CLINICAL PHARMACOLOGY REVIEW

NDA/Supplement	21304/S-011 and 22257/S-005
Submission Type	Efficacy supplement
Applicant Name	Roche
Submission Date	3/31/2014
Brand Name	Valcyte
Generic Name	Valganciclovir
Dosage Form (strength)	450 mg tablets (NDA 21304) and 50 mg/mL oral solution (NDA 22257)
Proposed Indication	Prevention of CMV disease in heart transplant recipients aged (b) (4) to 16 years
Review Team	Mario Sampson, PharmD, Jeffry Florian, PhD, Ping Zhao, PhD, and Islam R. Younis, PhD

CLINICAL PHARMACOLOGY REVIEW

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1 Executive summary

1.1 Background

Valganciclovir has several approved indications for prevention and treatment of cytomegalovirus (CMV) disease. Post-marketing requirement (PMR) 1533-2 (issued 8/28/2009) required the sponsor to "Perform a pharmacokinetic and safety study in pediatric heart transplant recipients <4 months of age in order to determine appropriate dosing in this age group and submit dosing recommendations for inclusion in the package insert". To fulfill the PMR, the sponsor conducted pharmacokinetic (PK) study NP 22523 in pediatric heart transplant recipients <4 months of age. As only two subjects <6 weeks of age were enrolled in study NP 22523, the sponsor also submitted physiologically-based PK modeling (PBPK) to support valganciclovir dosing recommendations for infants <6 weeks of age. In addition, supportive safety data was submitted from study CASG 112 in infants with symptomatic congenital CMV. This review concerns the PK data from studies NP 22523 and CASG 112, in addition to the PBPK modeling.

Valganciclovir is an ester prodrug of ganciclovir, which is a CMV nucleoside analogue DNA polymerase inhibitor. It is administered with food, and ganciclovir PK is dose proportional under fed conditions. The major route of valganciclovir elimination is by renal excretion as ganciclovir through glomerular filtration and active tubular secretion. The half-life is 3-7 h depending on the disease state. In adults, doses are adjusted according to renal function. Based on exposure-response relationships for efficacy (prevention of CMV viremia) and safety (hematologic adverse events), target ganciclovir $AUC_{0\text{-}24h}$ is 40-60 µg*h/mL.

In pediatrics, valganciclovir is approved for kidney or heart transplant patients aged 4 months to 16 years. Valganciclovir dose is calculated by the formula 7 x body surface area (BSA, m^2) x Schwartz creatinine clearance (CrCL, mL/min/1.73 m^2), where Schwartz CrCL (refers to CrCL estimated using the Schwartz equation) = k x height (cm) / serum creatinine (SCR, mg/dL). K values depend on age group, and k=0.45 for age <1 year. The Schwartz equation was developed based on Jaffe methodology for measurement of SCR, which is now known to be less specific compared to newer enzymatic methods.

1.2 Summary of clinical pharmacology findings

1.2.1 Study NP22523

Study NP22523 enrolled heart transplant recipients < 4 months of age at risk of developing CMV disease and being treated with ganciclovir or valganciclovir for prevention of CMV. A single dose of valganciclovir oral solution was given on each of two consecutive days according to the formula 7 x BSA x Schwartz CrCL. Valganciclovir and ganciclovir concentrations were measured by LC/MS/MS. As a sparse plasma sampling design was used, population PK modeling was used to estimate exposures and determine if the AUC target was reached. As this study only enrolled two subjects <6 weeks of age, PBPK modeling was done to support dosing recommendations in infants <6 weeks of age.

Bioanalytical and population PK methods were acceptable. The impact of SCR method (Jaffe vs enzymatic) could not be assessed as this data was not collected.

Sixteen subjects contributed 80 ganciclovir PK samples. An existing population PK model from prior pediatric transplant studies was used. The final ganciclovir model contained two compartments, and significant model covariates were weight on clearance and volume (central and peripheral), and Schwartz CrCL on clearance. Model-estimated median (range) ganciclovir steady-state $AUC_{0\text{-}24h}$ was 67.3 (33.8 – 123.2) µg*h/mL, with one (6%) subject below, six (38%) subjects within, and nine (56%) subjects above the AUC target range 40-60 µg*h/mL, respectively. In comparison, mean $AUC_{0\text{-}24}$ in adult heart transplant recipients is 40.2 ± 11.8 µg*h/mL, and the fraction of adult transplant recipients in the pivotal trial PV16000 with $AUC_{0\text{-}24h}$ values below, within, and above the target range was 38%, 48%, and 14%, respectively. Thus observed exposures in study NP22523 were higher than observed in adults. Ganciclovir exposures simulated using the population PK model, NP22523 dataset ("internal dataset"), and a larger dataset of patient covariates from prior valganciclovir pediatric studies ("external dataset") predicted a wide range of AUC values, but with the median closer to the target range [median (range) $AUC_{0\text{-}24h}$ for the internal and external datasets were 54.0 (12.3-169) and 54.5 (11.8-160) µg*h/mL, respectively].

1.2.2 Physiologically-based PK analysis

PBPK modelling was submitted to support valganciclovir dosing recommendations for infants <6 weeks of age. The PBPK model was initially developed for nonclinical species and then for human adults by incorporating physiological and drug-specific (valganciclovir and ganciclovir) parameters. The model was verified for adults by comparing model predictions to observed PK data from four studies in adult transplant or CMV-positive patients. The model was scaled from adults to children by incorporating age-specific changes in physiological parameters, and was evaluated in comparison to observed data from three studies in pediatric transplant recipients and one study in infants with congenital CMV disease. Several drug-specific and physiological parameters had to be "fit" and/or adjusted in order to obtain adequate PK predictions in adults and children >1 month of age. An additional assumption of very low kidney transporter expression (Figure 9), which was not verified, was required to obtain adequate predictions in neonates. Due to the uncertainty (in particular for neonates) in model predictions, we conclude that the model is insufficiently verified to support dosing recommendations in infants <1 month of age.

1.2.3 Study CASG112

As study NP 22523 only consisted of two days of valganciclovir dosing, study CASG 112 was intended to provide supportive safety information. In this trial, 96 infants with congenital CMV infection received oral valganciclovir therapy for 6 weeks or 6 months and ganciclovir exposure was estimated via population PK modeling. To support use of study CASG 112 for supportive safety, we requested population PK and bioanalytical reports from the sponsor. This study was conducted by an academic collaborator and a population PK report was not available. The information about the population PK model in the study report was insufficient to allow assessment of model development and evaluation.

1.2.4 Evaluation of the valganciclovir dosing formula

Using the valganciclovir dosing formula 7 x BSA x Schwartz CrCL in study NP22523, higher than target ganciclovir AUC values were observed in infants < 4 months of age. As the factor "7" in the formula is empiric, we asked the sponsor to predict exposures using a factor of "6" and

report the fraction of subjects within the ganciclovir AUC target range (40-60 µg*h/mL) for both factors ("6" and "7"). The factor "6" was chosen because it is a whole number that would not further complicate the dosing formula. The sponsor conducted this analysis using two datasets: 1) dataset 1 consisting of patients from studies using the valganciclovir dosing formula of 7 x BSA x Schwartz CrCL (studies NP22523 and WV16726); and 2) a much larger dataset (dataset 2) consisting of patient covariate records from valganciclovir studies in pediatric transplant and congenital CMV populations. Using the population PK model, ganciclovir PK parameters were simulated and AUC was calculated (bioavailability x dose / clearance) using valganciclovir dosing formula 7 x BSA x Schwartz CrCL and 6 x BSA x Schwartz CrCL.

Using a factor of "6" for the <4 month age group and dataset 1, the fraction of subjects with lower than targeted AUC values was unchanged, the fraction predicted to be within the AUC target range was increased, and the fraction with higher than targeted AUC values was decreased. However, across all pediatric age groups, the impact of changing the formula to 6 x BSA x Schwartz CrCL was predicted to increase the fraction of subjects with AUC values below the target, have no effect on the fraction within the target range, and decrease the fraction above the target. Using a factor of "6" with dataset 2, predictions for infants <4 months and for ages birth to 16 years were a greater fraction of patients below the target, similar fraction within the target, and decreased fraction above the target.

Using a factor of "6" with both datasets, the fraction of subjects with below target ganciclovir AUC was predicted to increase. As there was greater concern within the review team regarding the risk of CMV viremia versus hematological adverse effects, our conclusion was to not alter the current valganciclovir dosing formula of 7 x BSA x Schwartz CrCL.

1.3 Recommendations

- Approve valganciclovir for pediatric heart transplant patients aged 1-4 months based on the PK results for study NP22523.
- Do not approve valganciclovir for pediatric heart transplant patients aged <1 month due to lack of observed data in this age group from study NP22523 and unverified assumptions in the PBPK modeling.
- PMR 1533-2 can be considered fulfilled and the sponsor should be released from this requirement.

1.4 Labeling recommendations

Section	Labeling change
2.3 Dosing in pediatric patients	Add "The k values provided are based on the Jaffe method of measuring serum creatinine, and may require correction when enzymatic methods are used." Elaborate on the following statement proposed by the sponsor: (b) (4)
8.4 Pediatric use	Add "A physiologically based pharmacokinetic (PBPK) model was developed based on the available pharmacokinetic data from pediatric and adult patients to support dosing in heart transplant patients less than 1 month of age. However, due to uncertainty in model predictions for neonates, Valcyte is not indicated for prophylaxis in this age group.
12.3 Pharmacokinetics	Add "Relative to adult transplant patients (Table (b) (4), AUC values in pediatric patients were somewhat increased, but were within the range considered safe and effective in adults.
14.2 Pediatric patients	Update and/or add the underlined text: "The mean daily ganciclovir exposures in pediatric patients were somewhat increased relative to those observed in adult solid organ transplant patients receiving Valcyte 900 mg once daily, but were within the range considered safe and effective in adults.

2 Background

2.1 Regulatory background

Valganciclovir has several approved indications for prevention and treatment of CMV disease. PMR 1533-2, issued 8/28/2009, required the sponsor to "Perform a pharmacokinetic and safety study in pediatric heart transplant recipients <4 months of age in order to determine appropriate dosing in this age group and submit dosing recommendations for inclusion in the package insert". This review concerns the following submissions in response to the PMR:

- PK data from study NP22523, A study on the PK and safety of Valcyte (Valganciclovir) in pediatric heart transplant recipients less than 4 months of age
  - Population PK modeling to describe PK in this study
  - PBPK to predict exposures in infants <6 weeks of age
- Supportive safety data from study CASG112, a multi-center, prospective, international, Phase III, randomized, and blinded investigation of 6 weeks versus 6 months of oral valganciclovir therapy in babies with symptomatic congenital CMV disease.
  - Population PK modeling to describe PK in this study

2.2 Valganciclovir clinical pharmacology

Valganciclovir is an ester prodrug of ganciclovir, which is a CMV nucleoside analogue DNA polymerase inhibitor. Valganciclovir pharmacokinetic (PK) properties are summarized in Table 1.[1]

2.2.1 PK in special populations

Decreased renal function results in decreased clearance of ganciclovir and a corresponding increase in terminal half-life. Dosage adjustment is required for patients with impaired renal function.[1] At a dose of 900 mg oral valganciclovir daily with food, ganciclovir mean area under the curve (AUC) is increased in transplant versus non-transplant patients (Table 2).[1]

Table 1. Valganciclovir PK properties.

<table>
<tr><th>PK property</th><th>Result</th></tr>
<tr><td>Absorption</td><td>• Should be administered with food (Area under the curve increased 30% when administered with food)
• Absolute bioavailability of ganciclovir (Valcyte tablets) administered with food is ~60% in adults and in pediatric solid organ transplant patients aged 4 months to 16 years
• Ganciclovir (Valcyte tablets) median Tmax is 1-3 h</td></tr>
<tr><td>Distribution</td><td>• Ganciclovir protein binding is 1-2%
• V_d is 0.70 L/kg</td></tr>
<tr><td>Metabolism</td><td>• Valganciclovir is metabolized in the intestinal wall and liver to ganciclovir
• Ganciclovir is the only metabolite of valganciclovir
• Systemic AUC and C_{max} of valganciclovir are 1% and 3% of ganciclovir, respectively</td></tr>
<tr><td>Elimination</td><td>• Under fed conditions, ganciclovir PK is dose proportional
• The major route of elimination of valganciclovir is by renal excretion as ganciclovir through glomerular filtration and active tubular secretion
• Ganciclovir half-life
<table>
<tr><th>Population</th><th>Half-life (h)</th></tr>
<tr><td>Healthy, HIV-positive, and CMV-positive subjects</td><td>4</td></tr>
<tr><td>Transplanted adults</td><td>6.5</td></tr>
<tr><td>Pediatric solid organ transplant patients aged 4 months to 16 years in different age/organ groups</td><td>~3-5 h</td></tr>
</table>
</td></tr>
</table>

Source: Valcyte label.

