EVALUATION OF THE PHARMACOKINETICS AND PHARMACODYNAMICS OF GANCICLOVIR IN PREMATURE INFANTS RECEIVING TREATMENT FOR CYTOMEGALOVIRUS INFECTION

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DMID Medical Monitor: **Venus Shahamatdar, M.D.**

Version Number: **2.0**

14 August 2015

STATEMENT OF COMPLIANCE

Each investigator must adhere to the protocol as detailed in this document. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria. This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice guidelines (ICH-E6), and the applicable regulatory requirements, including:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations, if under IND, 21 CFR 312).
- Completion of Human Subjects Protection Training. Refer to http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html; http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp

SIGNATURE PAGE

The signature below constitutes the approval of this protocol "**Evaluation of the Pharmacokinetics and Pharmacodynamics of Ganciclovir in Premature Infants Receiving Treatment for Cytomegalovirus Infection**" and attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines. It is understood that no deviations from the protocol may be made without permission of the Sponsor.

Site Investigator:

Signed: _____ Date: _____

Name

_____

Title

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AESI	Adverse Event Special Interest
AUC	Area Under the Curve
CASG	Collaborative Antiviral Study Group
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
DAIDS	Division of AIDS
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GI	Gastrointestinal
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
KG	Kilogram
MG	Milligram
mL	Milliliter
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics

PRBC	Packed Red Blood Cell
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
US	United States

PROTOCOL SUMMARY

Title:	Evaluation of the Pharmacokinetics and Pharmacodynamics of Ganciclovir in Premature Infants Receiving Treatment for Cytomegalovirus Infection
Phase:	Not Applicable (clinical sampling study)
Population:	Male and female premature infants < 32 weeks gestation who are being treated for medical reasons with intravenous ganciclovir for proven [e.g., culture- or polymerase chain reaction (PCR)-confirmed] CMV infections. Subjects will be stratified according to age (gestational and chronologic)
Sample Size:	32
Number of Sites:	15
Study Duration:	5 years
Subject Participation Duration:	Up to 7 weeks
Description of Agent or Intervention:	None. This is not an interventional study. It is a clinical sampling study, and ganciclovir will not be administered as part of the protocol.
Objectives:	The goals of the study are to determine ganciclovir pharmacokinetic and pharmacodynamic parameters in premature infants who receive IV ganciclovir as part of medical care to treat CMV infections. Primary: • To define the pharmacokinetics of ganciclovir in premature (< 32 weeks gestational age at birth) infants Secondary:

- To assess changes in quantitative viral DNA in whole blood as a function of ganciclovir pharmacokinetics
- To assess clearance of CMV in urine as a function of ganciclovir pharmacokinetics

Exploratory:
- To assess development of neutropenia as a function of ganciclovir pharmacokinetics
- To determine the potential for the development of resistance to ganciclovir as a function of pharmacokinetics, dose, age, and duration of therapy

Outcome Measures

Primary Endpoint:
- Plasma pharmacokinetics parameters for ganciclovir AUC12

Secondary Endpoints:

- Plasma pharmacokinetics parameters for ganciclovir, including maximum serum concentration (Cmax), half-life (T1/2), CL, and Vd
- Correlation of ganciclovir plasma concentrations with CMV whole blood viral load
- Correlation of ganciclovir plasma concentrations with clearance of CMV in urine

Exploratory Endpoints:

- Correlation of ganciclovir plasma concentrations with neutropenia
- Detection of resistance to ganciclovir

Description of Study Design:

This is an open-label, multi-center, clinical sampling study to assess ganciclovir pharmacokinetics and pharmacodynamics in premature infants undergoing antiviral therapy for CMV. Only those subjects who receive ganciclovir for clinical reasons will be enrolled. The decision to initiate ganciclovir therapy will be

made by the attending physician based upon his/her clinical decision to treat virologically-confirmed CMV infection; infants receiving such therapy and meeting entry criteria will then be eligible for this study. Therefore, ganciclovir will not be provided under this protocol.

Subjects meeting enrollment criteria will be entered into this clinical trial. Following enrollment, subjects will be stratified by gestational age and by chronologic age as follows: 1) ≤ 27 weeks 6 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 2) ≤ 27 weeks 6 days gestational age at birth and > 30 days chronologic age at study enrollment; 3) ≥ 28 weeks 0 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 4) ≥ 28 weeks 0 days gestational age at birth and > 30 days chronologic age at study enrollment. The total sample size across these four cohorts is 32 subjects. Subjects in each cohort with inadequate pharmacokinetic data for analysis (e.g., due to dropping out of the study before PK assessments are performed, or blood sampling obtained but is inadequate for analysis) may be replaced and will not count toward the total of 32 subjects. Additionally, enrollment of an additional 2-3 subjects may be allowed for operational reasons.

A full pharmacokinetic profile will be obtained following at least one of the ganciclovir doses received after study enrollment. The PK samples will be obtained after the subject has received study assessment dose 3, 4, 5, 6, 7, or 8 of intravenous ganciclovir. Typically, ganciclovir is administered twice per day in these infants for a duration in excess of 10 days. Specimens will be shipped for processing at that time. The pharmacokinetic data will then be provided to the study site, including the AUC and CL values for information purposes.

