h}$ to estimate the number of inaccurate classifications.

2.6.1 Validation Dataset 1

The first validation dataset included pediatric recipients of SOT and SCT between August 2015 and February 2019 (subsequent patients) at CHU Sainte-Justine, who received GCV (5 mg/kg/every 12 h) or VGCV (10 mg/kg/every 12 h) as preemptive therapy, had available GCV concentrations per standard of care and were enrolled in the CHU Sainte-Justine Clinical Pharmacology Database. Blood samples were drawn and GCV concentrations determined in a similar manner to the development dataset.

2.6.2 Validation Dataset 2

The second validation dataset included children who underwent a SOT or a SCT between February and July 2019 (subsequent patients) at CHU Sainte-Justine with similar conditions to the previous validation dataset.

2.6.3 Validation Dataset 3

The third external validation dataset included child recipients of a kidney or a liver transplant and enrolled in a study previously published [10]. All the patients received 2 days of IV GCV followed by 2 days of VGCV. The GCV and VGCV daily doses of 260 mg/m^2 and 520 mg/m^2, respectively, were based on adult recommendations with dose adjustment for estimated CrCL by Schwartz. Pharmacokinetic plasma samples were collected on day 2 of GCV (pre-dose and 1; 2–3; 5–7; and 10–12 h) and on days 1 and 2 of VGCV (pre-dose; 0.25–0.75; 1–3, 5–7; and 10–12 h). Plasma concentrations were determined using another validated, high-performance liquid chromatography-tandem mass spectrometry method [22, 23].

3 Results

3.1 Patient Population

A total of 27 children treated with IV GCV (209 PK samples, 31 PK profiles, median of seven samples per profile) and 32 children treated with enteral VGCV (293 PK samples, 43 PK profiles, median of seven samples per profile) were included in the development dataset. The patient characteristics of the development and validation datasets are described in Table 1. No significant difference was observed between the development and validation datasets, except that children were younger in the second validation set and that there was no SCT patients in the third validation dataset.

3.2 PK Modeling and Model Evaluation

For both drugs, a two-compartment model with linear elimination best described the pharmacokinetics of ganciclovir. A double gamma distribution best described the absorption phase of VGCV for the enteral model. The parameters estimated by the model were: the apparent central volume of distribution, the apparent clearance, the intercompartmental transfer constants (k_{12}, k_{21}), and the absorption parameters: shape and scale of the two gamma distributions (a1, b1 and a2, b2 for the first and second, respectively) and the fraction of VGCV absorbed during the first gamma distribution (*r*) [20]. A combined analytical error model was used to describe the residual error (error = 0.02 + 0.05 × C for VGCV, error = 0.1 + 0.05 × C for GCV). The estimates of the individual PK parameters are presented in Table 2.

No association between covariates (WT, CrCL) and PK parameters was observed (no decrease in the Akaike information criterion and Bayesian information criterion; Table S2 of the ESM). The final models without covariate were therefore retained.

The diagnostic plots of the models are shown in Fig. 1. The prediction-corrected visual predictive checks show that the average prediction of the simulated data matched the observed concentration–time profiles and that the variability was reasonably estimated (Fig. 2).

3.3 Correlation Between $AUC_{0-12/24\,h}$ and C_0

The relationships between BEs of GCV $AUC_{0-12/24\,h}$ and observed C_0 after enteral VGCV or IV GCV are shown in Fig. 3. Thirty-four and 30 GCV profiles after VGCV and GCV, respectively included C_0 values. The coefficients of determination were 0.36 and 0.27 for VGCV and GCV, respectively.

3.4 BE and LSS

For both models, the mean relative bias, SD and range of bias, relative RMSE, and numbers of $AUC_{BE,0-24\,h}$ estimates outside ± 10, 15, and 20% intervals of $AUC_{trap,0-24\,h}$ are summarized in Table 3 for each combination of two or three sampling times. The scatterplots of the $AUC_{BE,0-24\,h}$ estimates vs the $AUC_{trap,0-24\,h}$ are shown in Fig. 4.

Table 1 Characteristics of the patients in the development and validation databases

	Development CAN	Validation 1 CAN	Validation 2 CAN	Validation 3 NOR
VGCV				
Patients (*n*)	32	17	5	18
Sex				
F	16 (50%)	6 (35%)	3 (60%)	8 (44%)
Age (years)	7.3 [0.5–16.8]	7.3 [0.7–18.6]	2.7 [1.3–11.5]	8 [0.5 –16]
Weight (kg)	26.7 [6.6–87]	24 [8.8–90.2]	30 [17–43]	20.5 [5.7–52]
Transplant				
SCT	17 (53%)	14 (82%)	2 (40%)	0 (0%)
SOT	15 (47%)	3 (18%)	3 (60%)	18 (100%)
PK profiles (*n*)	43	23	6	23
Creatinine clearance (mL/min/1.73 m^2)[a]	152 [64–335]	112 [57–158]	110 [87–171]	104 [74–242]
Dose (mg/kg)	10.1 [5.2–46.9]	11.1 [7.2–19.1]	9 [7.8–12.4]	13.5 [5.5–19.6]
IV GCV				
Patients (*n*)	27	6	4	22
Sex				
F	8 (30%)	1 (17%)	2 (50%)	8 (36%)
Age (years)	7.1 [0.5–17.4]	3.5 [0.6–12.4]	3.1 [0.8–18]	11 [0.8–16]
Weight (kg)	26.2 [6.3–72.5]	15.7 [6.6–35.8]	14.8 [8.8–64.4]	26 [6.5–81.6]
Transplant				
SCT	14 (52%)	4 (67%)	3 (75%)	0 (0%)
SOT	13 (48%)	2 (33%)	1 (25%)	22 (100%)
PK profiles (*n*)	31	8	5	22
Creatinine clearance (mL/min/1.73 m^2)[a]	152 [25–345]	117 [71–139]	98 [66–136]	118 [51–234]
Dose, mg/kg	5 [0.9–9.3]	4.8 [4.5–5.6]	5.3 [2.3–5.7]	7 [3.7–10]

Values are given as median [minimum–maximum] for continuous variables and *n* (%) for categorical variables

CAN Canada, *NOR* Norway, *F* female, *IV GCV* intravenous ganciclovir, *PK* pharmacokinetic, *SCT* stem cell transplant, *SOT* solid organ transplant, *VGCV* valganciclovir