Table 2. Ganciclovir mean AUC in transplant vs. non-transplant patients.

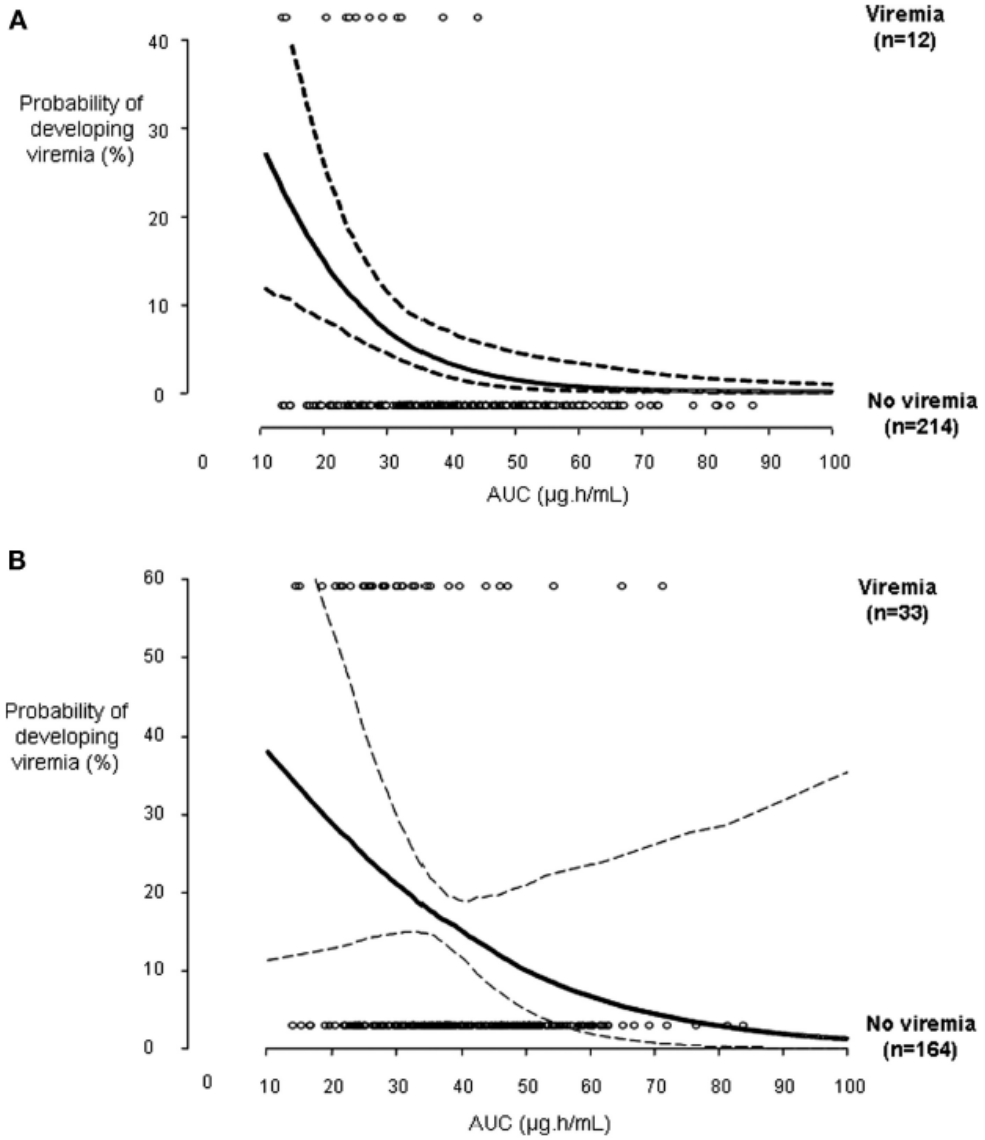
Population	N	Mean $AUC_{0\text{-}24h}$ (µg*h/mL)
Healthy volunteers and HIV+/CMV+ patients	57 (3 studies)	29.1
Heart transplant	17	40.2
Liver transplant	75	46.0
Kidney transplant	68	48.2

Source: Valcyte label.

2.2.2 Exposure-response

The sponsor chose a ganciclovir $AUC_{0\text{-}24h}$ target of 40-60 µg*h/mL in study NP22523. This is the same target that was used in study WV16726 in support of dosing in pediatric transplant patients aged 4 months to 16 years.[2] This target is consistent with mean exposures associated with efficacy and safety in transplant patients (Table 2), and is supported by a study of 372 solid organ transplant recipients aged >13 years.[3] In this study, subjects received oral valganciclovir 900 mg once daily or oral ganciclovir 1000 mg three times daily for 100 days for prevention of CMV. PK samples were available for 240 patients. PK parameters were estimated using population PK modeling. Exposure-response analysis was conducted using estimated PK parameters and logistic regression. The predicted probability of viremia decreased with increasing ganciclovir $AUC_{0\text{-}24h}$, and all instances of viremia during prophylaxis and the majority of instances 3 weeks after cessation of prophylaxis occurred at ganciclovir $AUC_{0\text{-}24h}$ <45 µg*h/mL (Figure 1). When analyzed by AUC categories, there was no clear relationship between the fraction of patients with hematological adverse events and ganciclovir $AUC_{0\text{-}24h}$ measured up to 4 months posttransplant (3 weeks after cessation of prophylaxis, Figure 2); however, when analyzed continuously using Monte Carlo simulations, there was an increasing predicted probability of neutropenia and leukopenia with increasing ganciclovir $AUC_{0\text{-}24h}$ over an $AUC_{0\text{-}24h}$ range of 10-100 µg*h/mL (Figure 3).

Figure 1. CMV viremia as a function of ganciclovir AUC.[3]



A = during prophylaxis; B = 4 months posttransplant (3 weeks after cessation of prophylaxis).

Figure 2. Hematological adverse events 4 months posttransplant (3 weeks after cessation of prophylaxis) as a function of ganciclovir AUC.[3]

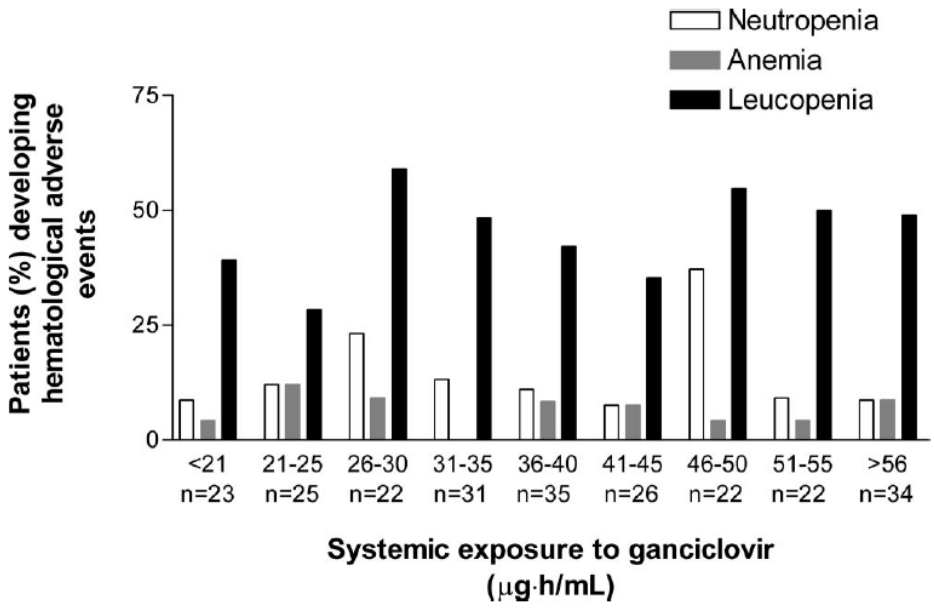
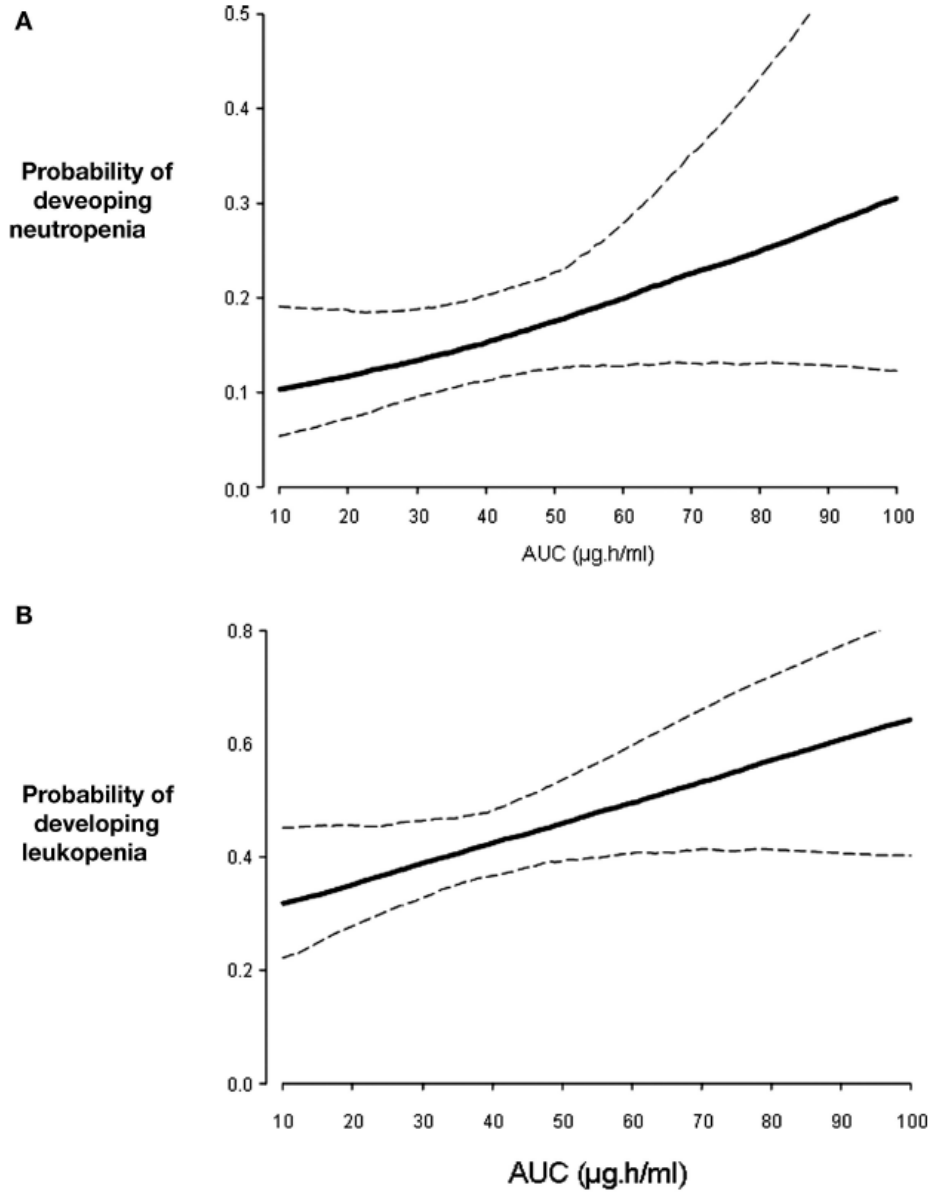


Figure 3. Probability of neutropenia and leukopenia 4 months posttransplant (3 weeks after cessation of prophylaxis) in relation to ganciclovir AUC.[3]



2.3 Rationale for pediatric dose selection

Pediatric dosing was based on matching adult valganciclovir exposures at 900 mg daily. Prior pediatric valganciclovir PK studies WP16296 (enrolled ages 1-16 years) and WP16303 (enrolled ages 6 months – 16 years) used BSA-based dosing, adjusted for Schwartz CrCL if <70 mL/min/1.73m^2.[4, 5] This algorithm led to ~2-fold under-dosing of children <6 years of age. Based on simulations, the following formula was selected for use in study WV16726 (enrolled ages 4 months – 16 years): valganciclovir dose = 7 x BSA x Schwartz CrCL, where there was no restriction on maximum Schwartz CrCL values. This formula produced exposures close to the target range and was approved by FDA for pediatric solid organ transplant patients aged 4 months to 16 years.[2, 6] Based on simulations done to address a theoretical risk of overdosing

children with BSA and SCR values in the lower range, the dosing formula was later modified to allow a maximum Schwartz CrCL value of 150 mL/min/1.73m^2.[7] This formula was then used for study NP22523, which is the subject of this review.[8]

3 Study NP22523

3.1 Study conduct

3.1.1 Study design

The study design of valganciclovir PK study NP22523 in pediatric heart transplant recipients < 4 months of age is summarized in Table 3 and is acceptable.