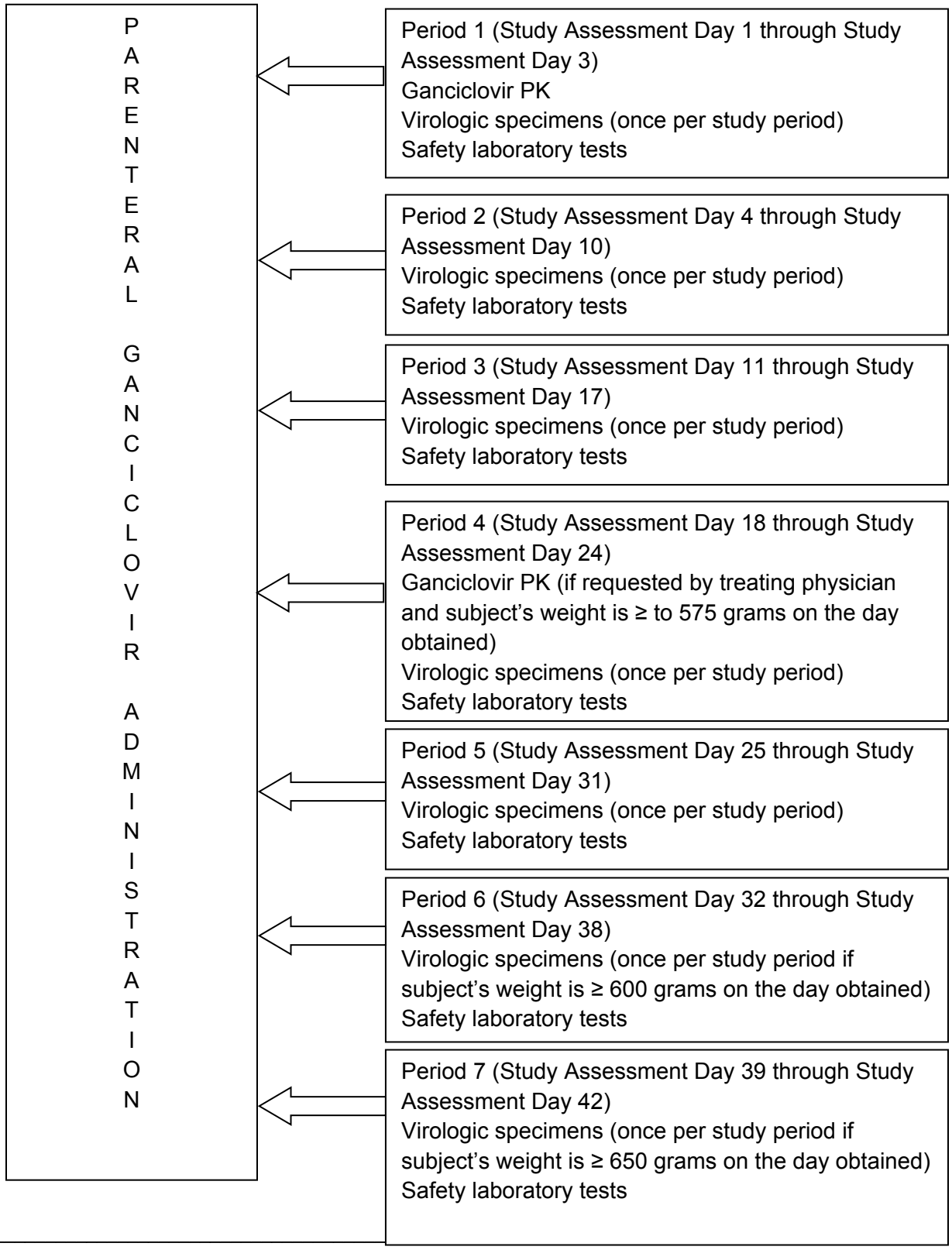
Dose and duration of intravenous ganciclovir therapy is at the discretion of the treating physician and will not be dictated by the research protocol. Both whole blood for CMV PCR and urine for CMV detection will be obtained once in each study period as long as the subject is receiving intravenous ganciclovir therapy. These specimens will be used to determine viral load and ganciclovir resistance. Since ganciclovir is a renally

excreted drug, serum creatinine will be drawn for the research protocol on the day that the ganciclovir pharmacokinetic specimens are obtained in order to calculate creatinine clearance using a method such as the modified Schwartz formula, and thus correlate ganciclovir clearance with renal function. Otherwise, data from hematology assessments (WBC count and differential, hemoglobin, platelet count) and from chemistry labs (serum creatinine, AST, and ALT) will be recorded on the study case report forms during each study period if they are being obtained for clinical reasons, but will not be drawn only for the purposes of the study. Ganciclovir dosing information (mg/dose, dosing interval, and subject weight) will be recorded on the day of the pharmacokinetic blood draws, and weekly from Period 1 through Period 7 as long as the subject is receiving intravenous ganciclovir therapy.

If the subject continues to receive intravenous ganciclovir from Study Assessment Day 18 through Study Assessment Day 24 (Period 4), a second PK assessment may be performed at the request of the treating physician if the subject weighs 575 grams or more at the time of specimen collection.

Estimated Time to Complete Enrollment: 4 years from enrollment of the first study subject

***Schematic of Study Design**:



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The natural history of congenitally acquired CMV infection is well described.[1-7] In contrast, outcomes of perinatally and postnatally acquired CMV infections are less well characterized. It is generally agreed that postnatal acquisition of CMV in term infants does not lead to symptomatology or disease.[8] In preterm infants, initial case reports suggested that peri- and postnatally acquired CMV infections could produce severe disease.[9-14] Larger series and case-controlled trials more recently suggest that symptomatic disease in preterm babies is less common than asymptomatic infection, and long-term sequelae are rare.[15-19] Nevertheless, severe disseminated CMV disease can occur in premature infants, including life-threatening pneumonitis, hepatitis, and thrombocytopenia.[20] Antiviral therapy in such patients is frequently used, albeit without either controlled data to demonstrate benefit or knowledge of proper dosing regimens.[12] In patients with symptomatic congenital CMV disease, six weeks of intravenous ganciclovir improves hearing outcomes in early childhood,[21] and may benefit neurodevelopmental outcomes as well.[22] In premature babies with visceral disease from postnatally acquired CMV, the goal of antiviral therapy is different; namely, it is to treat the end-organ disease, which is occurring at that time, rather than to prevent or improve long-term sequelae. Without efficacy data, the duration of treatment is variable, but anecdotal experience suggests that it generally is in the range of two to four weeks of total antiviral therapy. Approximately two-thirds of treated neonates receiving six weeks of intravenous ganciclovir develop Grade 3 or 4 neutropenia (utilizing the DAIDS Toxicity Tables) during therapy.[21]

The appropriate dose of intravenous ganciclovir for premature babies has not been studied. Previous studies of ganciclovir have indicated that the area-under-the-concentration curve (AUC) of ganciclovir is most closely related to virologic treatment success.[23] Data from an National Institute of Allergy and Infectious Diseases (NIAID) CASG Phase II study of intravenous ganciclovir (6 mg/kg/dose q 12 hrs) in term neonates with symptomatic congenital CMV disease revealed a median AUC_{12} of 27 μgxh/mL (mean AUC_{12}=32.3+13.7 μgxh/mL, range 17.2 to 55.9 μgxh/mL, n=13).[24] A more recent CASG investigation of oral valganciclovir included comparative pharmacokinetic data following 6 mg/kg/dose every 12 hrs of intravenous ganciclovir. The mean AUC_{12} in young neonates (median age: 16.5 days) on the second day following study enrollment was 38.2 μgxh/mL (median: 25.5 μgxh/mL), but the coefficient of variation was large (112).[25] In five neonates in whom pharmacokinetic sampling was performed on day 4 and day 34 of intravenous ganciclovir therapy, ganciclovir clearance nearly doubled and AUC_{12} reduced by almost half over the 30 day interval,[25] suggesting that maturational changes may be occurring rapidly in early life which may affect ganciclovir dosing. No data exist on ganciclovir dosing in very premature babies, who are at greatest risk for CMV disease

due to the absence of transplacental antibodies. Notably, acyclovir must be dose-adjusted based upon renal function such as that which can occur with prematurity.[26]

The CASG and the UAB Central Unit have had significant success in performing multi-center pharmacokinetic and pharmacodynamic studies with both ganciclovir and valganciclovir in infants during the first month of life.[21,22,25,27-30] In this study, this experience will be utilized to further define the utility of this drug in very premature children.