[a]Creatinine clearance calculated using the modified Schwartz formula

For enteral VGCV, the best LSS was $LSS_{1,2,6}$, leading to a mean/SD [range] bias of 0.7%/13.5% [− 40 to 24%] and a RMSE of 13.3%. The Bland–Altman plots of the difference between $AUC_{BE,0–24\,h}$ and $AUC_{trap,0–24\,h}$ vs the average of $AUC_{BE,0–24\,h}$ and $AUC_{trap,0–24\,h}$ are shown in Fig. 5A. Three $AUC_{BE,0–24\,h}$ estimates were outside the ± 20% interval. Among them, only one was misclassified as within the efficacy target range ($AUC_{BE,0–24\,h}$ = 46.2 mg h/L) while $AUC_{trap,0–24\,h}$ would have classified it as "underexposure" ($AUC_{trap,0–24\,h}$ = 33.0 mg h/L) (Fig. 4A). The $LSS_{1,6}$ showed a mean/SD bias of − 0.1%/12.1% [− 30% to 15%] and a RMSE of 12.1%. Two $AUC_{BE,0–24\,h}$ estimates were outside the ± 20% interval, one of them being misclassified ($AUC_{BE,0–24\,h}$ = 46.2 mg/L vs $AUC_{trap,0–24\,h}$ = 33.0 mg/L). Additionally, two other patients, while having bias below the ± 20% interval, would have had misclassified exposure ($AUC_{BE,0–24\,h}$ = 37.9 mg h/L vs $AUC_{trap,0–24\,h}$ = 44.5 mg h/L and $AUC_{BE,0–24\,h}$ = 39.7 mg h/L vs $AUC_{trap,0–24\,h}$ = 44.5 mg h/L).

For IV GCV, the best LSS was $LSS_{1,2,3}$ with a mean/SD bias of − 1.4%/9.5% [− 18% to 19%] and a RMSE of 9.1%. None of the $AUC_{BE,0–24\,h}$ estimates were outside the ± 20% interval. The Bland–Altman plots of the difference between $AUC_{BE,0–24\,h}$ and $AUC_{trap,0–24\,h}$ vs the average of $AUC_{BE,0–24\,h}$ and $AUC_{trap,0–24\,h}$ are shown in Fig. 5B. Only one patient had a misclassified exposure ($AUC_{BE,0–24\,h}$ = 39.3 mg h/L vs $AUC_{trap,0–24\,h}$ = 41.4 mg h/L) (Fig. 4B). The $LSS_{1,3}$ showed a mean/SD bias of 3.5%/12.2% [− 35% to 19%] and a RMSE of 12.3%. Only one $AUC_{BE,0–24\,h}$ estimate was outside the ± 20% interval but with a correct classification of exposure. However, one patient had inaccurate classification of exposure ($AUC_{BE,0–24\,h}$ = 39.3 mg h/L vs $AUC_{trap,0–24\,h}$ = 41.4 mg h/L) while its absolute bias was less than 20%.

Table 2 Final pharmacokinetic parameter estimates for VGCV and GCV

	VGCV		IV GCV	
	Median	Range	Median	Range
a1	5.98	1.85–14.47	NA	NA
b1 (1/h)	7.01	2.13–15.86	NA	NA
a2	11.03	4.29–27.44	NA	NA
b2 (1/h)	4.86	2.58–6.98	NA	NA
r	0.43	0.10–0.81	NA	NA
V1/F^a (L)	21.20	3.33–48.04	7.59	1.00–18.96
CL/F^a (L/h)	17.04	3.47–43.66	6.55	1.00–14.21
k_{12} (1/h)	1.02	0.10–1.67	1.09	0.88–1.47
k_{21} (1/h)	0.58	0.10–1.23	1.02	0.40–1.46

a1, b1, a2 and b2 shape and scale of the two gamma distributions, *CL* clearance, *CL/F* apparent clearance, *h* hour, *IV GCV* intravenous ganciclovir, *k12, k21* intercompartmental transfer constants, *NA* not applicable, *r* the fraction of VGCV absorbed during the first γ-distribution, *V1/F* apparent central volume of distribution, *VGCV* valganciclovir

^{a}Absolute CL and V1 for IV GCV (F = 1)

3.5 Validation

For the three validation datasets, the good performances were observed (Table 4). The scatterplots of the 3-point and 2-point $AUC_{BE,0–24\,h}$ estimates vs the $AUC_{trap,0–24\,h}$ for each model are shown in Fig. 6. The performances of the BEs in the pooled validation sets overall and split by the type of transplantation (SOT or SCT) are presented in the ESM Table S3.

As a sensitivity analysis, data from the development, validation 1 and 2 sets (validation set 3 has only a few C_{4h} available) were pooled to compare the performances of the $LSS_{1,2,6}$ chosen to the $LSS_{1,4,6}$ that exhibited better performances but a lower number of patients available in the development set (Table 3). The overall performances were largely better with $LSS_{1,2,6}$ (mean bias/RMSE = 2.0%/14.6%) in comparison to $LSS_{1,4,6}$ (− 14.4%/30%).

The individual profiles with the best and worst performances in each dataset are shown in Fig. 7. For enteral VGCV, 7/52 (13%) GCV $AUC_{BE,0–24\,h}$ had exposure misclassification (Fig. 6A). In detail, 4 were classified as below instead of within the efficacy target range; 1 within instead of below; 1 above instead of within; and 1 within instead of above the efficacy target range (ESM Table S4). Among the misclassified patients, assuming the GCV pharmacokinetics is linear and the $AUC_{0–24\,h}$ targeted for dose individualization is 50 mg h/L, only two patients would have had a dose proposal resulting in AUC_{trap} > 60 mg h/L ($AUC_{BE,0–24\,h}$/$AUC_{trap,0–24\,h}$ = 38.6/47.7 mg h/L, leading to 50.0/61.8 mg h/L; $AUC_{BE,0–24\,h}$/$AUC_{trap,0–24\,h}$ = 39.8/50.0 mg h/L, leading to 50.0/62.8 mg h/L). Conversely, two patients would have had an exposure within the target, resulting in no dose change.

For IV GCV, 5/35 GCV (14%) $AUC_{0–24\,h}$ were misclassified, all from the third validation dataset (Fig. 6B), with three patients classified as below instead of within the efficacy target range and two patients within instead of above the range (ESM Table S4). Among the misclassified patients, two would have had a dose proposal resulting in $AUC_{trap,0–24\,h}$ > 60 mg h/L ($AUC_{BE,0–24\,h}$/$AUC_{trap,0–24\,h}$ = 35.0/42.0 mg h/L, leading to 50.0/61.1 mg h/L; $AUC_{BE,0–24\,h}$/$AUC_{trap,0–24\,h}$ = 35.7/43.6 mg h/L, leading to 50.0/62.8 mg h/L). Conversely, two patients would have had exposure within the target, resulting in no dose change.