Table 3. Design of study NP22523.

Type	Multi-center, open-label, PK and safety study
Population	Pediatric heart transplant recipients < 4 months of age at risk of developing CMV disease and being treated with ganciclovir or valganciclovir for prevention of CMV
Study Rationale	To characterize the PK of ganciclovir
Treatments	A single dose of valganciclovir oral solution given on each of two consecutive days. Dose (mg) = 7 x BSA (m^2) x Schwartz CrCL (mL/min/1.73 m^2). • The final concentration of the oral solution when constituted per bottle was 50 mg/mL. • BSA = body surface area = sqrt[length(cm) x weight (kg)/3600]. • Schwartz CrCL = Schwartz creatinine clearance = 0.45 x Length (cm) / serum creatinine (mg/dL). • If the calculated dose was >900 mg, the dose administered was 900 mg. • If the calculated Schwartz creatinine clearance exceeded 150 mL/min/1.73 m^2, then a maximum value of 150 mL/min/1.73 m^2 was used in the equation.
Dose Selection Rationale	The pediatric dosing equation was derived based on matching exposures in adults receiving oral valganciclovir 900 mg once daily
Administration	☐ Fasted ☑ Fed
Formulation	Valganciclovir for oral solution. The formulation number was RO 107-9070/F01. The bulk batch numbers were B1010, B1020 and N0011.
Interfering Substances Excluded	Acyclovir, valacyclovir, famciclovir, cidofovir, foscarnet, lobucavir, and probenecid. Extreme caution was advised for agents that interfere with renal function, along with imipenem-cilastatin.
Sampling Times	-0.5 h pre-dose, and 1-3, 3-7, 7-12, and 24 h post-dose
PK Parameters	C_{max} and AUC were used to compare pediatric vs. adult exposures
PK Analysis	• Population PK modeling was used to estimate exposures and determine if the AUC target was reached. • PBPK modeling was used to predict exposures in infants and neonates for different dosing regimens.

3.1.2 Valganciclovir and ganciclovir bioanalytical methods

The LC/MS/MS bioanalytical methods for valganciclovir and ganciclovir were acceptable according to the criteria in the FDA Bioanalytical Guidance. There was a significant carryover effect for valganciclovir; none was observed for ganciclovir. Valganciclovir carryover was addressed by placement of samples within runs (for example, by placing blank samples after test samples), and absence of carryover was demonstrated by lack of contamination in blank wells. Also, apparent interference in the ganciclovir chromatograms due to the internal standard was addressed by an analysis showing that subtracting out interference and re-integrating chromatograms resulted in unchanged concentrations. See the appendix for a detailed review of method validation, cross-validation at another site, and analysis of study samples.

The bioanalytical inspection found the analytical portion of study NP22523 to be acceptable.[9]

3.1.3 Serum creatinine bioanalytical methods

This submission utilized the original Schwartz equation for estimating glomerular filtration rate (GFR). This equation was derived from a population of full-term infants <1 year of age without renal disease. The equation is as follows: estimated GFR (mL/min/1.73m^2) = k x length (cm) / serum creatinine (SCR, mg/dL), where k is a proportionality constant equal to 0.45 for ages <1 year.[10] The creatinine assay underlying development of the formula used Jaffe methodology. Using Jaffe methodology, precise estimates of SCR are difficult at concentrations <1 mg/dL.[11] As the study NP 22523 population consisted largely of full-term infants without renal disease (see study conduct results below), the use of the original Schwartz equation is appropriate.

The two main methods for measuring SCR are Jaffe and enzymatic. Enzymatic methods are more specific and tend to result in values around 30% lower in adults.[11, 12] However, the difference between Jaffe and enzymatic measures are unknown in children.[12] Serum creatinine reference ranges are low in pediatrics, increasing bias when Jaffe methods are used. Hence enzymatic methods are preferred for pediatrics in Europe.[13] Updated values for k must be determined for use of the original Schwartz equation with enzymatic methods.[11, 14]

Isotope dilution mass spectrometry (IDMS) is now considered the gold standard method for determining SCR concentrations because of excellent analytical performance.[15, 16] All creatinine methods are recommended to be calibrated to an IDMS method and all major laboratory instrument manufacturers in the US claim to have IDMS-traceable methods.[17, 18] There is a revised Schwartz equation intended for use with IDMS-traceable creatinine methods; however, this study only enrolled children >1 year of age with kidney disease.[19]

In this submission, we were unable to assess the impact of SCR method (Jaffe vs enzymatic) as this information was not collected. As FDA policy on SCR methods is not yet established and this issue affects all drugs dosed according to SCR, we will defer action on this issue.

3.2 Results

3.2.1 Study conduct

The study conduct was acceptable. Seventeen subjects were enrolled and sixteen contributed PK samples (Table 4). Two subjects, both with postnatal age >6 weeks at the time of their last PK sample, were born prematurely. Two subjects (one aged <6 weeks) had Schwartz CrCL<60 mL/min/m^2, indicative of possible kidney disease.[20] There were three protocol violations, none of which affected the results: one serious adverse event was reported after the pre-specified time period of 24 hours, one patient received a lower dose on day two than day one, and one patient who discontinued early did not have all laboratory assessments.

Table 4. Demographics of subjects who contributed PK samples.*

	Birth to <6 weeks	6 weeks to <4 months	Total
N patients	2 (13%)	14 (88%)	16 (100%)
N samples	10 (13%)	70 (88%)	80 (100%)
Female	1 (6%)	6 (38%)	7 (44%)
Gestational age (weeks)	39 (39-39)	39 (35-41)	39 (35-41)
Postnatal age (days)	31 (26-37)	93 (44-124)	89 (26-124)
BSA (m^2)	0.25 (0.23-0.26)	0.29 (0.22-0.32)	0.28 (0.22-0.32)
Schwartz creatinine clearance (mL/min/1.73 m^2)	57 (49-66)	86 (45-119)	86 (45-119)

Source: reviewer's analysis.
Values are number (%) or median (range) at time of last PK sample.
* Here PK data from all subjects are combined, whereas the study report presents data separately for subjects included in model development (n=14) versus an additional two subjects enrolled after model development.

3.2.2 Population PK analysis

3.2.2.1 Model development

The model development methods were acceptable. The population PK model used was previously developed based on data from prior pediatric transplant patients in studies WP16296, WP16303, and WV16726.[4-6] Modeling was conducted using the First Order Conditional Estimation with Interaction estimation method implemented in NONMEM V software. The existing final ganciclovir model was a 2-compartment model including fixed effect parameters for first order formation of ganciclovir from oral valganciclovir (K_f), lag time, and relative bioavailability (F) of oral valganciclovir compared to IV ganciclovir. Inter-subject variability parameters were included for K_f, CL, central volume (V_{cent}), peripheral volume (V_{periph}), and F. Covariates included were CrCL and height on CL, and height on V_{cent} and V_{periph}.[2]

In this submission, the existing 2-compartment base model was used and covariate analysis was undertaken using a dataset consisting of the three prior pediatric studies combined with the first

14 subjects enrolled in study NP22523. The prior review of study WV16726 identified bioanalytical issues which necessitated excluding 37 samples. However, the dataset used by the sponsor for this submission contained these 37 samples. Thus, we requested that the sponsor repeat the population PK analysis using the correct dataset. This review is of the revised population PK results.[21] The dataset available for modeling consisted of 948 ganciclovir samples from 119 subjects (Table 5). 225 samples were taken following administration of IV ganciclovir and 723 following oral valganciclovir administration. For model building, 10 samples from 6 subjects were excluded. Reasons for sample exclusion included lack of adequate dose or sample information (n=3 samples) or significant differences between observed concentrations after a particular dose in comparison to the typical profile in a given patient (n=7 samples). Thus 938 samples from 117 subjects were included for estimation of PK parameters.

Table 5. Summary of data included in the population PK analysis.

Study	Type	Number of patients	Number of ganciclovir samples	Transplant population	Formulation	Ages (years)
NP22523	PK, safety	14	80	Heart	VOS	< 0.33
WP16296		25	372	Renal	GIV, VOS	1-16
WP16303		18	165	Liver		0.5-16
WV16726	PK, safety, efficacy	62	331	Solid organ	VS	0.33-16

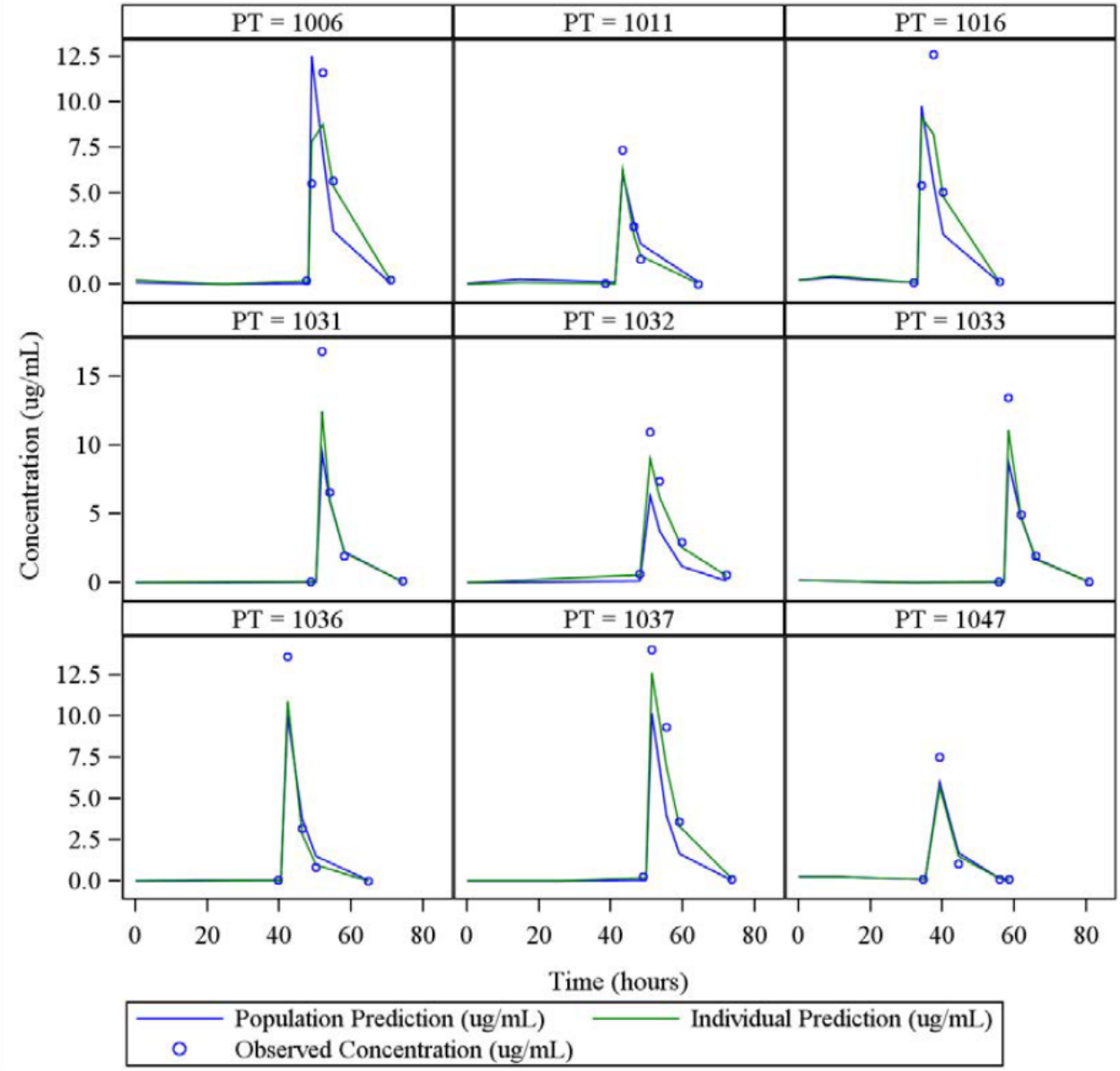
Source: reviewer's analysis.
GIV=IV ganciclovir; VOS=valganciclovir for oral solution; VS=valganciclovir syrup.

The sponsor arrived at a final model with the following covariates: Schwartz CrCL on CL, allometric weight (exponent of 0.75) on CL and intercompartmental CL, and linear weight (exponent of 1) on V_{cent} and V_{periph}. The effect of Schwartz CrCL on ganciclovir CL is physiologically plausible as ganciclovir is predominantly renally cleared, and the significant effect of body size on CL and V is consistent with many pediatric population PK studies.