2.2 Rationale

Parenteral ganciclovir is used regularly in premature neonates to treat end-organ disease, but the appropriate dose to use has never been studied or defined. This is a clinical sampling study, and premature infants who receive intravenous ganciclovir as part of clinical care will be eligible for participation. A prospective treatment trial is not feasible at this time, but this study will inform dosing parameters for a subsequent prospective study to evaluate dose and effectiveness. At the completion of the study, appropriate dosing of intravenous ganciclovir in premature infants will be defined. While this will not allow for conclusions to be drawn regarding efficacy, these data will help minimize toxicity for future premature babies treated with ganciclovir. Importantly, all pharmacokinetic assays will be performed in the UAB Antiviral Pharmacology Laboratory. Of relevance to this trial, this laboratory has developed the methodology to process microspecimens of blood for ganciclovir drug concentrations.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

As a sampling study, the potential risks relate to blood draws (e.g., bruising at the site, infection, anemia possibly requiring packed red blood cell transfusion). Requiring a baby to be at least 500 grams at the time of study enrollment will ensure that blood volumes withdrawn will be acceptable by research standards. Additionally, virtually all of the subjects will have long-term intravenous access for clinical care, through which blood can be withdrawn without risk of discomfort from the needle stick or bruising at the site during or after the blood drawing and rarely an infection. Transfusions are expected in hospitalized premature infants. One study reported that extremely premature neonates receive an average of 5.2 ± 4.2 transfusions over a six week period.[31] Other trials have reported similar numbers of transfusions in this population.[32,33] It is possible that even higher numbers of transfusions might be experienced in the at-risk population in this sampling study since they are receiving the myelosuppressive drug ganciclovir as part of clinical care. Transfusions are not associated with long-term sequelae.[33] Nevertheless, throughout the conduct of this study, transfusion rates will be carefully monitored.

2.3.2 Known Potential Benefits

Knowledge of a given subject's pharmacokinetic profile may allow for rational clinical adjustments in treatment dose, thereby minimizing potential drug toxicity and maximizing potential antiviral efficacy.

3 OBJECTIVES

3.1 Study Objectives

Primary:

- To define the pharmacokinetics of ganciclovir in premature (< 32 weeks gestational age at birth) infants

Secondary:

- To assess changes in quantitative viral DNA in whole blood as a function of ganciclovir pharmacokinetics
- To assess clearance of CMV in urine as a function of ganciclovir pharmacokinetics

Exploratory:

- To assess development of neutropenia as a function of ganciclovir pharmacokinetics
- To determine the potential for the development of resistance to ganciclovir as a function of pharmacokinetics, dose, age, and duration of therapy

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Primary Endpoint:

- Plasma pharmacokinetics parameters for ganciclovir AUC12

3.2.2 Secondary Outcome Measures

Secondary Endpoints:

- Plasma pharmacokinetics parameters for ganciclovir, including maximum serum concentration (Cmax), half-life (T1/2), CL, and Vd
- Correlation of ganciclovir plasma concentrations with CMV whole blood viral load
- Correlation of ganciclovir plasma concentrations with clearance of CMV in urine

3.2.3 Exploratory Outcome Measures

Exploratory Endpoints:

- Correlation of ganciclovir plasma concentrations with neutropenia
- Detection of resistance to ganciclovir

4 STUDY DESIGN

This is an open-label, multi-center, clinical sampling study of ganciclovir pharmacokinetics and pharmacodynamics of ganciclovir in premature infants undergoing antiviral therapy for CMV. Only those subjects who receive ganciclovir for clinical reasons will be enrolled. The decision to initiate ganciclovir therapy will be made by the attending physician based upon his/her clinical decision to treat virologically-confirmed CMV infection; infants receiving such therapy and meeting entry criteria will then be eligible for this study. Therefore, ganciclovir will be dispensed from the institution's pharmacy and not provided under this protocol.

Subjects meeting enrollment criteria will be entered into this clinical trial. Following enrollment, subjects will be stratified by gestational age and by chronologic age as follows: 1) ≤ 27 weeks 6 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 2) ≤ 27 weeks 6 days gestational age at birth and > 30 days chronologic age at study enrollment; 3) ≥ 28 weeks 0 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 4) ≥ 28 weeks 0 days gestational age at birth and > 30 days chronologic age at study enrollment. The total sample size across these four cohorts is 32 subjects. Subjects in each cohort with inadequate pharmacokinetic data for analysis (e.g., due to dropping out of the study before PK assessments are performed, or blood sampling obtained but is inadequate for analysis) may be replaced and will not count toward the total of 32 subjects. Additionally, enrollment of an additional 2-3 subjects may be allowed for operational reasons.

Treatment Day 1 is the calendar day when the first dose of intravenous ganciclovir was administered to the study subject for clinical care, and Treatment Dose 1 corresponds to the first dose of intravenous ganciclovir the patient receives under clinical care. Study Assessment Dose 1 is the first dose of intravenous ganciclovir the subject receives following enrollment on the trial, and Study Assessment Day 1 is the calendar day when Study Assessment Dose 1 is administered. Both Treatment Day 1 and Treatment Dose 1 can coincide with or precede study enrollment; Study Assessment Day 1 and Study Assessment Dose 1 by definition will follow study enrollment, and may or may not equate with Treatment Day 1 and Treatment Dose 1. Typically, ganciclovir is administered twice per day in these infants.

With the receipt of one of the Study Assessment Doses (dose 3, 4, 5, 6, 7, or 8) of intravenous ganciclovir, pharmacokinetic specimens will be obtained at 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h. This complete set of pharmacokinetic time points will allow for the AUC and clearance (CL) to be calculated in each subject. Specimens will be shipped to the UAB Antiviral Pharmacology Laboratory for processing at that time. The pharmacokinetic data will then be provided to the study site, including the AUC and CL values,

for information purposes. The decision of whether or not to adjust the dose will be a clinical one to be decided by the treating physician, and will not be dictated by the research protocol. The parameters for AUC and CL from prior CASG ganciclovir[27,30] and valganciclovir[25,29] studies demonstrate that these PK parameters may be useful in guiding dose adjustments. Duration and dose of intravenous ganciclovir therapy is made at the discretion of the treating physician and will not be dictated by the research protocol.

Both whole blood for CMV PCR and urine for CMV assessment will be obtained serially through Study Assessment Period 7 as long as the subject is receiving intravenous ganciclovir therapy (that is, until the ganciclovir is permanently discontinued). These specimens will be used to determine viral load and, as indicated, ganciclovir resistance. At each sample collection time, urine will be split into two aliquots. These aliquots will be available for CMV detection as appropriate, based upon whole blood viral loads. Specimens will then be available for subsequent testing for ganciclovir resistance in the UAB Virology Resistance Laboratory. Since ganciclovir is a renally excreted drug, serum creatinine will be drawn for the research protocol on the day that the ganciclovir pharmacokinetic specimens are obtained in order to calculate creatinine clearance using a method such as the modified Schwartz formula, and thus correlate ganciclovir clearance with renal function. Otherwise, data from hematology assessments (WBC count and differential, hemoglobin, platelet count) and from chemistry labs (serum creatinine, ALT, and AST) will be recorded on the study case report forms each study assessment period if they are being obtained for clinical reasons, but will not be drawn only for the purposes of the study. Ganciclovir dosing information (mg/dose, dosing interval, and subject weight) will be recorded on the day of the pharmacokinetic blood draws. Once a subject is successfully enrolled into the study the ganciclovir dosing information (mg/dose and dosing interval) will be recorded from the start of clinical treatment of ganciclovir until date of enrollment. After enrollment ganciclovir dosing information (mg/dose and dosing interval) will be captured from Study Assessment Period 1 through the Study Assessment Period 7; as long as the subject is receiving intravenous ganciclovir therapy.

If the subject continues to receive intravenous ganciclovir from Study Assessment Day 18 through Study Assessment Day 24 (Period 4), and at the request of the treating physician, an additional set of blood samples will be obtained for PK assessments if the subject is 575 grams or more at time of collection. The additional samples will be analyzed as they are received in the UAB Antiviral Pharmacology Laboratory and the results will be provided to the treating physician for information purposes. Specimens would be obtained around one dose administered during this window, using the same sampling times as with the first pharmacokinetic draws.