4 Discussion

In this study, we developed POPPK models for enteral VGCV and IV GCV and MAP-BE based on 3-point or 2-point LSSs that accurately estimated GCV $AUC_{0–24\,h}$ in pediatric transplant recipients. Afterwards, we thoroughly validated them in three independent external datasets. The determination of GCV $AUC_{0–24\,h}$ is challenging in children because it requires many blood samples causing pain and stress, a prolonged hospital stay, or multiple visits. The main goal of this study was to develop MAP-BEs that can be used for routine care with LSSs including sampling times compatible with day hospital admissions, and not to perform a thorough POPPK study to characterize the sources of variability, for which a non-linear mixed-effect approach would have been preferred.

The size of the validation sets 1 and 2 were small and could have been grouped together. However, the data were available sequentially and gathering them would have led to the loss of the real design of the study. However, sensitivity analyses were performed in the pooled validation set (ESM Table S3) and the results were similar to those initially obtained.

To the best of our knowledge, only one MAP-BE based on a LSS for children has been reported, specifically for VGCV in kidney transplant recipients using three PK samples (0 h/2 h/4 h). It showed good performances in the development stage and an independent validation datasets (MPE_{dev} = 2.6 ± 7.7%, MAPE = 5.5 ± 5.9%; MPE_{val} = 3.1 ± 13.8%, $MAPE_{val}$ = 10.5 ± 9.1%) [18]. Another study assessed the correlation between single concentrations or 2-point trapezoidal AUC and $AUC_{0–24\,h}$ and concluded that the 2-point $AUC_{2–5\,h}$ was well correlated with the 4-point trapezoidal $AUC_{0–24\,h}$ (coefficient of determination = 0.846). The authors developed a formula for the calculation of GCV $AUC_{0–24\,h}$ based on trapezoidal $AUC_{2–5\,h}$ but no validation was performed [12].