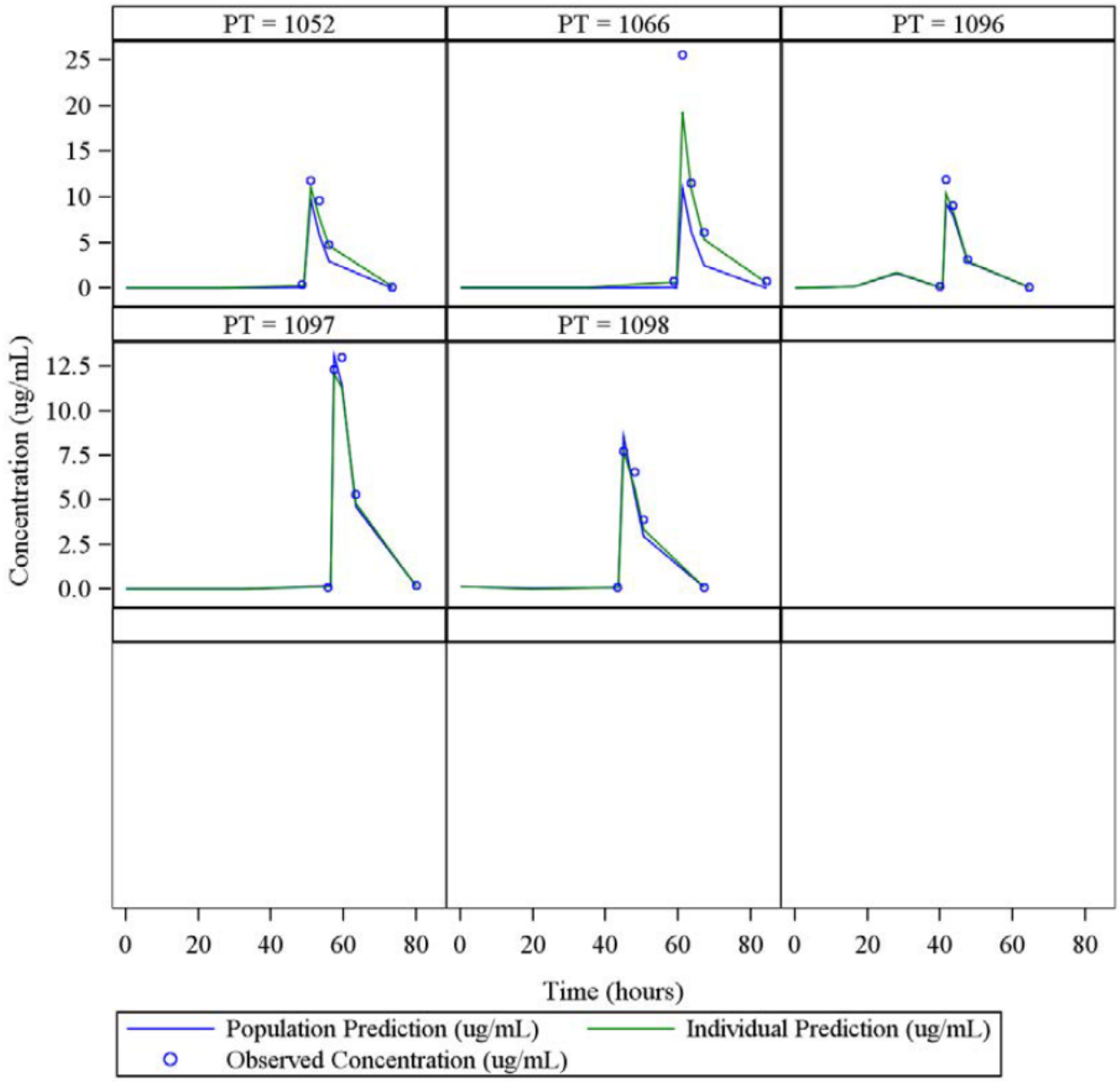
3.2.2.2 Model evaluation

The sponsor conducted sufficient model evaluation that demonstrated acceptability of the final model. Goodness-of-fit plots (observed vs population and vs individual predicted concentrations, conditional weighted residuals vs time and vs population predictions) for the base and final models were without significant bias (data not shown). For subjects in study NP22523, population and individual predictions agreed with most of the observed data points for all subjects; however, individual predictions better captured observed peak concentrations (Figure 4). Simulations of the final model (visual predictive check) demonstrated adequate overlap with observed data from study NP22523 (Figure 5).

Figure 4. Ganciclovir observed and predicted concentrations for subjects in study NP22523.

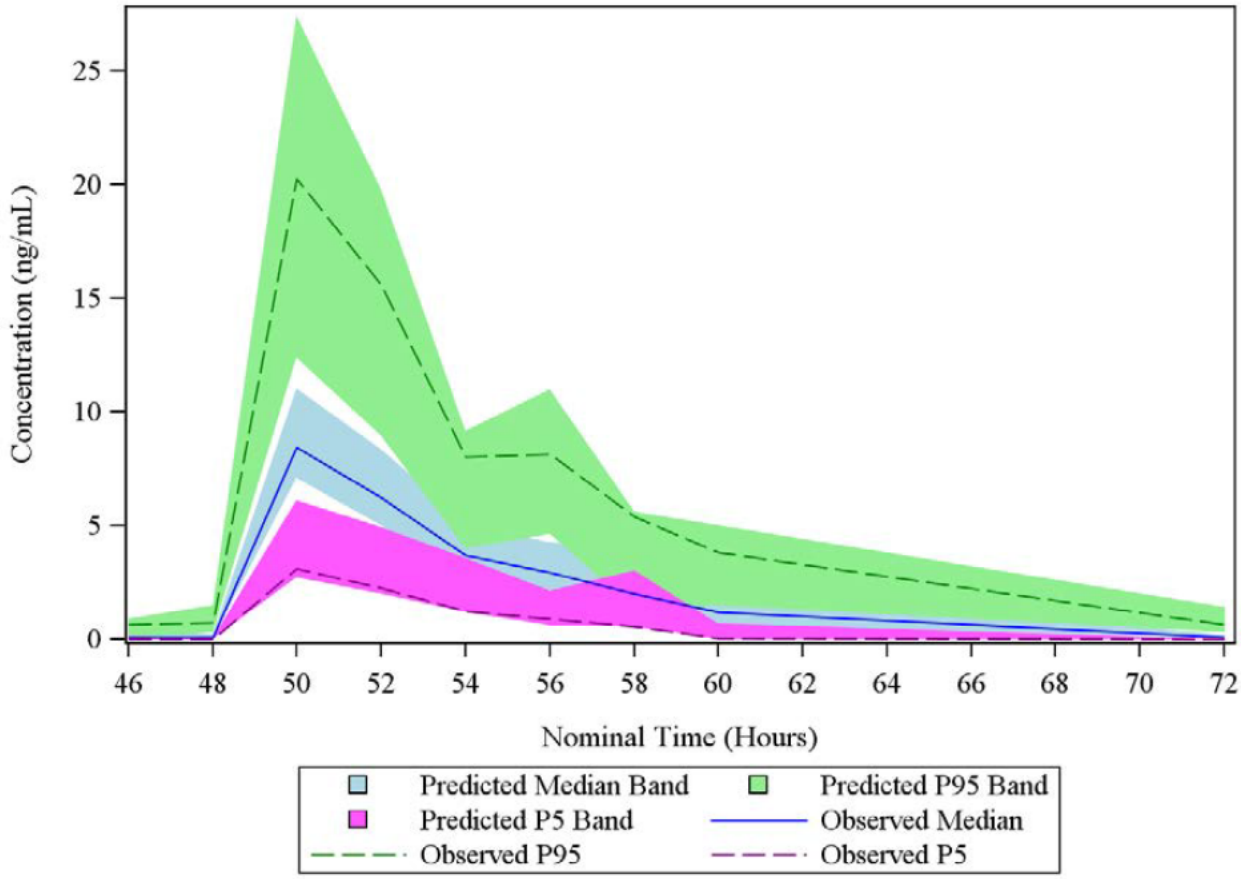


Source: sponsor's analysis.



Source: sponsor's analysis.

Figure 5. Visual predictive check.



Lines representing observed data percentiles are for study NP22523.[21]
Source: sponsor's analysis.

3.2.2.3 PK parameter estimates

Population PK parameter estimates for the first 14 subjects enrolled in study NP22523 are shown in Table 6. Steady-state $AUC_{0\text{-}24h}$ was calculated from each subject's model-estimated parameters according to $AUC_{0\text{-}24h}$ = Dose x F / CL. Compared to older children where median AUC was within the target range of 40-60 µg*h/mL, median AUC in study NP22523 (64.6 µg*h/mL) was above the upper limit of the target (Table 7). As there were only two subjects in the <6 week age group in study NP22523, it is not possible to draw conclusions about exposures across age groups (Table 8). Two additional subjects were enrolled in study NP22523 after model development; when these subjects were included in the AUC analysis and AUC values were averaged for subjects with more than one value, median (range) $AUC_{0\text{-}24h}$ was 67.3 (33.8 – 123.2) µg*h/mL (Figure 6).

Table 6. Population PK parameter estimates for study NP22523.

Parameter	Population Mean		Interindividual Variability	
	Estimate	CV (%)	Estimate (%)	CV (%)
Kf(h^{-1})	0.63	9.7	46.9	32.8
CL (L/h)	4.03	4.8	26.4	18.2
V_{cent} (L)	8.54	11.7	39.4	52.8
V_{periph} (L)	8.31	9.0	39.9	32.5
Q (L/h)	2.82	15.2	----	----
F	0.587	4.7	23.1	22.4
Lag Time (h)	0.217	2.7	----	----
Multiplicative Error (%)	31.7 %	4.9	----	----
Additive Error (μg/mL)	0.0144	62.8	----	----
Influence of Creatinine Clearance on CL	0.681	16.0	----	----
CV = standard error expressed as coefficient of variation in %				

Source: sponsor's analysis.

Table 7. Model-estimated ganciclovir steady-state $AUC_{0\text{-}24h}$ across age groups in all studies.

PK Parameter	NP22523[a] (<4 Months)	Pooled Studies (<2 years)	Pooled Studies (2–6 years)	Pooled Studies (6–12 years)	Pooled Studies (>12 years)
$AUC_{0\text{-}24h}$ (µg•h/mL)	n=18	n=102	n=48	n=63	n=101
Mean	68.1	54.5	50.3	46.1	46.9
Median	64.6	56.4	49.6	46.5	47.3
%CV	29.0	37.4	37.6	32.8	37.7
C_{max} (µg/mL)	n=14	n=28	n=14	n=20	n=43
Mean	10.5	11.1	9.09	8.87	8.38
Median	10.7	11.1	9.62	9.25	8.65
%CV	31.9	38.2	27.8	40.5	36.9

$AUC_{0\text{-}24h}$ = area under the concentration–time curve over the dosing interval; n = number of observations; CV = coefficients of variation; PK = pharmacokinetic.
[a] n = 14 patients

Source: sponsor's analysis.
[a]Reviewer's note: Several subjects received different dose amounts while in the study and thus had more than one AUC value.

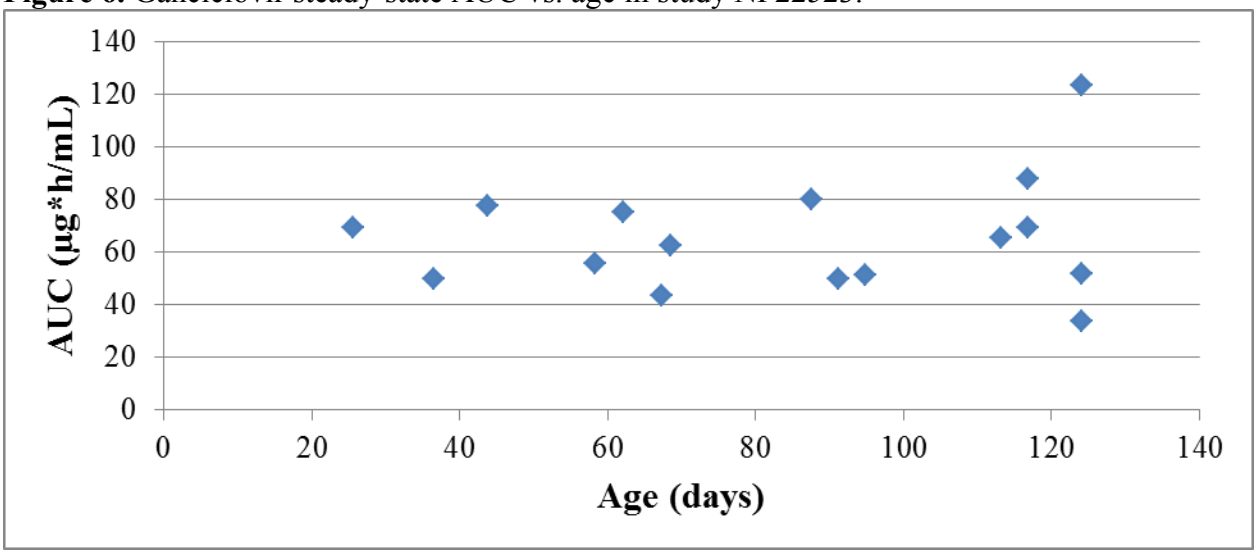
Table 8. Model-estimated ganciclovir steady-state $AUC_{0\text{-}24h}$ across age groups in study NP22523.