5 STUDY ENROLLMENT AND WITHDRAWAL

Male and female premature infants < 32 weeks gestation of any ethnicity who are being treated with intravenous ganciclovir for proven [e.g., culture- or polymerase chain reaction (PCR)-confirmed] CMV infections are eligible for enrollment. Participating clinical sites will identify potential study subjects. Patients meeting study eligibility criteria may be offered enrollment into the trial. Informed consent signed by study subject's parent(s) or guardian(s) must be obtained prior to study enrollment. Site investigators are all clinicians and also care providers for the neonatologists at their respective institutions, and therefore have direct access to the study population. The study population will be drawn from the in-hospital setting. Potential subjects will be identified by the site investigators and the study coordinators.

5.1 Subject Inclusion Criteria

1. Signed informed consent from parent(s) or legal guardian(s)
2. Confirmation of CMV infection from urine, blood, or saliva by culture, shell vial, or PCR tests (local lab)
3. Receiving intravenous ganciclovir, prescribed by the patient's physician and anticipated to receive at least 3 more doses after consenting
4. < 32 weeks gestational age at birth
5. ≥ 500 grams at study enrollment

5.2 Subject Exclusion Criteria

1. Imminent demise
2. Current receipt of valganciclovir or foscarnet
3. Receiving breast milk from a mother who is being treated with ganciclovir or valganciclovir
4. Current receipt of other investigational drugs
5. Major congenital anomaly that in the site investigator's opinion may impact drug metabolism or the patient's volume of distribution

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Since this is a sampling study with no therapeutic intervention or investigation, there are no randomization procedures.

5.3.2 Reasons for Delaying Study-Related Procedures

Study related laboratory draws will be delayed or deferred if the investigator believes that obtaining them might increase subject risk [e.g., at risk of a packed red blood cell (PRBC)

transfusion due to the removal of blood for the study; however, if the subject would receive a transfusion of packed red blood cells even if the study-related labs were not obtained, then this would not be considered a situation of increasing subject risk]. Additionally, a study-related laboratory specimen that is unevaluable due to clotting or laboratory related issues will not be repeated. If a study related laboratory draw is delayed or deferred, study staff will document this on a source document.

5.3.3 Reasons for Withdrawal

The criteria for discontinuations during the study include:

- Study subject (parent/legal guardian) wishes to withdraw
- Non-compliance with study procedures
- Trial termination (by UAB, , FDA, DMID, NIAID, NIH, or agreement of all investigators)
- Any other reason which, in the opinion of the investigator, precludes the study subject's participation in the study. The principal investigator must call the Protocol Chair prior to discontinuing a study subject for this reason.

5.3.4 Handling of Withdrawals

Study subjects may withdraw voluntarily from participation in the study at any time. If a study subject withdraws or is discontinued from the study at any time prior to completion of the study, the reason for this decision will be recorded on the eCRFs. If subject withdraws before collection of the pharmacokinetic collection, this subject may be replaced (see Section 4).

5.3.5 Termination of Study

The UAB Central Unit and NIH have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Study subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

None.

This is a sampling study with no therapeutic intervention. Only subjects who receive intravenous ganciclovir as practice of medicine will be eligible to enroll. Intravenous ganciclovir will not be provided under this protocol.

7 STUDY SCHEDULE

7.1 Screening

There are no screening procedures for this study. Potential subjects will be identified by the site investigators and the study coordinators. Informed consent signed by study subject's parent(s) or guardian(s) must be obtained prior to study enrollment.

7.2 Enrollment/Baseline

7.2.1. Baseline Assessment (Period 1: Study Assessment Day 1 through Study Assessment Day 3, unless broader window specified)

- Confirm that informed consent has been obtained from parent or legally authorized representative

- Document baseline demographics (see Section 8.1.1; Window: Birth through Study Assessment Day 3)
  - Gestational age at delivery
  - Date of birth
  - Day of life at initiation of IV ganciclovir therapy
  - Gender
  - Race
  - Ethnicity
  - Birth weight
  - Weight at enrollment
  - Length in centimeters at enrollment
  - Reason ganciclovir being administered
  - Ganciclovir dose and dosing frequency
  - Manufacturer of the ganciclovir lot utilized at the treating facility
  - Date ganciclovir therapy was initiated

- Hematology labs (see Section 8.2; Window: Birth through Study Assessment Day 3)
  - WBC with differential, hemoglobin, and platelet count, if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

- Chemistry labs (see Section 8.2; Window: Birth through Study Assessment Day 3)
  - AST and ALT, if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

- Serum Creatinine (see Section 8.2)
  - To be drawn on the calendar day that ganciclovir PKs are being drawn (Window: Study Assessment Day 1 through Study Assessment Day 5)
  - Required amount of whole blood for serum creatinine is 0.25 mL

- Ganciclovir pharmacokinetics (see Section 8.2.2.2.)
  - Ganciclovir concentrations will be obtained with one of the Study Assessment Dose 3, 4, 5, 6, 7, or 8 (Window:Study Assessment Day 1 through Study Assessment Day 5)
  - Timepoints for pharmacokinetic draws: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h
  - Required amount of whole blood for plasma ganciclovir determination at each timepoint is 200 μL (0.2 mL)

- Virology specimens (see Sections 8.2.2.1.)
  - Blood for CMV quantitative PCR will be obtained (required amount of whole blood for CMV PCR is 0.5 mL)
  - If obtainable, urine for CMV detection will be collected non-invasively (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)

- Clinical treatment ganciclovir dosing information
  - mg/dose
  - Dosing interval
  - Patient weight

- PRBC transfusion documentation on eCRF

- Study Assessment ganciclovir dosing information
  - mg/dose
  - Dosing interval
  - Subject weight

7.3 Follow-up

7.3.1. Period 2 (Study Assessment Day 4 through Study Assessment Day 10)

Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

Required assessments [following confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)]: 1) virology blood

specimens for CMV viral load (required amount of whole blood for CMV PCR is 0.5 mL); 2) CMV detection from noninvasive urine specimen (if obtainable); 3) ganciclovir dosing information (mg/dose, dosing interval, subject weight).

Optional assessments (if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value): 1) hematology labs (WBC with differential, hemoglobin, platelet count); 2) chemistry labs (AST, ALT); 3) serum creatinine.

Study Assessments (as described)	**Optional Assessments***
Virology blood specimens for CMV viral load	Hematology labs (WBC with differential, hemoglobin, platelet count)
If obtainable, CMV detection from noninvasive urine specimen (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)	Chemistry labs (AST, ALT)
Ganciclovir dosing information (mg/dose, dosing interval, subject weight)	Serum creatinine

* If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

7.3.2. Period 3 (Study Assessment Day 11 through Study Assessment Day 17)

Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

Required assessments [following confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)]: 1) virology blood specimens for CMV viral load (required amount of whole blood for CMV PCR is 0.5 mL); 2) CMV detection from noninvasive urine specimen (if obtainable); 3) ganciclovir dosing information (mg/dose, dosing interval, subject weight).