Fig. 1 Diagnostic plots of enteral (1) and intravenous models (2): **A** individual-predicted concentrations and **B** population-predicted concentrations vs observed concentrations; **C** weighted residuals error vs time after last dose; and **D** vs individual-predicted concentrations
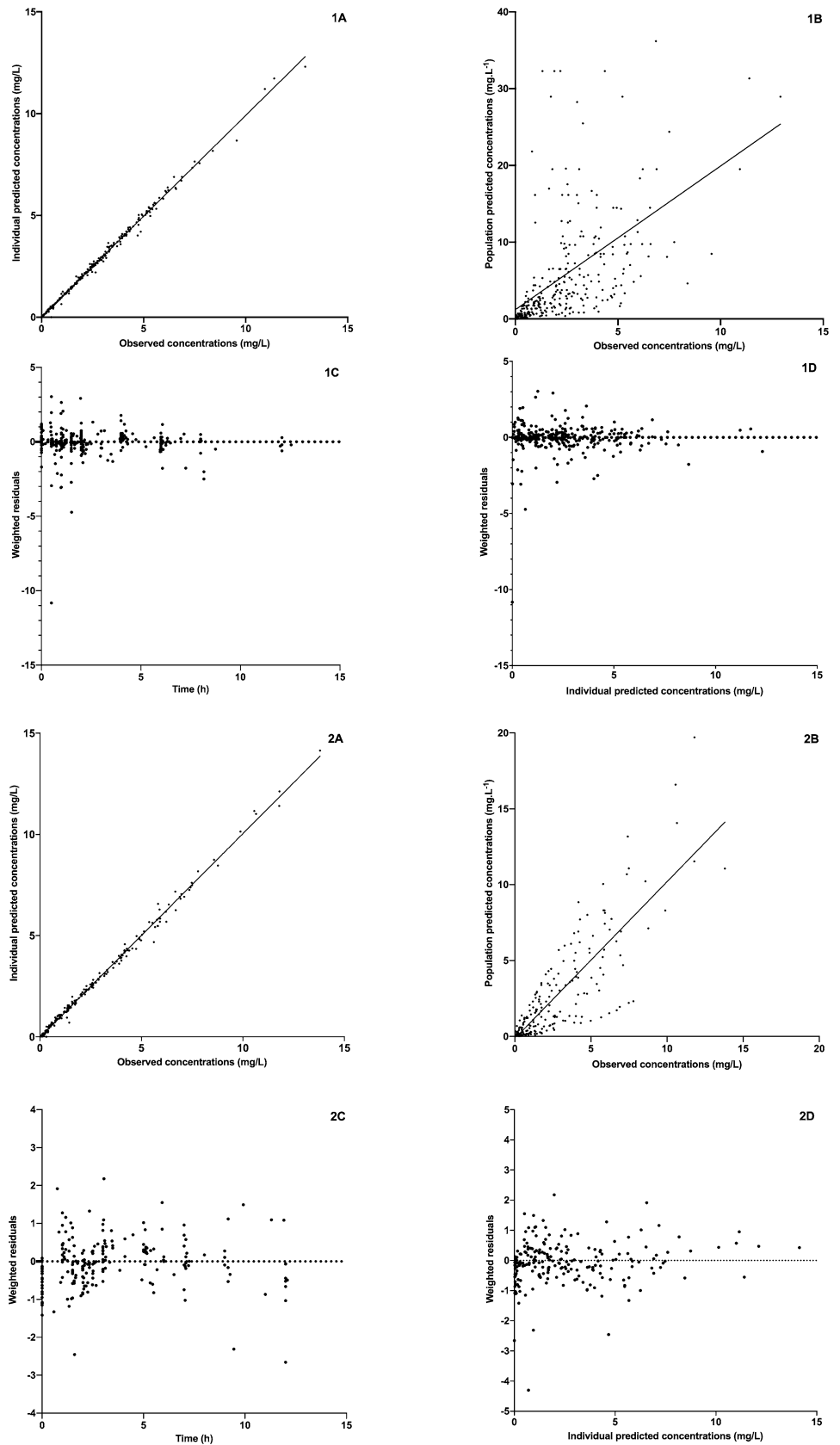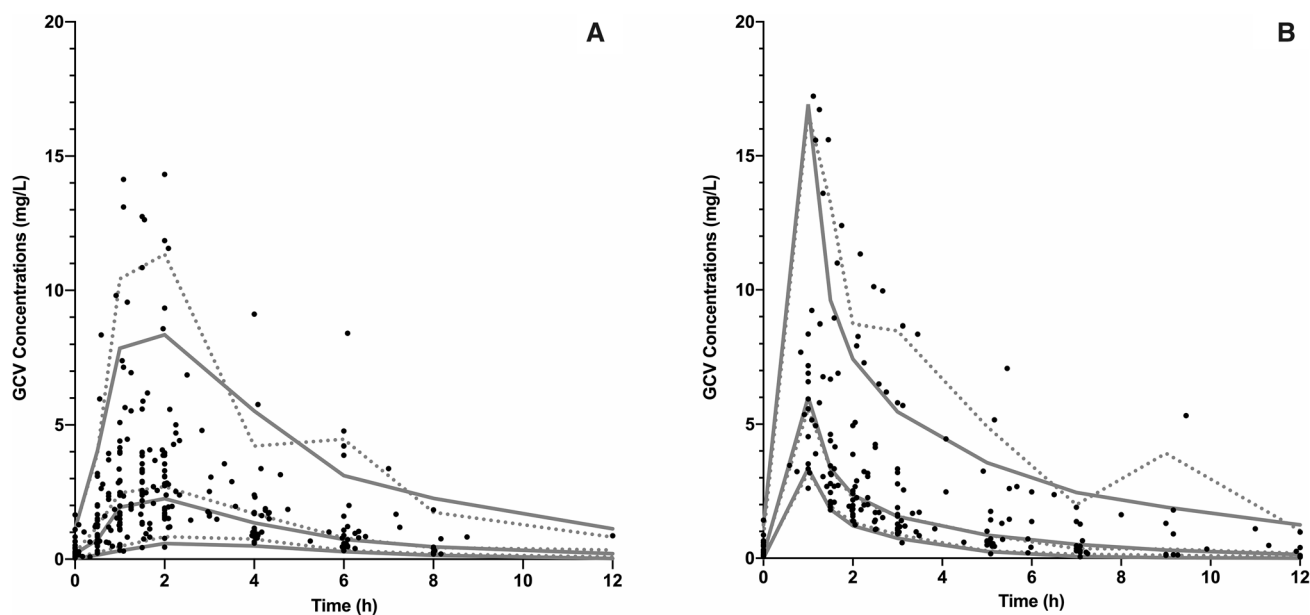
Fig. 2 Prediction-corrected visual predictive checks of the enteral (**A**) and intravenous (**B**) models. Percentiles (5%, 50%, and 95%) of observations (gray dashed lines) and predictions (gray solid lines) are overlaid with the observations (symbols). *GCV* ganciclovir
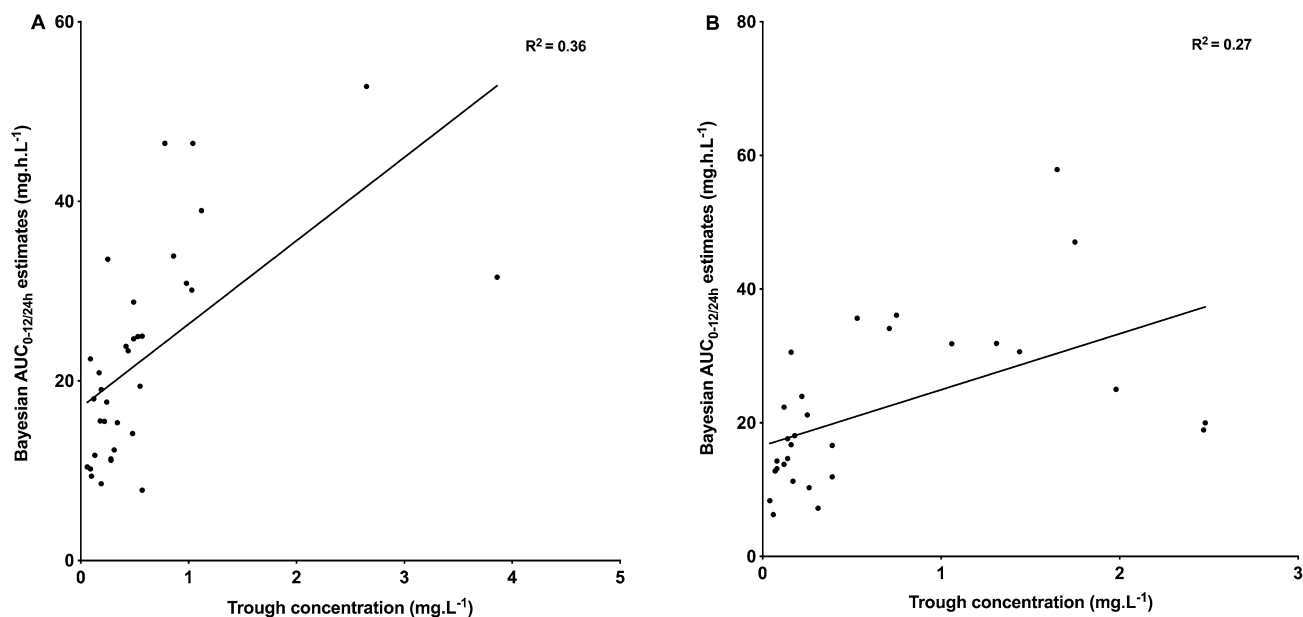
Fig. 3 Association between trough concentration and area under the concentration–time curve from 0 to 12/24 h ($AUC_{0-12/24\ h}$) among pediatric solid organ transplant and stem cell transplant recipients receiving enteral valganciclovir (**A**) or intravenous ganciclovir (**B**). R^2 coefficient of determination

Additionally, no MAP-BE has been reported for enteral VGCV in pediatric SCT, or for IV GCV. The tools that we developed in the present work are innovative as they also allow the estimation of GCV $AUC_{0-24\ h}$ in pediatric SCT recipients given VGCV and in either SOT or SCT pediatric recipients given IV GCV.