PK Parameter	Age Group (<6 wks)	Age Group (6 wks–4 Months)
$AUC_{0\text{-}24h}$ (µg•h/mL)	(n=3)	(n=15)
Mean	64.7	68.8
Median	57.3	67.4
%CV	22.1	30.7
C_{max} (µg/mL)	(n=2)	(n=12)
Mean	8.33	10.8
Median	8.33	11.0
%CV	10.8	32.4

[a] n = 14 patients

Source: sponsor's analysis.
[a]Reviewer's note: Several subjects received different dose amounts while in the study and thus had more than one AUC value.

Figure 6. Ganciclovir steady-state AUC vs. age in study NP22523.

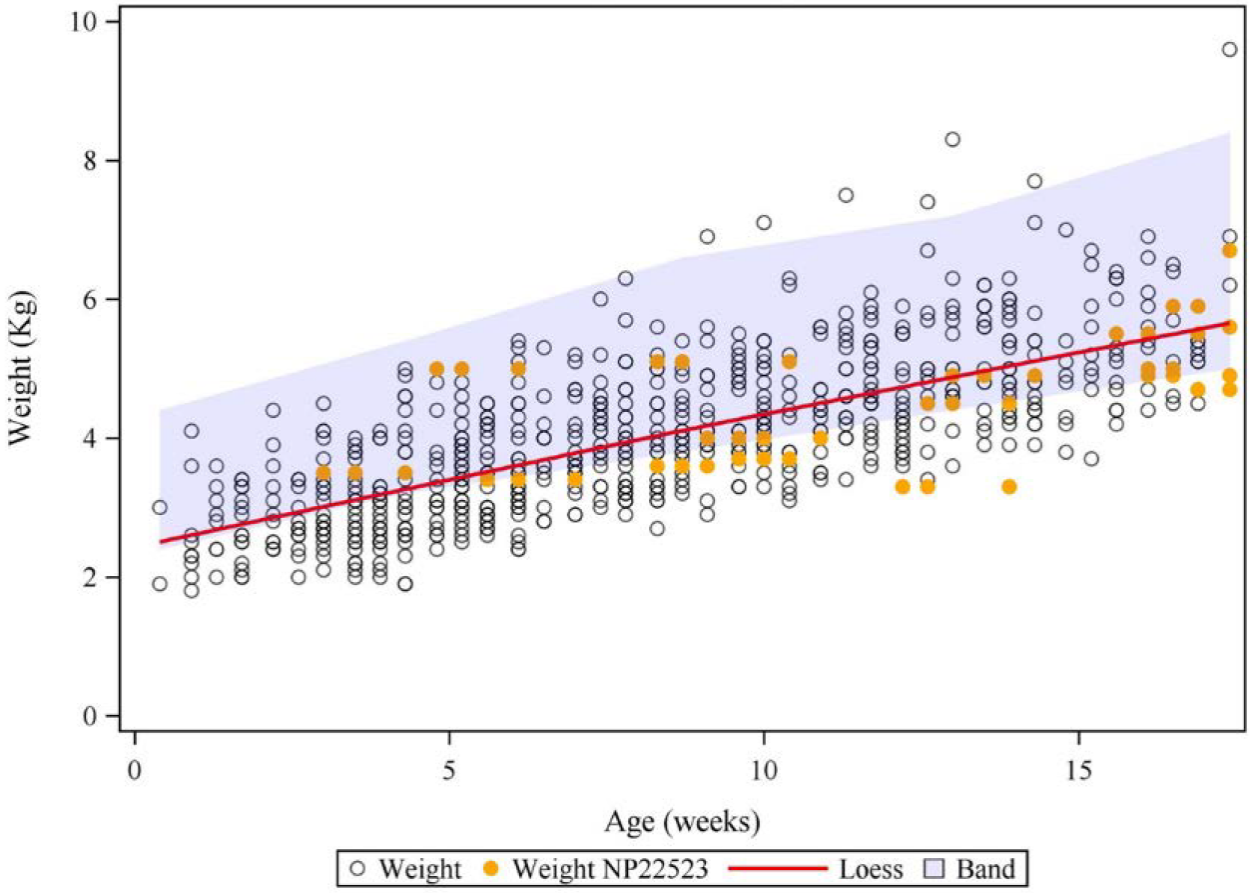


Source: reviewer's analysis.

3.2.2.4 Model-simulated ganciclovir exposures in children <4 months of age

In response to an Information Request, the sponsor simulated ganciclovir exposures for children <4 months of age using the population PK model.[22] Simulations were conducted using an internal dataset consisting of the initial 14 subjects enrolled in study NP22523, and a second "external" dataset consisting of patient records from studies NP22523 and WV16726, in addition to investigator-initiated studies CASG109 and CASG112. These datasets contained patient demographics needed to calculate dose (SCR, height, BSA) and to simulate the final model (Schwartz CrCL and weight). The sponsor found that body weights and heights in the external dataset (infants up to 4 months of age) were lower compared to the CDC Growth Charts for the United States, perhaps because the CASG studies contained many infants with congenital CMV disease, who are often born prematurely and with lower body weights than healthy infants (Figure 7). When the proposed dosing regimen was simulated (50 times for each dataset) for these populations using the final model, median (range) $AUC_{0\text{-}24h}$ for the internal and external datasets were 54.0 (12.3-169) and 57.8 (11.8-160) µg*h/mL, respectively (Table 9, Table 10). Thus, compared with the observed data (median AUC = 67.3), simulated AUC for subjects <4 months of age are closer to the target range (Figure 8).

Figure 7. Body weight vs age for the external dataset in comparison to CDC Growth Charts.



Source: sponsor's analysis. Band = data from CDC US Growth Charts.

Table 9. Simulated AUC for subjects in study NP22523.

	Simulation Results			Model Estimated Results		
	< 6 weeks	6 weeks to 4 months	All Patients	< 6 weeks	6 weeks to 4 months	All Patients
$AUC_{0\text{-}24h}$ (μg • h/mL)	n = 2 (50 times)	n = 12 (50 times)	n = 14 (50 times)	n = 2 (3 obs) [a]	n = 12 (16 obs) [a]	n = 14 (19 obs) [a]
Mean	53.9	58.2	57.6	62.80	66.94	66.29
Median	53.1	54.2	54.0	57.32	64.72	61.44
CV (%)	33.8	36.4	36.1	26.05	32.30	30.98
Range	23.2–136	12.3–169	12.3–169	49.9–81.2	33.7–123	33.7–123
C_{max} (μg/mL)	n = 2 (50 times)	n = 12 (50 times)	n = 14 (50 times)	n = 2 (1 obs) [b]	n = 12 (11 obs) [b]	n = 14 (12 obs) [b]
Mean	6.73	9.35	8.98	7.76	11.11	10.83
Median	6.66	8.72	8.43	7.76	11.03	10.96
CV (%)	32.9	38.0	39.2	N/A	29.81	30.49
Range	2.45–12.4	1.86–24.5	1.86–24.5	N/A	6.25–19.3	6.25–19.3

N/A = not applicable.

[a] Some patients provided more than one set of pharmacokinetic samples (observations) for the estimation.

[b] Evaluation of C_{max} was not possible at all occasions because the sparse samples were not sufficiently close to T_{max} for the estimation using popPK in those cases.

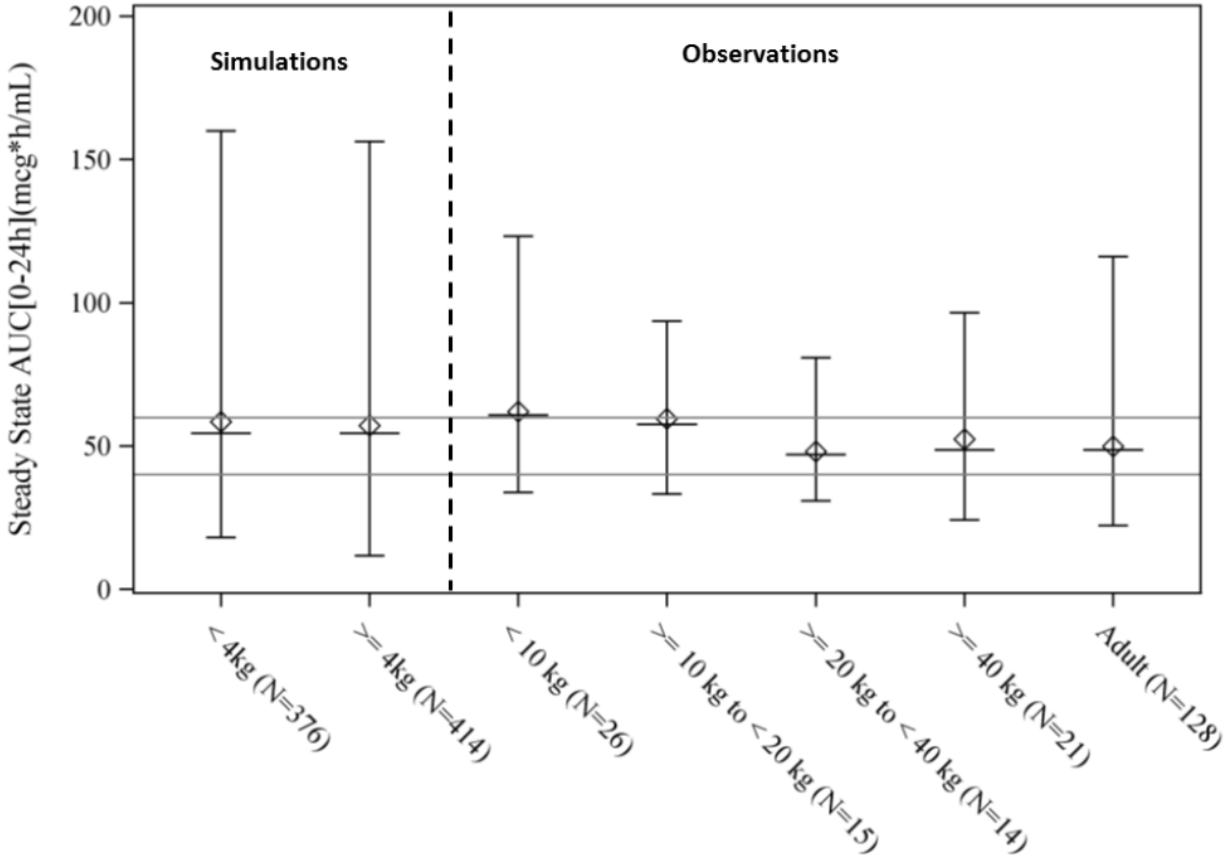
Source: sponsor's analysis.

Table 10. Simulated AUC for subjects in external dataset.

	Simulation Results		
	< 6 weeks	6 weeks to 4 months	All Patients
$AUC_{0\text{-}24h}$ (μg • h/mL)	n = 249	n = 541	n = 790
Mean	56.8	58.2	57.8
Median	54.5	54.5	54.5
CV (%)	35.5	36.6	36.2
Range	11.8–122	18.1–160	11.8–160
C_{max} (μg/mL)	n = 249	n = 541	n = 790
Mean	8.42	9.26	8.99
Median	7.86	8.75	8.41
CV (%)	41.2	38.4	39.4
Range	2.6–21.4	2.1–25.6	2.1–25.6

Source: sponsor's analysis.

Figure 8. Distribution of simulated and observed ganciclovir AUCs across age groups.



Note: The graph represents mean (diamond) median (line) and minimum and maximum values for each cohort. The lines at 40 and 60 μg • h/mL represent the target exposure. Data to the left of the dashed line are simulations based on demographic data (range of estimates is *generally* wider because the simulation is based on a much larger number of patient records). Data to the right of the dashed line are estimates based on population PK model.

Source: sponsor's analysis.

3.2.3 Safety

All seventeen patients enrolled were included in the safety analysis. Sixteen patients received treatment on two consecutive days as planned and one patient received only one dose. During the study, there were two serious adverse events and no deaths. The serious adverse events consisted of one case of dehydration and one post-operative wound infection, which resulted in death. Neither serious adverse event was considered related to study drug.

3.2.4 Conclusions

Based on ganciclovir's exposure-response relationships, there is more concern within the review team regarding CMV viremia due to below target ganciclovir AUC versus hematological adverse effects due to above target AUC. The valganciclovir dosing regimen applied to the infants enrolled (~1-4 months of age) in study NP 22523 resulted in acceptable exposures, with all but one subject having an AUC value >40 μg*h/mL.