Optional assessments (if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal

value): 1) hematology labs (WBC with differential, hemoglobin, platelet count); 2) chemistry labs (AST, ALT); 3) serum creatinine.

Study Assessments (as described)	Optional Assessments*
Virology blood specimens for CMV viral load	Hematology labs (WBC with differential, hemoglobin, platelet count)
If obtainable, CMV detection from noninvasive urine specimen (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)	Chemistry labs (AST, ALT)
Ganciclovir dosing information (mg/dose, dosing interval, subject weight)	Serum creatinine

* If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

7.3.3. Period 4 (Study Assessment Day 18 through Study Assessment Day 24)

- Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

- Confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)

- Hematology labs (see Section 8.2)
  - WBC with differential, hemoglobin, and platelet count, if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

- Chemistry labs (see Section 8.2)
  - AST and ALT, if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

- Serum creatinine (see Section 8.2)
  - Obtain if the ganciclovir pharmacokinetics are being obtained during this study assessment period

- To be drawn on the calendar day that ganciclovir PKs are being drawn

- Ganciclovir pharmacokinetics, if desired by the treating physicians and the subject weighs ≥ 575 grams at that time (see Section 8.2.2.2.)
  - Ganciclovir concentrations may be obtained around one dose in this assessment window (Study Assessment Day 18 through Study Assessment Day 24)
  - Timepoints for pharmacokinetic draws: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h
  - Required amount of whole blood for plasma ganciclovir determination at each timepoint is 200 μL (0.2 mL)
  - If subject weighs < 575 grams, the second set of PK draws cannot be performed

- Virology specimens (see Sections 8.2.2.1.)
  - Blood for CMV quantitative PCR will be obtained (required amount of whole blood for CMV PCR is 0.5 mL)
  - If obtainable, urine for CMV detection will be obtained non-invasively (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)

- Ganciclovir dosing information
  - mg/dose
  - Dosing interval
  - Subject weight

7.3.4. Period 5 (Study Assessment Day 25 through Study Assessment Day 31)

Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

Required assessments [following confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)]: 1) virology blood specimens for CMV viral load (required amount of whole blood for CMV PCR is 0.5 mL); 2) CMV detection from noninvasive urine specimen (if obtainable); 3) ganciclovir dosing information (mg/dose, dosing interval, subject weight).

Optional assessments (if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value): 1) hematology labs (WBC with differential, hemoglobin, platelet count); 2) chemistry labs (AST, ALT); 3) serum creatinine.

Study Assessments (as described)	Optional Assessments*
Virology blood specimens for CMV viral load	Hematology labs (WBC with differential, hemoglobin, platelet count)
If obtainable, CMV detection from noninvasive urine specimen (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)	Chemistry labs (AST, ALT)
Ganciclovir dosing information (mg/dose, dosing interval, subject weight)	Serum creatinine

* If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

7.3.5. Period 6 (Study Assessment Day 32 through Study Assessment Day 38)

Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

Required assessments [following confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)]: 1) virology blood specimens for CMV viral load (required amount of whole blood for CMV PCR is 0.5 mL); 2) CMV detection from noninvasive urine specimen (if obtainable); 3) ganciclovir dosing information (mg/dose, dosing interval, subject weight).

Optional assessments (if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value): 1) hematology labs (WBC with differential, hemoglobin, platelet count); 2) chemistry labs (AST, ALT); 3) serum creatinine.

Study Assessments (as described)	Optional Assessments*
Virology blood specimens for CMV viral load (NOTE: if subject weighs < 600 grams, this virology specimen will not be obtained)	Hematology labs (WBC with differential, hemoglobin, platelet count)
If obtainable, CMV detection from noninvasive urine specimen (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)	Chemistry labs (AST, ALT)
Ganciclovir dosing information (mg/dose, dosing interval, subject weight)	Serum creatinine

* If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

7.4 Final Study Visit

7.4.1. Period 7 (Study Assessment Day 39 through Study Assessment Day 42)

Documentation of receipt of PRBC transfusion(s) will be provided on an eCRF. If the study subject has NOT received ≥ 10 PRBC transfusions (see Section 9.3.1), proceed with required assessments.

Required assessments [following confirmation that subject is still receiving ganciclovir (e.g., that the ganciclovir has not been permanently discontinued)]: 1) virology blood specimens for CMV viral load (required amount of whole blood for CMV PCR is 0.5 mL); 2) CMV detection from noninvasive urine specimen (if obtainable); 3) ganciclovir dosing information (mg/dose, dosing interval, subject weight).

Optional assessments (if being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value): 1) hematology labs (WBC with differential, hemoglobin, platelet count); 2) chemistry labs (AST, ALT); 3) Serum creatinine.

Study Assessments (as described)	Optional Assessments*
Virology blood specimens for CMV viral load (NOTE: if subject weighs < 650 grams, this virology specimen will not be obtained)	Hematology labs (WBC with differential, hemoglobin, platelet count)
If obtainable, CMV detection from noninvasive urine specimen (e.g., bagged, from indwelling foley catheter, excess clinical specimen, etc.)	Chemistry labs (AST, ALT)
Ganciclovir dosing information (mg/dose, dosing interval, subject weight)	Serum creatinine

* If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value

7.5 Early Termination Visit

Study subjects may withdraw voluntarily from participation in the study at any time. If a study subject withdraws or is discontinued from the study at any time prior to completion of the study, the reason for this decision will be recorded on the eCRFs. The remaining follow-up evaluations will be conducted if patient consent is obtained. SAEs and AEs will be followed according to guidelines in Section 9.

Should a study subject's therapy be discontinued prematurely, all clinical and laboratory evaluations scheduled during the next study visit will be completed on the day the study subject is discontinued. All key endpoints will be evaluated and all randomized study subjects will continue to be followed as long as possible and included in the final analysis.

7.6 Unscheduled Visit

All subjects enrolled on the study are hospitalized. As such, there will not be unscheduled visits.

8 STUDY PROCEDURES/EVALUATIONS

The study procedures and evaluations are summarized in Appendix A. In the conduct of this study, hospital staff may perform research activities (e.g., pharmacokinetic blood draw collection, serum creatinine collection, blood CMV viral load collection, urine collection for CMV), under the direction of the site Principal Investigator or designee.

8.1 Clinical Evaluations

8.1.1. Baseline Demographics

To characterize the study subjects, information will be recorded at the baseline study visit (Period 1) following the obtaining of informed consent. Data collected will include basic demographics and birth history (date of birth; gender; race; ethnicity; birth weight; weight at enrollment; length in centimeters at enrollment; reason ganciclovir is being administered, gestational age at birth; ganciclovir dose and dosing frequency at time of enrollment; detailed ganciclovir dose and dosing frequency since ganciclovir therapy was initiated; manufacturer of the ganciclovir lot utilized at the treating facility; and date ganciclovir therapy was initiated).

8.2 Laboratory Evaluations

Chemistry labs (serum creatinine) will be drawn on the calendar day that ganciclovir PKs are being drawn; required amount of whole blood for serum creatinine is 0.25 mL. All other chemistry labs (serum creatinine, AST, and ALT) and hematology labs (WBC with differential, hemoglobin, platelet count) will be recorded on eCRFs if they have been drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value.

8.2.1 Special Assays or Procedures

Research assays (CMV viral load/resistance and pharmacokinetics will be conducted at the respective UAB Central labs).