A two-compartment model with first-order elimination best described the GCV concentrations, consistent with previously published POPPK models in pediatric transplantation [10, 11, 22–26]. However, for the enteral formulation, we found that a double-gamma distribution better described the absorption of VGCV and its hydrolyzation to GCV by

Table 3 Predictive performance of different limited sampling strategies for Bayesian estimation of GCV AUC_{0-24h} after enteral VGCV or IV GCV

Time (h)	n	Mean bias (%)	SD (%)	Range [minimum to maximum]	RMSE (%)	Number of AUC_{0-24h} estimates outside of the reference interval		
						± 20%	± 15%	± 10%
Enteral VGCV								
0/1/4	23	− 10.9	29.8	[− 84% to 18%]	31.1	5	6	13
0/1/6	24	− 6.2	17.5	[− 63% to 15%]	18.0	4	7	10
0/1.5/4	20	− 15.8	33.8	[− 111% to 16%]	36.5	5	7	8
0/1.5/6	22	− 4.0	19.2	[− 62% to 17%]	19.2	3	6	12
0/2/4	22	− 15.6	32.6	[− 110% to 26%]	35.5	10	10	13
0/2/6	24	− 3.7	18	[− 48% to 24%]	18.0	6	8	13
1/2/4	24	0.2	17	[− 64% to 28%]	16.7	3	4	9
1/2/6	26	0.7	13.5	[− 40% to 24%]	13.3	3	6	9
1/4/6	16	1.8	7.6	[− 16% to 13%]	7.6	0	1	3
1/6	26	− 0.1	12.1	[− 30% to 15%]	11.8	2	3	12
2/6	26	− 1.1	19.9	[− 64% to 37%]	19.5	6	9	12
1/2	26	− 15.5	40.5	[− 127% to 42%]	42.7	11	15	18
IV GCV								
0/1/2	23	− 15.5	21.6	[− 91.8% to 5.4%]	26.2	6	7	11
0/1/3	19	− 7.8	14.5	[− 53% to 10%]	16.1	2	2	6
0/1/5	12	− 3.1	6.5	[− 18% to 4.3%]	7.0	0	1	2
1/2/3	16	− 1.4	9.3	[− 18% to 19%]	9.1	0	3	3
1/2/5	12	− 3.1	4.4	[− 9.9% to 3.5%]	5.3	0	0	0
1/3/5	10	− 1.1	3.9	[− 8.4% to 4.2%]	3.9	0	0	0
1/2	19	− 8.7	18.8	[− 38% to 33%]	20.3	8	10	11
1/3	17	− 3.5	12.2	[− 35% to 19%]	12.3	1	4	5
2/5	13	− 16.5	40.7	[− 149% to 10%]	42.4	0	1	1
1/5	12	− 2.8	6.3	[− 18% to 5.2%]	6.6	2	2	5

AUC_{0-24h} area under the concentration–time curve from 0 to 24 hours, *h* hour, *IV GCV* intravenous ganciclovir, *RMSE* root mean square error, *SD* standard deviation, *VGCV* valganciclovir

the intestinal esterases than a lagtime. Population PK models without covariates were retained for enteral VGCV and IV GCV as they showed the best performances in terms of likelihood (Akaike information criterion and Bayesian information criterion) and individual plots. Thus, despite renal elimination of GCV through glomerular filtration and tubular secretion and the inclusion of CrCL in several previous studies [10, 22–26], CrCL was not included as a covariate for GCV CL in our model. We think that covariates are useful in the case of simulation or for a priori estimation of the first dose [27]. In the case of BEs based on a LSS, the information is carried by the concentrations themselves (i.e., a patient with an altered renal function will have higher concentrations).

In any event, the VGCV and the GCV BEs were developed in patients with CrCL above 64 and 25 mL/min/1.73 m^2, respectively, and should not be used for patients with lower CrCL. However, this limitation would have been the same if the CrCL had been included in the model as extrapolations require assumption of linearity that has to be

evaluated. The influence on PK parameters of other covariates such as the type of transplant (SOT vs SCT) was not investigated because they were not significant in previously reported POPPK models.

The selection of the LSS times was based on clinical feasibility in addition to performance, to avoid patient hospital stay and to accurately describe GCV PK parameters. For VGCV, the $LSS_{1,2,6}$ was selected even though the $LSS_{1,4,6}$ showed better performances in term of relative bias and RMSE. This is because of the lower number of patients with a blood sample at 4 h than at 2 h after VGCV administration (16 vs 26 patients, respectively), leading us to select the latter. Moreover, on a theoretical viewpoint, the selection of C_{2h} instead of C_{4h} is more relevant to obtain information about the peak (i.e., the median time to maximum concentration is 2.18 h [1.7–3.0 h]) [8]. The selection of C_{6h} in the VGCV LSS seems inconvenient for outpatients, but a late PK sample was required to accurately estimate GCV CL/F (which might have only been partly compensated by using CrCL as a covariate). Analysis on the pooled development
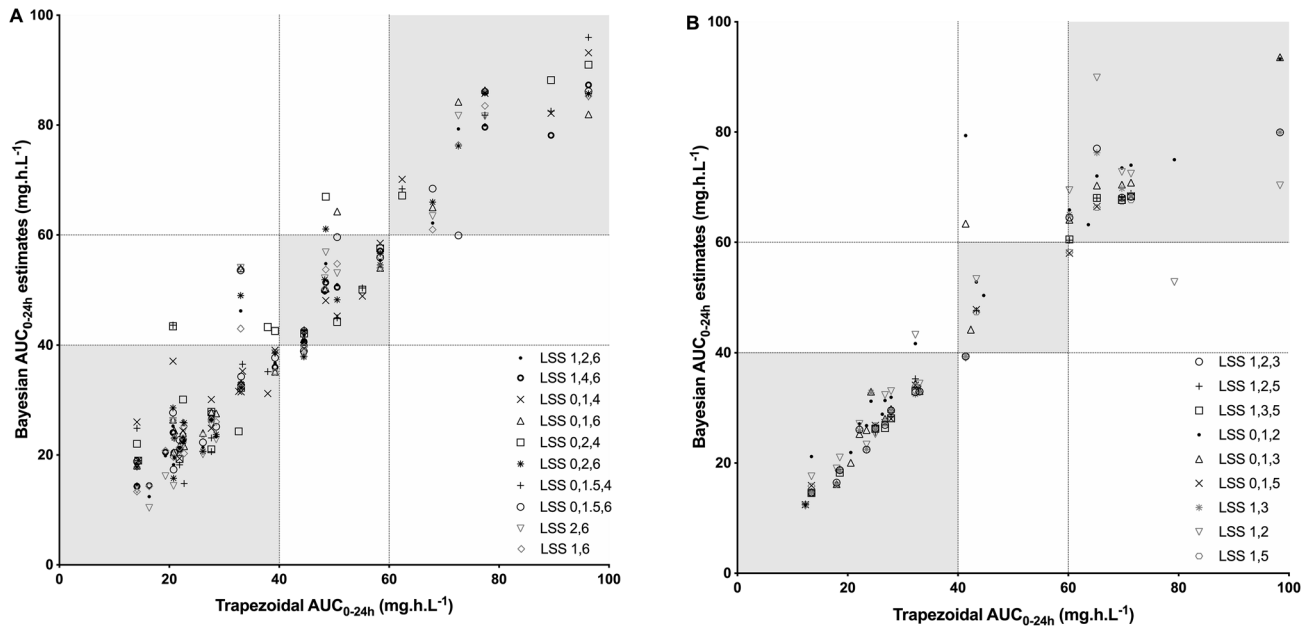
Fig. 4 Scatterplots of the Bayesian area under the concentration–time curve from 0 to 24 h ($AUC_{0-24\ h}$) estimates vs trapezoidal $AUC_{0-24\ h}$ for the combinations of two or three sampling times with the enteral (**A**) and intravenous (**B**) models. Gray dashed lines correspond to the