4 Physiologically-based PK analysis

4.1 Regulatory background

In comments on the study NP22523 protocol in 2010, FDA stated that enrolling four subjects aged birth to six weeks would not be a sufficient sample size.[23] The sponsor subsequently agreed to perform an interim analysis upon enrolling four subjects in the birth to six week age group in order to determine the need for enrolling additional subjects. In 2012, due to enrollment difficulty, the sponsor requested to remove the requirement for enrolling at least four subjects in the birth to six weeks age group, and to instead provide ganciclovir PK information for this age group via PBPK analysis.[23] In response, FDA requested that the sponsor continue enrolling and to submit all available data, including PBPK analysis.[23] Ultimately two subjects were enrolled in the birth to six weeks age group.

4.2 Model development and evaluation

Due to lack of data supporting adjustments of kidney transport expression (Figure 9) parameters in the neonate model, the model is not acceptable as a basis for supporting valganciclovir dosing recommendations in infants younger than one month of age (the approximate youngest age enrolled in study NP22523). PBPK modeling was performed by Simulations Plus for the sponsor using a custom version of GastroPlus 8.0.0016 software. Initially a preclinical ganciclovir PBPK model was developed. This model informed development of adult human valganciclovir and ganciclovir PBPK models, and finally development of pediatric PBPK models for increasingly younger ages (down to neonates).[24] Active transport of valganciclovir and ganciclovir was assumed in the gut, liver, and kidney (Figure 9). Based on the preclinical PBPK model, permeability-limited tissue penetration was assumed. As the model was applied sequentially to younger age groups, multiple parameters were optimized (i.e. fit to observed data) or adjusted in order to obtain good predictions (Table 11). Although good predictions were obtained for infants with heart transplant under 4 months of age (Figure 10) and a single 25-day-old neonate (Figure 11), respectively, these predictions relied on unvalidated assumptions of disease-related and age-related alterations in transport expression.

Figure 9. Ganciclovir and valganciclovir PBPK model.[24]

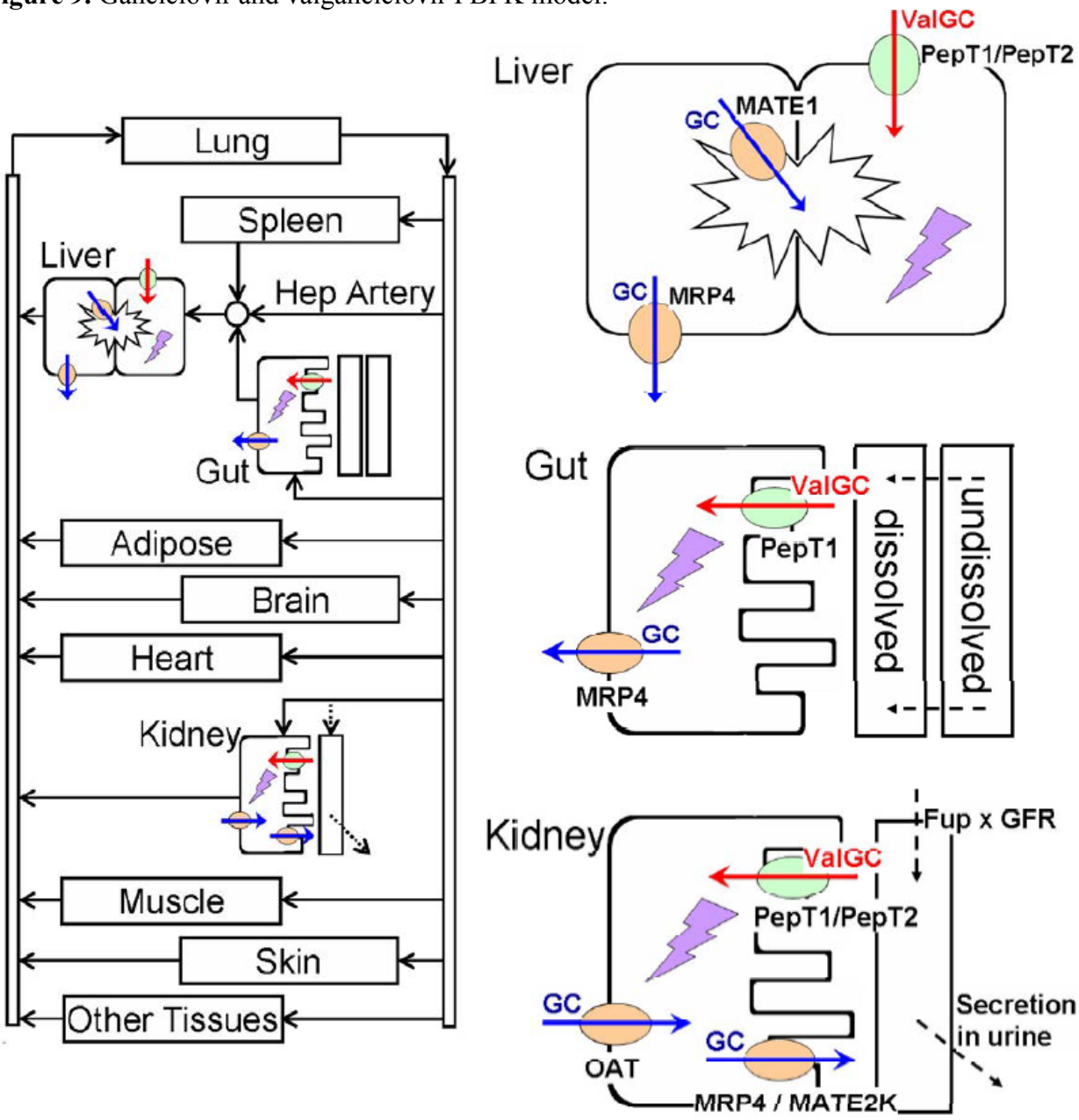
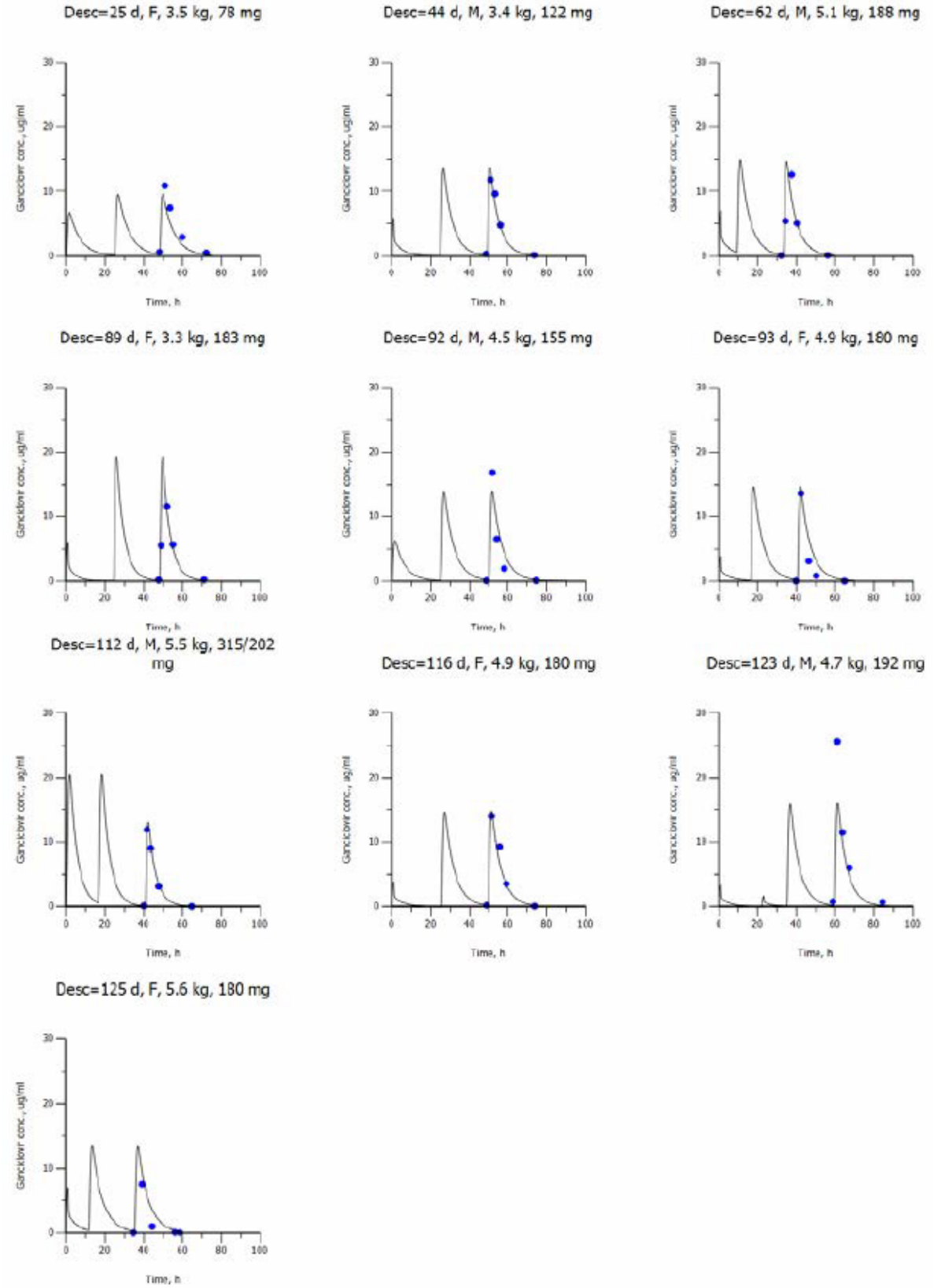


Table 11. Key modeling steps taken to obtain good predictions across age groups.

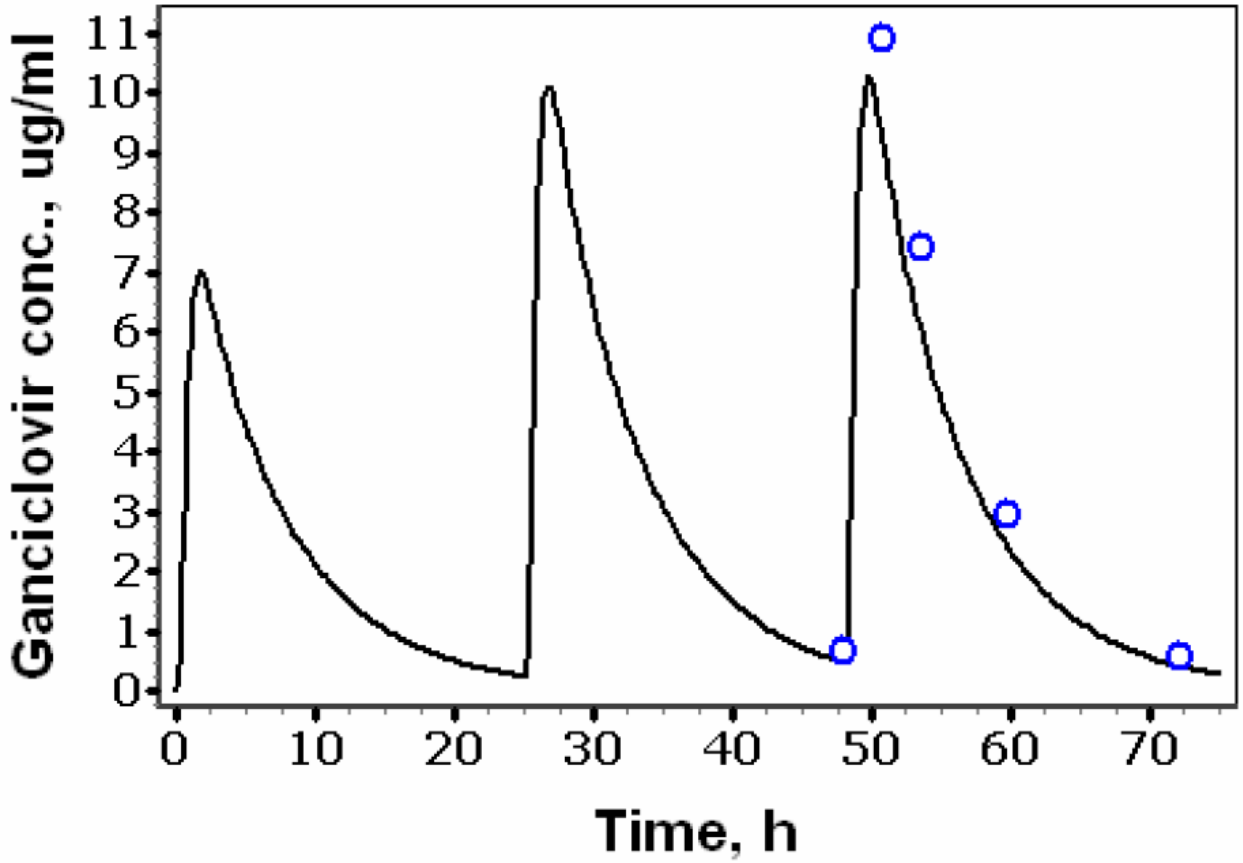
Age group	Initial model	Initial predictions sufficient?	Features incorporated in model to obtain adequate predictions
Adults	Preclinical	No	Enzyme and transporter expression parameters fit to human in vivo ganciclovir IV data from HIV-infected subjects and transplant recipients
13-16 years	Adult	Yes	None
5-12 years	13-16 years	No	Adjustment of the specific permeability-surface-area parameter (important for describing permeability-limited uptake)
1-3 years	5-12 years	Yes	None
1 month - <1 year	NR	NR	Growth-related changes in tissue compartment volumes and composition
<1 month	1 month - <1 year	No (underpredicted)	Very low kidney transporter expression

Figure 10. Simulated ganciclovir PK profiles in infants <4 months of age with heart transplant.