8.2.1.1. CMV Viral Load/Resistance Testing

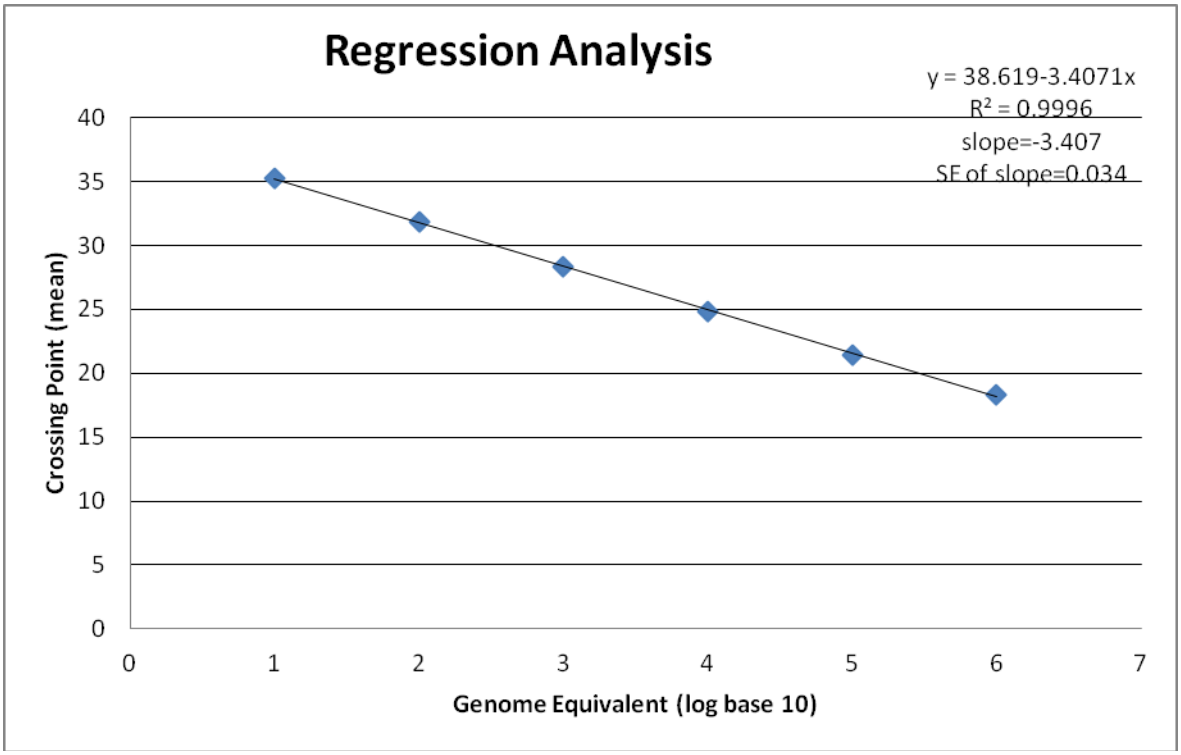
Assessment of CMV viral load in whole blood will be conducted at the UAB Central Laboratory. The required amount of whole blood for CMV PCR is 0.5 mL. Whole blood will be collected at each study visit from Period 1 through Period 5. Whole blood will also be collected at the Period 6 study visit if the

subject weighs at least 600 grams, and at the Period 7 study visit if the subject weighs at least 650 grams.

Urine collection by noninvasive mechanism (e.g., bagged, indwelling foley catheter, excess clinical specimen, etc.) will be attempted at each study visit. If urine is obtainable, it will be frozen and sent to the UAB Central Laboratory for processing. If an attempt to obtain urine for CMV detection is unsuccessful, study staff will document the failed attempt on a source document. All specimens collected will be sent to the UAB Central Laboratory.

Viral detection assays will be performed in the UAB Central Laboratory. These assays and tests are in clinical and research use already in the Central Laboratory. The detection of CMV DNA will be performed using the CMV two primer Taqman assay already employed regularly in our laboratory. The detection limit of our real-time PCR assay, as determined by the sensitivity titration analysis, is 10genomic equivalents per reaction. The results of 10 different runs on different days are shown in the Table below. Regression analysis shows a very high correlation coefficient (0.9996) for the 6 log concentration range (see Figure below).

genome equivalents	log(10)	# +/10	ct(mean)	std
1000000	6	10/10	18.362	0.085609
100000	5	10/10	21.476	0.155863
10000	4	10/10	24.855	0.187157
1000	3	10/10	28.321	0.305449
100	2	10/10	31.869	0.2283
10	1	10/10	35.283	0.606045



The amplification efficiency is calculated to be 1.95 with the theoretical maximum of 2.0, which would indicate a doubling with each PCR cycle. Each assay contains a No Target Control and inhibition is monitored using a house-keeping gene, human albumin. This 2 primer set assay has been used for several previous studies and has been utilized in the Children's Diagnostic Virology Laboratory for 2 years, primarily to monitor transplant patients. The Diagnostic Laboratory participates in the College of American Pathologists, CAP, quantitative unknowns for CMV, EBV and BK viruses. Over the last 3 cycles, the CMV assay has been within 4 fold of the mean for these unknowns and has improved with each group.

Antiviral resistance will be performed in the UAB Viral Resistance Laboratory. Lab testing of viral isolates for ganciclovir resistance will be conducted by a standing SOP with a fully characterized assay.

8.2.1.2. Ganciclovir Pharmacokinetics/Adherence

Assessment of ganciclovir plasma concentrations will be conducted at the UAB Central Pharmacokinetic Laboratory. Ganciclovir concentrations will be obtained with one of the Study Assessment Doses 3, 4, 5, 6, 7, or 8 (Window: Study Assessment Day 1 through Study Assessment Day 5). Timepoints for

pharmacokinetic draws are as follows: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h. The required amount of whole blood for plasma ganciclovir determination at each timepoint is 200 μL (0.2 mL). Specimens will be processed to separate the plasma, which will be shipped to the UAB Central Pharmacokinetic Laboratory.

If desired by the treating physicians and the subject weighs ≥ 575 grams at that time, ganciclovir concentrations may be evaluated again around one dose in this assessment window (Study Assessment Day 18 through Study Assessment Day 24). Timepoints for pharmacokinetic draws are as follows: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h. The required amount of whole blood for plasma ganciclovir determination at each timepoint is 200 μL (0.2 mL). Specimens will be processed to separate the plasma, which will be shipped to the UAB Central Pharmacokinetic Laboratory. If subject weighs < 575 grams, the second set of PK draws cannot be performed.

A rapid, sensitive and specific high performance liquid chromatographic (HPLC) assay is used for the determination of ganciclovir in 200uL of human plasma. The UV detector, set to monitor the 270nm wavelength, provides adequate sensitivity with minimal interference from endogenous matrix components. The calibration curves are linear for ganciclovir over the range of 10 to 10,000 ng/mL.

8.2.2 Specimen Preparation, Handling, and Shipping

8.2.2.1 Instructions for Specimen Preparation, Handling, and Storage

Specific instructions on specimen preparation, handling and storage will be provided in the Manual of Procedures for this study.

8.2.2.2 Specimen Shipment

Specific instructions on specimen shipping will be provided in the Manual of Procedures for this study.