$AUC_{0-24\ h}$ threshold; *gray shades* correspond to the exposure area: underexposure: $AUC_{0-24\ h} < 40$ mg h/L, within the efficacy target: $AUC_{0-24\ h} = 40$–60 mg h/L, overexposure: $AUC_{0-24\ h} > 60$ mg h/L. *LSS* limited sampling strategy
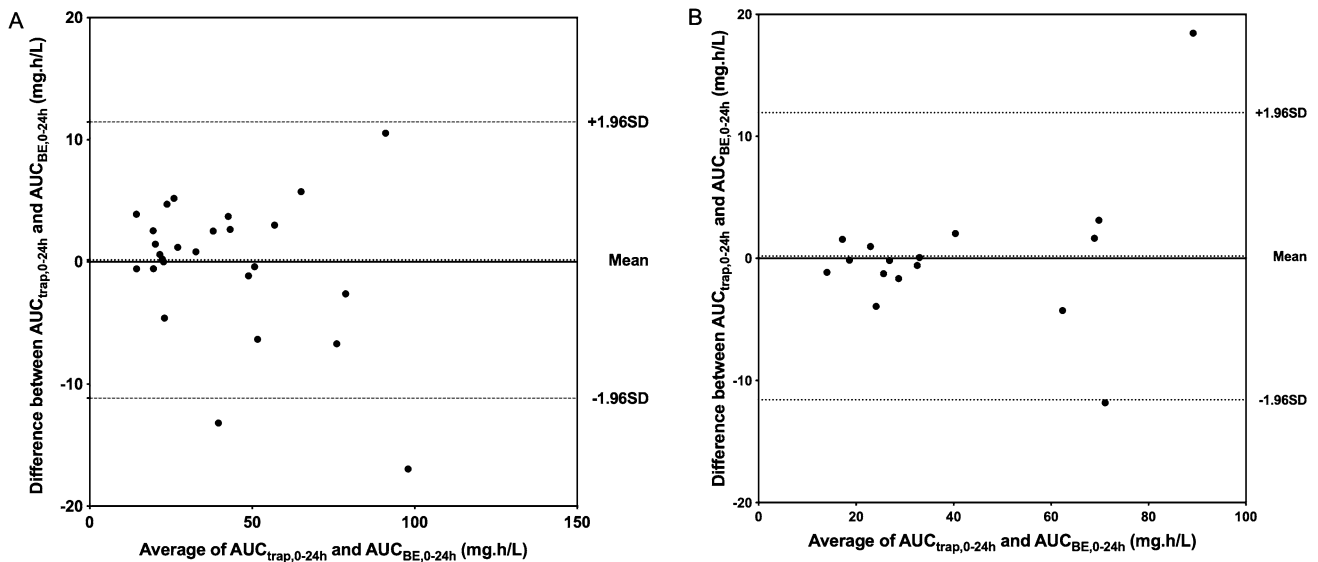
Fig. 5 Bland–Altman plots of the difference between Bayesian area under the concentration–time curve from 0 to 24 h ($AUC_{BE,0-24\ h}$) estimates and $AUC_{trap,0-24\ h}$ vs the average of $AUC_{BE,0-24\ h}$ and AUC

$_{trap,0-24\ h}$ for the selected three-point limited sampling strategy with the enteral (**A**) and intravenous (**B**) models. *BE* Bayesian estimator, *trap* trapezoidal

and validation 1 and 2 datasets confirmed that the $LSS_{1,2,6}$ was the best option. Similarly, Padullés Caldés et al. in their optimal LSS building found a higher bias and imprecision for GCV CL estimation with LSSs including only early time points (up to 5 h) as compared with those including a PK sample between 6 and 8 h [28].

While during the development of the BE phase, the sampling time selection was restricted to $\pm\ 0.25$ h, during the validation phase, this cut-off would have led to only a small number of PK profiles available for validation (only five and three PK profiles in pooled datasets for VGCV and GCV, respectively). Thus, for each PK profile, the nearest

Table 4 Predictive performance of Bayesian estimators using the selected LSS in three validation datasets

	Validation 1	Validation 2	Validation 3
Enteral VGCV			
n	23	6	23
t1 (h) [minimum to maximum]	1.00 [1–1.58]	1.00 [0.97–1.03]	0.64 [0.50–0.90]
t2 (h) [minimum to maximum]	2.00 [1.97–2.18]	2.00 [1.97–2.02]	2.08 [1.16–3.02]
t3 (h) [minimum to maximum]	7.26 [3.88–8.25]	8.00 [6.00–8.00]	6.01 [5.00–7.00]
3 points (1h, 2h, 6h)			
Mean bias ± SD (%)	2.7% ± 8.4%	8.9% ± 13.4%	1.4% ± 9.2%
Range [minimum to maximum]	[− 14.3% to 16.8%]	[− 11.6% to 20.9%]	[− 14.9% to 19%]
RMSE	8.7%	15.2%	9.1%
$AUC_{0–12/24\,h}$ estimates outside ± 20%/15%/ 10% of the reference	0/1/7	2/3/4	0/1/9
2 points (1h, 6h)			
Mean bias ± SD (%)	6.7% ± 17.5%	13.1% ± 34.3%	0.5% ± 13.4%
Range [minimum to maximum]	[− 24.8% to 40.1%]	[− 28.9% to 75.7%]	[− 23.8% to 30.5%]
RMSE	18.4%	33.9%	13.1%
$AUC_{0–12/24\,h}$ estimates outside ± 20%/15%/ 10% of the reference	8/12/14	2/2/4	2/7/11
IV GCV			
n	8	5	22
t1 (h) [minimum to maximum]	1.14 [1.00–1.68]	1.13 [1.00–1.3]	0.95 [0.90–1.50]
t2 (h) [minimum to maximum]	2.02 [1.75–2.20]	1.67 [1.50–1.80]	2.13 [1.92–3.08]
t3 (h) [minimum to maximum]	3.32 [3.00–4.10]	5.08 [5.00–5.17]	5.01 [4.92–5.50]
3 points (1h, 2h, 3h)			
Mean bias ± SD (%)	− 7.9% ± 11.7%	− 5.4% ± 21%	8.7% ± 8.3%
Range [minimum to maximum]	[− 31.1% to 5.2%]	[− 42.9% to 7.9%]	[− 14.2% to 19%]
RMSE	13.5%	19.7%	11.9%
$AUC_{0–12/24\,h}$ estimates outside ± 20%/15%/10% of the reference	1/2/2	1/1/1	0/6/11
2 points (1h, 3h)			
Mean bias ± SD (%)	− 9.3% ± 12.2%	− 2.4% ± 23.7%	9.4% ± 9.3%
Range [minimum to maximum]	[− 32.2% to 8.8%]	[− 42.9% to 19.1%]	[− 13.8% to 18.1%]
RMSE	14.7%	21.3%	13.1%
$AUC_{0–12/24\,h}$ estimates outside ± 20%/15%/10% of the reference	1/2/4	1/2/2	0/9/15