Source: sponsor's analysis. Above each PK profile is the age, gender, weight, and valganciclovir or ganciclovir dose. Circles = observed data, lines = PBPK simulations.

Figure 11. Simulated ganciclovir PK profiles in 25-day-old infant with heart transplant.



Source: sponsor's analysis. Circles = observed data, lines = PBPK simulation.

4.3 Conclusions

The population PK analysis supports acceptable ganciclovir exposures in the infants enrolled (~1-4 months of age) using the valganciclovir dosing regimen. Due to unverified assumptions, the PBPK analysis is not sufficient to support valganciclovir dosing recommendations for infants <1 month of age.

5 Study CASG112

For the proposed indication in pediatric heart transplant recipients <4 months of age, study NP 22523 only involved two days of valganciclovir dosing. Study CASG 112 was included in the submission to provide supportive safety data. In this trial, 96 infants with congenital CMV infection received oral valganciclovir therapy for 6 weeks or 6 months and ganciclovir exposure was assessed via population PK modeling. To support use of study CASG 112 for supportive safety, we requested population PK and bioanalytical reports from the applicant.[25] A population PK report was not available for this study and the information about the population PK model in the study report was insufficient to allow assessment of model development and evaluation. The

bioanalytical report was not reviewed in depth due to lack of information regarding the population PK methods.

6 Evaluation of the valganciclovir dosing formula

As noted above, use of the valganciclovir dosing formula 7 x BSA x Schwartz CrCL in study NP22523 resulted in a median ganciclovir $AUC_{0\text{-}24h}$ (~64.6 µg*h/mL) that was above the AUC target range (40-60 µg*h/mL), suggesting higher than target exposures are expected in infants <4 months of age. As the factor "7" in the formula is empiric, we asked the sponsor to predict exposures using a factor of "6". The factor "6" was chosen because it is a whole number that would not further complicate the dosing formula. The fraction of subjects below, within, above the AUC target range was then computed for both factors ("6" and "7"). Reference values for adult transplant recipients in the pivotal trial PV16000 were mean $AUC_{0\text{-}24h}$ of 40.2 ± 11.8 µg*h/mL in adult heart transplant recipients, and across all transplanted organs the fraction of subjects with $AUC_{0\text{-}24h}$ values below, within, and above the target range was 38%, 48%, and 14%, respectively. [26]

6.1 Observed ganciclovir PK data

PK data from pediatric transplant recipients dosed with valganciclovir according to the formula 7 x BSA x Schwartz CrCL (studies NP22523 and WV16726, dataset 1) was used to evaluate the impact of changing the formula to 6 x BSA x Schwartz CrCL. For patients <4 months of age, using a factor of "6" was predicted to increase the fraction of subjects within the AUC target range and decrease the fraction with higher than targeted AUC values; the fraction below target was unchanged (Table 12, Table 13, Figure 12). Across all pediatric age groups, the impact of changing the formula to 6 x BSA x Schwartz CrCL was predicted to increase the fraction of subjects with below target AUC, have no effect on the fraction within the target range, and increase the fraction above the target (Table 12, Table 13, Figure 12).

Table 12. Target attainment from studies NP22523 and WV16726 using valganciclovir dosing formula 7 x BSA x Schwartz CrCL.

	< 4 months	≥ 4 months to ≤ 2 years	> 2 to < 6 years	≥ 6 to < 12 years	≥ 12 to ≤ 16 years	All Patients
No. patients	14	17	8	12	25	76
No. AUC estimates [a]	19	101	29	56	59	264
Median	61.4	59.5	54.9	53.1	49.0	55.1
Min	33.8	33.3	40.7	30.9	24.2	24.2
Max	123.2	107.1	82.0	93.6	96.6	123.2
P5	33.8	38.4	42.7	38.2	29.1	36.6
P95	123.2	89.7	80.1	77.6	79.8	83.2
Patients AUC < 40 μg • h/mL	1 (7%)	1 (6%)	—	—	8 (32%)	10 (13%)
Patients AUC 40–60 μg • h/mL	5 (36%)	8 (47%)	5 (62%)	9 (75%)	12 (48%)	39 (51%)
Patients AUC > 60 μg • h/mL	8 (57%)	8 (47%)	3 (38%)	3 (25%)	5 (20%)	27 (36%)

AUC = area under the plasma concentration-time curve; BSA = body surface area; CrCL = creatinine clearance; max = maximum; min = minimum.

[a] Some patients provided more than one set of pharmacokinetic samples (observations) for the estimation.

Source: Sponsor's analysis.

Table 13. Predicted target attainment from studies NP22523 and WV16726 based on a valganciclovir dosing formula of 6 x BSA x Schwartz CrCL.*

	<4 months	≥4 months to ≤2 years	>2 to <6 years	≥6 to <12 years	≥12 to ≤16 years	All Patients
No. patients	14	17	8	12	25	76
No. AUC estimates [a]	19	101	29	56	59	264
Median	52.6	51.0	47.1	45.5	42.0	47.2
Min	29.0	28.5	34.9	26.5	20.7	20.7
Max	105.6	91.8	70.3	80.2	82.8	105.6
P5	29.0	32.9	36.6	32.7	24.9	31.4
P95	105.6	76.9	68.7	66.5	68.4	71.3
Patients AUC <40 μg • h/mL	1 (7%)	5 (29%)	—	5 (42%)	11 (44%)	22 (29%)
Patients AUC 40–60 μg • h/mL	8 (57%)	9 (53%)	7 (87%)	6 (50%)	10 (40%)	40 (53%)
Patients AUC >60 μg • h/mL	5 (36%)	3 (18%)	1 (13%)	1 (8%)	4 (16%)	14 (18%)

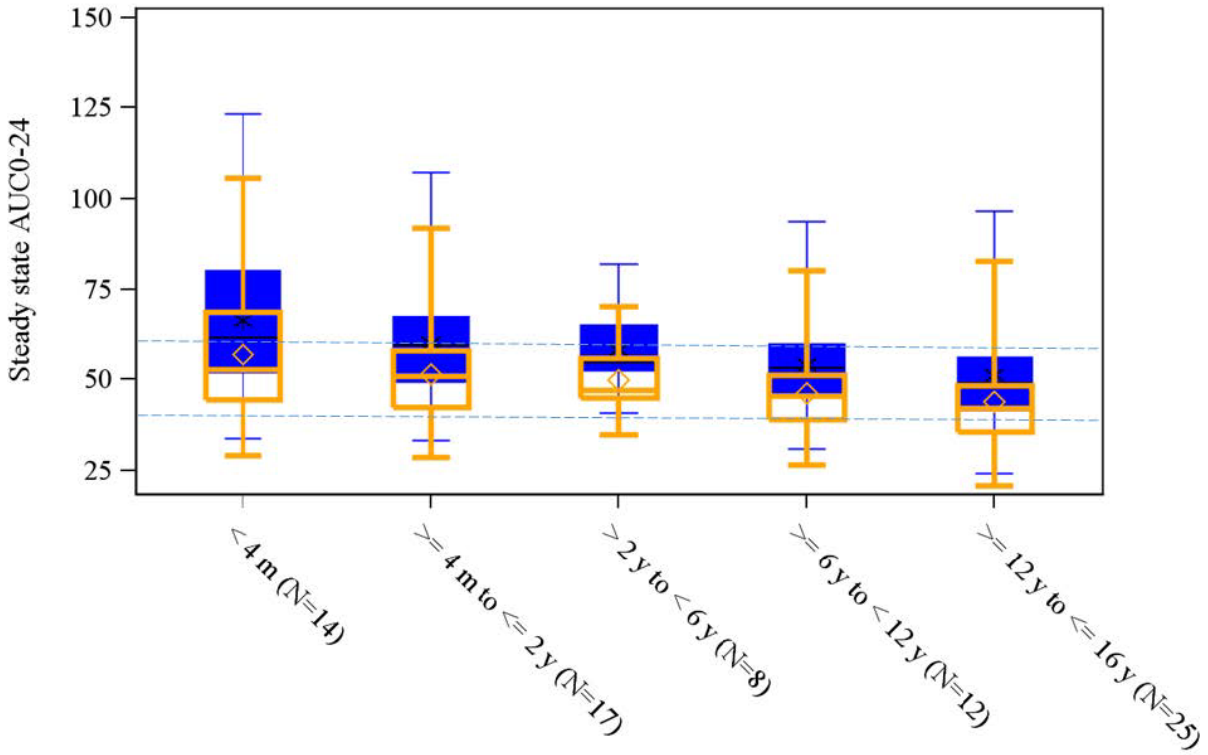
AUC = area under the plasma concentration-time curve; BSA = body surface area; CrCL = creatinine clearance; max = maximum; min = minimum.

[a] Some patients provided more than one set of pharmacokinetic samples (observations) for the estimation.

Source: Sponsor's analysis.

* Valganciclovir doses in these studies were calculated from 7 x BSA x Schwartz CrCL.

Figure 12. Distribution of AUC in studies NP22523 and WV16726 using valganciclovir dosing formula 7 x BSA x Schwartz CrCL versus 6 x BSA x Schwartz CrCL.



AUC = area under the plasma concentration-time curve; BSA = body surface area; CrCL = creatinine clearance.

Note: The graph represents mean (star/diamond) median (line), 25th percentile, 75th percentile, minimum and maximum values for each cohort. The lines at 40 and 60 μg • h/mL represent the target exposure.

Source: Sponsor's analysis. Blue = 7 x BSA x Schwartz CrCL; Orange = 6 x BSA x Schwartz CrCL.

6.2 Simulations using patient covariates and the population PK model

For a second approach to evaluating the valganciclovir dosing formula, the applicant assembled a dataset of patient covariates (dataset 2) from studies in pediatric transplant (NP22523, WV16726, and PV16000 [n=1 patient]) and congenital CMV treatment (CASG109 and CASG112) populations. Covariates included age, BSA, SCR, Schwartz CrCL, height, and weight. Each patient had multiple rows in the dataset corresponding to each visit. The covariate dataset included 201 patients aged <16 years and 1473 covariate records. For the <4 months age group, covariates were similar for transplant versus congenital CMV populations (Table 14). Ganciclovir PK parameters were simulated using the population PK model and AUC was calculated by F x dose / CL. The impact of changing the valganciclovir dosing formula to 6 x BSA x Schwartz CrCL for ages <4 months and for all pediatric ages generally was predicted to be a greater fraction of patients below the target, similar fraction within the target, and decreased fraction above the target (Table 15, Table 16, Figure 13).

Table 14. Comparison of covariates across transplant and congenital CMV populations for age <4 months.

Population (N covariate records)	Weight (kg)	Height (cm)	BSA (m^2)	Schwartz CrCL (mL/min/m^2)
Transplant (n=757)	4.7 (3.3-5.9)	57 (50-62)	0.27 (0.22-0.32)	86 (35-243)
Congenital CMV (n=68)	4.0 (1.8-8.3)	53 (41-65.5)	0.24 (0.15-0.37)	83 (24-282)

Source: Reviewer's analysis. Values are median (range).

Table 15. Simulation of ganciclovir AUC using a patient covariate dataset and a valganciclovir dosing formula of 7 x BSA x Schwartz CrCL.

	<4 months	≥4 months to ≤2 years	>2 to <6 years	≥6 to <12 years	≥12 to ≤16 years	All Patients
No. patient records	781	384	86	96	126	1473
Median	54.5	55.2	50.4	48.3	41.7	53.2
Min	11.8	19.8	9.4	13.1	19.9	9.4
Max	160.0	155.0	144.9	152.8	110.7	160.0
P5	31.3	32.4	24.3	23.4	26.1	29.2
P95	95.0	98.8	89.2	87.2	78.9	94.0
Patients AUC <40 µg • h/mL	148 (19%)	65 (17%)	21 (24%)	30 (31%)	60 (48%)	324 (22%)
Patients AUC 40–60 µg • h/mL	343 (44%)	168 (44%)	38 (44%)	38 (40%)	40 (32%)	627 (43%)
Patients AUC >60 µg • h/mL	290 (37%)	151 (39%)	27 (31%)	28 (29%)	26 (21%)	522 (35%)

AUC = area under the plasma concentration-time curve; BSA = body surface area; CrCL = creatinine clearance; max = maximum; min = minimum.