8.3 PRBC Transfusion Assessment

PRBC transfusions are commonly required in the management of critically ill premature babies. Because of the fragile nature of these subjects and the concern of risk of increasing the frequency of blood transfusions in participants in this sampling study, once during each Study Period the study team will record the number of PRBC transfusions received by the study subject since enrollment (for the Period 1 documentation) or since the last recording (for documentation for Periods 2-7). A PRBC transfusion is defined as ≥ 10 mL/kg of PRBCs within a 24 hour period.

9 ASSESSMENT OF SAFETY

This is a non-interventional sampling study. Risk to subjects is limited to risks associated with blood draws. In an effort to document only untoward medical events that have a greater likelihood of being study-related, only adverse events associated with blood draws will be considered adverse events for the purposes of this protocol. These events will be reported as adverse events of special interest (AESIs). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to blood draw (assessed only by those with the training and authority to make a diagnosis, which would include MD or DO), and time of resolution/stabilization of the event. AEs will be followed to adequate resolution.

Any other adverse events that meet the reporting requirements of the Institutional Review Board will be documented as AESIs, and reported to Protocol Chair, DMID Medical Officer, Medical Monitor and the Clinical Project Manager.

9.1 Safety Reporting: Adverse Events of Special Interest (AESI)

The investigator must also report the following AESI on the AESI case report form:

- AEs associated with blood draws
- All adverse events that meet the reporting requirements of the Institutional Review Board (which will also be reported to Protocol Chair, DMID Medical Officer, Medical Monitor and the Clinical Project Manager).
    -

9.2 Type and Duration of Follow-up of Subjects after Adverse Events

All AEs as described above will be followed until resolved or considered stable.

9.3 Halting Rules

9.3.1 Discontinue of Study participation for individual subject.

Subjects who experience significant problems with blood draws may be withdrawn from any future sampling. Transfusions are expected in hospitalized premature infants. One study reported that extremely premature neonates receive an average of 5.2 ± 4.2 transfusions over a six week period.[31] Other trials have reported similar numbers of transfusions in this population.[32,33] It is possible that even higher numbers of transfusions might be experienced in

the at-risk population in this sampling study since they are receiving the myelosuppressive drug ganciclovir as part of clinical care. Transfusions are not associated with long-term sequelae.[33] Nevertheless, if a study subject receives ≥ 10 PRBC transfusions during the course of the study, all future study-related blood draws will not be performed.

9.3.2 Discontinuation of Study Enrollment Pending Sponsor Review

DMID may interrupt study testing and/or study entry at any time if medically indicated. To minimize risk, the Protocol Chair, DMID Medical Officer, and Medical Monitor will review cumulative safety data.

Study enrollment and dosing will be stopped, and an ad hoc review will be performed if:

- X of the first Y subjects enrolled have more than 5 packed red blood cell (PRBC) transfusions during their period of time on the study, according to the table below. For a PRBC transfusion to be counted toward this halting rule, it must involve ≥ 10 mL/kg of PRBCs administered within a 24 hour period of time.

Number enrolled (Y)	Stop/Investigate when number with events equals this number (X)	type I error	power when mean=7
4	4	0.0218	0.2391
5	5	0.0084	0.1672
6	5	0.0341	0.4186
7	6	0.0151	0.3279
8	6	0.0406	0.5500
9	7	0.0196	0.4609
10	7	0.0438	0.6477
11	8	0.0225	0.5675
12	8	0.0450	0.7218
13	9	0.0242	0.6522
14	9	0.0450	0.7787
15	10	0.0251	0.7195
16	10	0.0442	0.8230
17	11	0.0254	0.7732
18	11	0.0429	0.8578
19	12	0.0253	0.8162
20	12	0.0414	0.8853

21	13	0.0248	0.8506
22	13	0.0397	0.9072
23	14	0.0242	0.8784
24	14	0.0378	0.9246
25	15	0.0234	0.9008
26	15	0.0360	0.9387
27	16	0.0226	0.9189
28	16	0.0342	0.9500
29	16	0.0496	0.9701
30	17	0.0324	0.9592
31	17	0.0465	0.9756
32	18	0.0307	0.9666

This halting rule is supported by an NICHD study that reported that extremely premature neonates receive 5.2 ± 4.2 transfusions per patient.[31] Other trials have reported similar numbers of transfusions in this population.[32,33] We therefore will anticipate up to 5 transfusions of PRBCs per subject. Subjects who would qualify for study enrollment are very sick, and frequent PRBC transfusions may be related to their underlying illness and not to the study-related blood draws. If following the review additional study enrollment is deemed safe, enrollment may be allowed to resume. Assuming the number of transfusions follows a Poisson distribution with mean of 5, then the probability of a subject receiving more than 5 PRBC transfusions is 0.384 (or 38.4%). If the halting rule is to stop the study when exactly 4 subjects receive more than 5 transfusions, then the probability of halting the study based on the given assumptions is 0.001 (or 0.1%) using a Binomial distribution with n=32 and probability of success equal to 0.384.

9.4 DMID Safety Oversight

DMID (medical officer, medical monitor and clinical project manager), the Protocol Chair, the UAB Central Unit, and an independent safety monitor (ISM) with expertise in pharmacology will:

- Monitor ganciclovir systemic exposures achieved with the clinical administration of ganciclovir.
- Review the safety information when a halting rule is met.
- Review AESIs on a regular basis.

9.5 FDA MedWatch Adverse Event Reporting Program

Reporting of adverse events related to drug administration is not part of the protocol. The reporting is in support of the FDA post-marketing safety surveillance program, and is encouraged.

The FDA MedWatch adverse event reporting program allows health care professionals to voluntarily report a serious adverse event, product quality problem or product use error that is suspected to be associated with the use of an FDA-regulated drug, biologic, medical device or dietary supplement. This system is for the reporting of adverse events noted spontaneously in the course of clinical care, and not for events that occur during clinical trials under an Investigational New Drug (IND) application.

The MedWatch Website, http://www.fda.gov/Safety/MedWatch/HowToReport/ucm085568.htm can be used to voluntarily report a serious adverse event, product quality problem or product use error that is suspected to be associated with the use of an FDA-regulated drug, biologic, medical device, dietary supplement or cosmetic. FDA uses these data to maintain safety surveillance of all FDA-regulated products. A health care professional report may be the critical action that prompts a modification in use or design of the product, improves the safety profile of the drug or device and leads to increased patient safety.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site Monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and study manuals. NIAID/ DMID, the sponsor of this study, or its designee will conduct site monitoring visits as detailed in the monitoring plan. A Clinical Monitoring Plan will be developed to include specifics about the monitoring, including a description of the number and type of subjects monitored, frequency of visits, tasks completed during the visits, and the different types of visits that will be used during the monitoring. The monitoring plan will be a risk based assessment.

Site visits typically may be made at standard defined intervals. More frequent sites visits may be made if needed. Monitoring visits will include, but are not limited to, review of regulatory files, case report forms, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

Every effort will be made to maintain the anonymity and confidentiality of subjects during this study. However, because of the experimental nature of this sampling study, the Investigator agrees to allow representatives of the sponsor as well as authorized representatives of the Food and Drug Administration, or other Regulatory agencies or the UAB Central Unit, to inspect the facilities used in this study and to inspect, for purposes of verification, the hospital or clinic records of all subjects enrolled into this.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

Our primary hypothesis is that ganciclovir pharmacokinetics and pharmacodynamics in premature infants with postnatally or congenitally acquired CMV infection differ from those in term neonates and older infants.