$AUC_{0–12/24\,h}$ area under the concentration–time curve from 0 to 12/24 h, *h* hour, *IV GCV* intravenous ganciclovir, *LSS* limited sampling strategy, *RMSE* root mean square error, *SD* standard deviation, *VGCV* valganciclovir

available sampling time was used, with accurate determination of GCV $AUC_{0–24\,h}$.

The two-point $LSS_{1,6}$ for VGCV showed very good performances as compared to the $LSS_{1,2,6}$ in the development dataset but not in the validation datasets. Moreover, it does not decrease the length of hospital stay but has the advantage of decreasing the number of samples collected.

For GCV LSSs, the inclusion of t_{5h} showed better performances but the low number of samples available led us to select t_{3h} in the final LSS (12 and 16 patients, respectively). In the validation datasets, the time of the third PK sample ranged between 3.0 and 5.5 h and was associated with good performances, which confirms that BEs are flexible with respect to the actual sampling times. The two-point $LSS_{1,3}$ for GCV showed comparable performances to the $LSS_{1,2,3}$

in all the development and validation datasets, which is in favor of the two-point $LSS_{1,3}$.

In addition to usual performance metrics, we aimed to select LSSs based on a minimum number of inaccurate exposure classifications that would have resulted in inaccurate dose adjustment in clinical practice. In the independent datasets, seven (13%) and five (14%) children would have had exposure misclassification using $LSS_{1,2,6}$ for VGCV and $LSS_{1,2,3}$ for IV GCV, respectively. Among them, two children for each LSS would have reached out-of-range exposure after dose adjustment. However, the surrogate efficacy target (40–60 mg h/L) is only correlated to a probability of breakthrough viremia, thus even if a GCV $AUC_{trap,0–24\,h} = 39.7$ mg h/L is considering as underexposure (vs $AUC_{trap,0–24\,h} = 44.5$ mg h/L, as presented above), the probability of breakthrough viremia is not very different than a
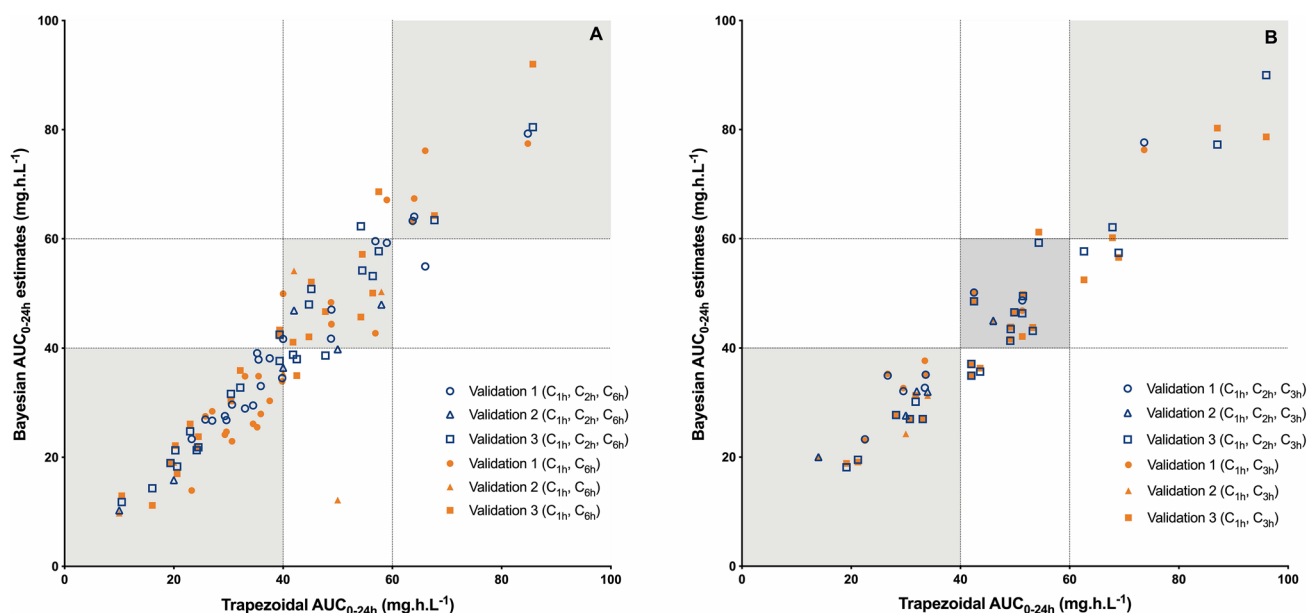
△ Adis

Fig. 6 Scatterplots of the Bayesian area under the concentration–time curve from 0 to 24 h ($AUC_{0-24\,h}$) estimates vs trapezoidal $AUC_{0-24\,h}$ for the selected 3-point and 2-point limited sampling strategies with the enteral (**A**) and intravenous (**B**) models in the different validation datasets. Blue dots correspond to the 3-point estimates, orange dots to the 2-point estimates; gray dashed lines correspond to the clinical decision $AUC_{0-24\,h}$ thresholds; gray shades correspond to the different exposure areas: underexposure: $AUC_{0-24\,h} < 40$ mg h/L, within the efficacy target: $AUC_{0-24\,h} = 40{-}60$ mg h/L, overexposure: $AUC_{0-24\,h} > 60$ mg h/L

GCV $AUC_{0-24\,h} = 40$ mg h/L. For both enteral VGCV and IV GCV, inaccurate classification of GCV exposure would only have led to an increase in the dose and increased $AUC_{trap,0-24\,h}$ up to 62.8 mg h/L, which is very close to the upper limit of the GCV target range. No dose adjustment based on exposure misclassification would have led to $AUC_{trap,0-24\,h} < 40$ mg h/L.