Source: Sponsor's analysis.

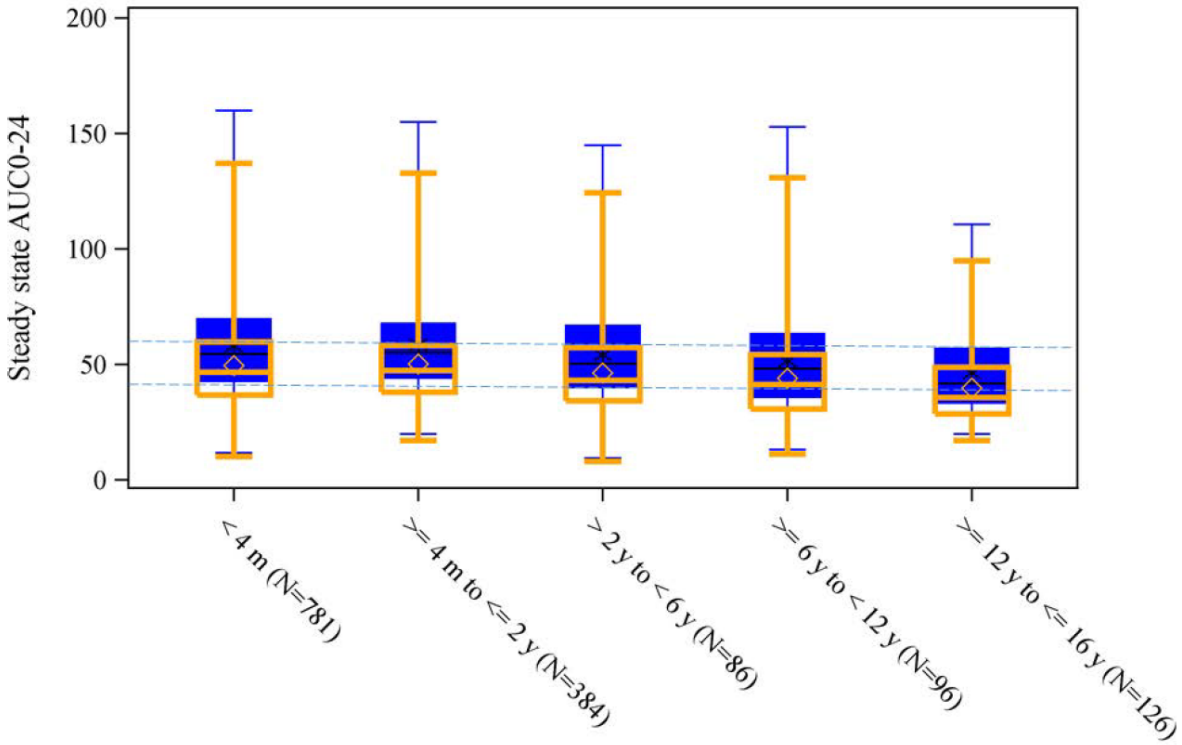
Table 16. Simulation of ganciclovir AUC using a patient covariate dataset and a valganciclovir dosing formula of 6 x BSA x Schwartz CrCL.

	< 4 months	≥ 4 months to ≤ 2 years	> 2 to < 6 years	≥ 6 to < 12 years	≥ 12 to ≤ 16 years	All Patients
No. patient records	781	384	86	96	126	1473
Median	46.7	47.3	43.2	41.4	35.7	45.6
Min	10.1	17.0	8.1	11.3	17.0	8.1
Max	137.1	132.8	124.2	130.9	94.9	137.1
P5	26.8	27.8	20.9	20.1	22.4	25.1
P95	81.4	84.7	76.4	74.8	67.6	80.6
Patients AUC < 40 μg • h/mL	255 (33%)	117 (30%)	32 (37%)	42 (44%)	73 (58%)	519 (35%)
Patients AUC 40–60 μg • h/mL	337 (43%)	182 (47%)	36 (42%)	38 (40%)	38 (30%)	634 (43%)
Patients AUC > 60 μg • h/mL	189 (24%)	85 (22%)	18 (21%)	16 (17%)	15 (12%)	323 (22%)

AUC = area under the plasma concentration-time curve; BSA = body surface area; CrCL = creatinine clearance; max = maximum; min = minimum.

Source: Sponsor's analysis.

Figure 13. Comparison of simulated ganciclovir AUC using a patient covariate dataset for valganciclovir dosing formula 7 x BSA x Schwartz CrCL versus 6 x BSA x Schwartz CrCL.



Source: Sponsor's analysis.

6.3 Conclusions

Based on ganciclovir exposure-response relationships, there was more concern within the review team regarding CMV viremia due to below target ganciclovir AUC versus hematological adverse effects due to above target AUC. With the exception of AUC predictions using the observed data for age <4 months, evaluation of the valganciclovir dosing formula 6 x BSA x Schwartz CrCL suggests it would result in a greater fraction of subjects below the AUC target. As a result, our conclusion is to not alter the current valganciclovir dosing formula of 7 x BSA x Schwartz CrCL.

7 Appendix

7.1 Valganciclovir and ganciclovir bioanalytical methods for study NP22523

7.1.1 Method validation

Method validation results are summarized in Table 17.[27] There were no major deficiencies.

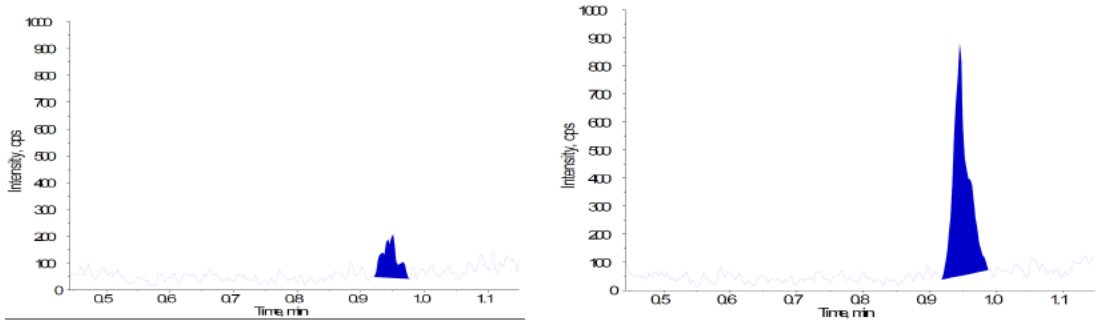
7.1.2 Minor deficiencies

A minor deficiency was that the plasma blank with ganciclovir internal standard consistently had a peak at the ganciclovir retention time whose peak area was an average of 17% of the LLOQ peak area (Figure 14). In a response to our Information Request regarding this issue (dated 7/23/14), the sponsor subtracted the blank + internal standard peak areas from all calibration standards, quality control, and study samples. After adjusting calibration curves and recalculating sample concentrations, sample concentrations were unchanged (mean percent difference of 0.04% [range -0.48% - 0.97%]). Thus this interfering peak did not affect the accuracy of ganciclovir quantification.

7.1.3 Method modifications

A cross-validation study was conducted to validate the modified method for use at a different laboratory.[28] However, as method validation and sample analysis for study NP22523 took place at Nuvisan, the cross-validation study in comparison with the Eurofins laboratory is not relevant to this submission.

Figure 14. Interference due to the ganciclovir internal standard.



Left – Validation run 1 blank + internal standard; Right – Validation run 1 LLOQ. Source: sponsor's 07/23/2014 response to Information Request, p 5.

Table 17. Summary of method validation.

		Ganciclovir	Valganciclovir
Method Type		LC/MS/MS	
Sample preparation		Protein precipitation	
Calibration Range		0.0151-37.6 µg/mL	0.00400-10.0 µg/mL
Stability		≤408 days at -75°C ± 15°C	
Number of samples		80	30
Criteria according to FDA Bioanalytical Guidance (Draft 2013)		Criteria satisfied?	
Calibration standards and QCs prepared from separate stock solutions. • Exception for spiking stock solution if stability verified. • Exception for blank matrix if no matrix effects.		Yes, ganciclovir stock solution SS1 for calibration samples and SS2 for QC samples	Yes, valganciclovir stock solution SS3 for calibration samples and SS2 for QC samples
Appropriate calibration samples	≥6 total	Yes, 8 samples	
	≥5 replicates for LLOQ (otherwise 2)	Yes, 6 replicates at each concentration	
	≥75% pass	Yes	
Appropriate QC samples	≥3 total	Yes, 4 samples	
	one within 3x of LLOQ, one midrange, one within 80% of ULOQ	Yes. The third QC is near the midrange when the range is converted to log scale	
	≥3 per run in duplicate	Yes, 3 in duplicate per run	
	≥67% pass overall (≥50% at each concentration)	Yes	
≥6 runs over multiple days		Yes, 7 runs done over a week	
Justification of complex methods for curve fitting, if used		Typical linear regression with $1/x^2$ weighting used	
Is LLOQ response ≥5x blank response		Yes, for 6/6 lots of blank plasma	

≥5 intra-run determinations per concentration		Yes. The least accurate or precise value at any concentration was 7.8%	Yes. The least accurate or precise value at any concentration was 7.1%
Validated stability • Accuracy and precision within 20% at LLOQ, otherwise 15% using 2 middle QCs	Room temperature stability in plasma	≤24 h	≤3 h
	Stability in plasma in ice water	≤3 h	
	Stability in plasma at -20°C ± 5°C	≤408 days	28 days
	Stability in plasma at -75°C ± 5°C	≤408 days	
	Processed samples	48 h at ~10°C in autosampler and 5 days at 5°C ± 3°C	
	Freeze-thaw	5 cycles	
	Analyte stock solutions	29 days at 5°C ± 3°C and 6 h at room temperature	
	Internal standard stock solutions	28 days at 5°C ± 3°C and 6 h at room temperature	
Matrix effect		No matrix effect up to 28.2 µg/mL	Consistent matrix effect of 16% up to 7.5 µg/mL
Recovery		Consistent; mean is 89% at 0.0452 µg/mL and 95% at 28.2 µg/mL	Consistent; mean is 88% at 0.0120 µg/mL and 91% at 7.50 µg/mL
Carryover	Two samples of solvent were run after an ULOQ sample in each of 7 runs	Minimal. The first and second blank after the ULOQ were mean 6.3% and 1.7% of the LLOQ peak	Pronounced. The first and second blank after the ULOQ were mean 51% and 19% of the LLOQ peak
Dilution		Met acceptance criteria at 4-fold dilution	
Interference		Effect of ganciclovir on valganciclovir and vice versa not assessed	
Chromatograms		• Blank response is small compared to response at LLOQ • IS contributes ~20% of peak height at LLOQ (addressed under minor deficiencies)	• Blank response is small compared to response at LLOQ
Deviations		None were significant	

7.1.4 Study Sample Analysis

Sample analysis results are summarized in Table 18.[8] There were no major deficiencies.

A minor deficiency was that after correcting for an interfering peak in the blank + internal standard at the ganciclovir retention time, sample concentrations were unchanged (see the method validation review above for more details).

Table 18. Summary of sample bioanalysis

		Ganciclovir	**Valganciclovir**
Criteria according to FDA Bioanalytical Guidance (Draft 2013)		Criteria satisfied?	
Analysis of study samples uses same methods as when validated?	Internal standard	Yes, D_5-ganciclovir	Yes, D_5-valganciclovir
	Anticoagulant	Yes, EDTA	
	Matrix	Yes, plasma	
	Instrumentation	Yes, LC/MS/MS with detection by a Sciex API 5000	
	Sample preparation	Yes, protein precipitation	
	Same calibration curve samples	Yes	
	Same QCs	Yes	
Analyzed within duration of stability	Freeze and thaw	Not discussed	
	Bench-top		
	Long-term	Yes	
	Analyte stock solution	Not discussed	
	IS stock solution		
	Processed samples		
QCs are ≥5% of unknown samples or 6, whichever greater		Yes, 4 QCs	Yes, 4 QCs
Dilutions within validated range		Dilutions not discussed	
Carryover addressed		Yes, no sample detected in water blanks	Yes, no sample detected in most water blanks (also, ISR passed)
Complete serial chromatograms from 5-20% of subjects provided (20% for pivotal studies)		Yes	
Incurred sample reanalysis	≥7% of total samples	Yes, 10%	Yes, 10%
	Coverage of entire PK curve	Timepoints not reported	
	67% of samples within 20% deviation for small molecules (30% for large)	Yes, 100% of samples within 20% deviation	
Deviations		None	

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