The main limitation of this study is its retrospective nature. Pharmacokinetic samples were collected during routine care, resulting in potential inaccuracies regarding dosing and/or sampling times, or associated clinical data. Another limitation is the use of the trapezoidal $AUC_{0-24\,h}$ as the reference to evaluate BEs, particularly in patients with less than seven PK samples (median value for both drugs). Indeed, the number of PK samples was highly variable in the development dataset (four to ten samples per child). To test the robustness of our conclusions, the performances of the LSSs were calculated using as references the BEs obtained with all the individual time points available and showed better results than with the trapezoidal AUCs as references (ESM Table S5). Both routes were modeled separately while IV information can be of added value to model oral data. However, ITSIM used for PK modeling was not able to model simultaneously both dosing routes. Last, the analytical method of GCV determination was different between cohorts and could have resulted in inaccuracy in GCV $AUC_{0-24\,h}$ as no cross-validation was performed. However, the goal of the third validation was to evaluate the BEs in a very different population to propose a model that can be used in routine care, which implies a wide range of different analytical methods.

5 Conclusions

In this study, we developed MAP-BEs based on three-point LSSs: 1 h/2 h/6 h and 1 h/3 h for VGCV and GCV, respectively, for both SOT and SCT pediatric recipients. The performances obtained in three independent datasets show that they accurately estimate GCV $AUC_{0-24\,h}$ and can be used clinically for the therapeutic drug monitoring of these two drugs. They are now available to the medical community at https://pharmaco.chu-limoges.fr/.
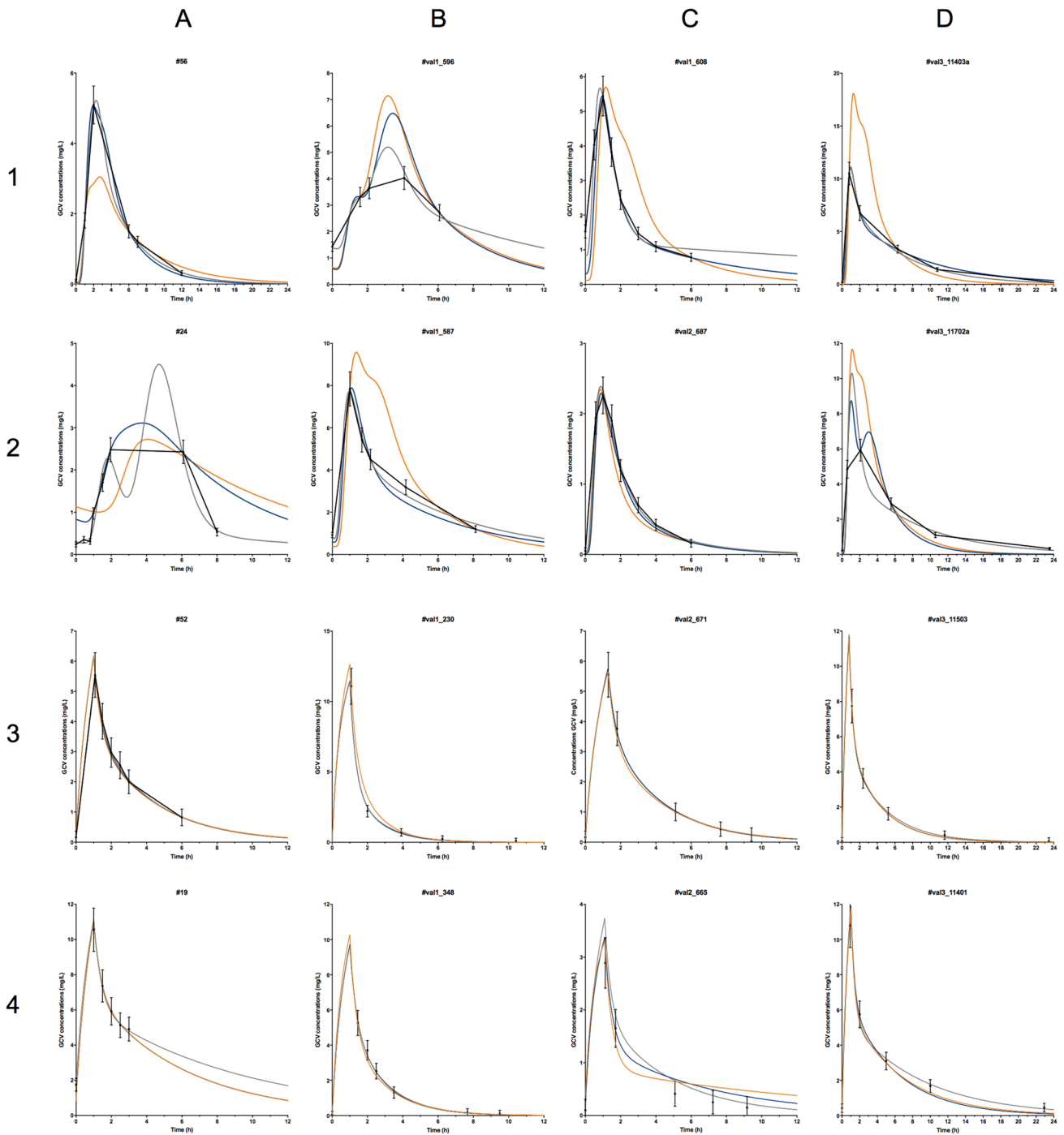
Fig. 7 Examples of individual modeled pharmacokinetic (PK) profiles for enteral valganciclovir (best fits: 1; worst fits: 2) and intravenous ganciclovir (best fits: 3; worst fits: 4) in the development (**A**) and validation (**B–D**) datasets. Black lines and plots correspond to the observed concentrations and PK profiles; gray lines correspond to the modeled PK profiles using all available time points; blue and orange lines correspond to the modeled PK profiles using a 3-point and 2-point limited sampling strategy, respectively

Declarations

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