11.2 Sample Size Considerations

The primary outcome used for power analysis is the area under the curve for 12 hours (AUC12) following 6 mg/kg of ganciclovir. In a one-way ANOVA design, we would like to compare the means of 4 groups (see below) with sample sizes of approximately 8, 8, 8, and 8 (32 total subjects enrolled across the four cohorts). If the means of the AUC12 of the 4 groups are 20, 20, 30, and 35 assuming a common standard deviation of 10, these mean differences (equivalent to an effect size of 0.72) will be detected at 83% power using an F test with 0.05 level of significance. In order to look at other possible scenarios, we provide the table below that looks at other standard deviation values, its corresponding effect size given the differences in means as described above, and the power of detecting such a difference/effect size. If estimating the AUC without regard to the groups, the margin of errors associated with a 95% confidence interval assuming different standard deviation values and a sample size of 32 are given in the table below.

Standard Deviation	**S=8**	**S=9**	**S=10**	**S=11**
Effect Size	0.81	0.72	0.64	0.59
Power	96%	91%	83%	75%
Margin of Error	2.33	2.62	2.91	3.20

The above parameter values were hypothesized based on results from other studies.[25,34] It is worth noting that there are no PK data in this population and the results are likely to be different from other studies due to the maturational changes.

Following enrollment, subjects will be stratified by gestational age and by chronologic age as follows: 1) ≤ 27 weeks 6 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 2) ≤ 27 weeks 6 days gestational age at birth and > 30 days chronologic age at study enrollment; 3) ≥ 28 weeks 0 days gestational age at birth and ≤ 30 days chronologic age at study enrollment; 4) ≥ 28 weeks 0 days gestational age at birth and > 30 days chronologic age at study enrollment. The total sample size across these four cohorts is 32 subjects. Enrollment of an additional 2-3 subjects may be allowed for operational reasons. If the 32

subjects enrolled are not balanced across the 4 groups, we instead will look at the correlation between gestational age at birth. With 32 subjects, the margin of errors of a 95% confidence interval for estimating the correlation given the observed correlation values are given below.

Correlation	0.4	0.5	0.6	0.7
Margin of Error	0.6	0.542	0.47	0.38

Data will be collected on standardized case report forms. Standard non-compartmental techniques will be used initially to estimate pharmacokinetic parameters derived from the ganciclovir concentration-time data. The resource utilized for pharmacokinetic analysis is WinNonlin version 5.3, Pharsight Corporation, Mountain View, CA.

11.3 Planned Interim Analyses (if applicable)

There are no planned interim analyses or safety reviews for this trial.

11.4 Final Analysis Plan

Primary analysis: To compare the ganciclovir pharmacokinetics and pharmacodynamics as measured by AUC12 in premature infants with postnatally or congenitally acquired CMV infection differ from those in term neonates and older infants, we will construct 95% confidence intervals around the mean AUC_{12} for the subjects in this study and compare these means across the groups. If there are not enough subjects in the groups, we will use the actual gestational age and correlate it with the AUC_{12} by computing correlation coefficients and a fitting regression model. Additional pharmacokinetic parameters that compose analyses for secondary endpoints include Cmax, T1/2,CL, and VD. For measures that are repeated over time, we will utilize mixed models for repeated measures. In addition to being able to compare these outcomes for the different groups, we can also look into how these measures change over time and adjust for covariates.

Ganciclovir pharmacokinetic parameters will initially be determined using non-compartmental analysis (WinNonlin v5.3) for each individual subject. The parameters (AUC_{12}, CL, Vd, etc.) will be summarized and further used for exploratory pharmacodynamic analysis. We will also apply pharmacokinetic models to the concentration-time data, either individually or using a population approach. We may also pool these data with prior CASG 109 PK data in order to have a more robust covariate analysis across a wider range of ages.

The second pharmacokinetic analysis will utilize either population pharmacokinetic or individual modeling approaches, depending upon how much data we actually are able to collect and the nature of those data. Concentration-time data will be analyzed using nonlinear mixed-effects in ADAPT 5.0. If a population approach is employed, this will give us the opportunity to explore

relationships between ganciclovir exposure and multiple covariates, such as gestational age, weight, renal function, etc.

A 95% confidence interval will be constructed to evaluate the mean changes in the log of the viral load between baseline (day 1) and weeks 1, 2, 3, 4, 5, and 6. Area under the curve will be calculated for each subject's CMV viral load measurement utilizing the trapezoidal rule. These AUCs will be calculated over the entire course of drug administration. Spearman's correlations will be given for each AUC estimation and also for the AUC after baseline viral load (VL) adjustment. Analysis of variance will be used to investigate whether grouping AUCs into groups (undetected, 100-1000, 1000-10000 and ≥ 10000) would indicate any PD relationships. In addition, changes in CMV viral load from baseline over time (weeks 1 through 6) will be correlated with PK parameters. These data will provide the framework for all PD analyses, where ganciclovir exposures variables will be correlated with the response variables using linear or maximum effect PD models.

Secondary analyses: To investigate the association of secondary outcomes namely, neutropenia, CMV in urine, viral load and resistance to ganciclovir, with plasma concentration, we will utilize inference methods based on Generalized Estimating Equations (GEE) which models repeated binary data. As in the primary analysis, GEE will enable us to compare groups, investigate changes of outcomes over time, and adjust for any covariates.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored study, the site will permit authorized representatives of the sponsor(s), DMID, the UAB Central Unit and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations, and other activities during a clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, Source Document Worksheets, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Source documentation serves to substantiate the integrity of trial data, confirms observations that are recorded, and confirm the existence of study participants. This standard also serves to ensure data quality by creating audit trails and enabling verification that data are present, complete, and accurate. Sites that are participating in this trial should consult the MOP, and DMID/NIAID Source Document Standards (most current version) for specific instruction and forms.

13 QUALITY CONTROL AND QUALITY ASSURANCE

The study site will implement a quality management plan. The quality management procedures are described herein, as well as in the Manual of Procedures and the study site quality management plan (QMP). Per the QMP, data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The study will be conducted in accordance with procedures identified in the protocol. Items to be reviewed include, but are not limited to: eligibility (including informed consent), AE reporting, study/clinical endpoints, follow-up visits, regulatory documents, missed visits, and review of clinical records. Data that will be reviewed, who is responsible for implementation, and the schedule for internal reviews will be specified or referenced in the quality management plan.

The study monitors will verify that the clinical trial is conducted and data are generated, documented, and reported in compliance with the protocol, ICH E6(R1), and the applicable regulatory requirements. Reports will be submitted to DMID and UAB on monitoring activities. The study site research team will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor and their designees.

The UAB Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database within 24 hours of new data entered into the system. Full documentation of these checks will be provided to the UAB Central Unit so that any resulting queries can easily be understood and transmitted to the respective site for resolution within a short period of time. These processes are validated by a second programmer and also tested with faulty test data. All of these procedures and processes are described in detail in the DMID 11-0067 Data Management Plan (DMP) specific for this protocol. The DMP defines and provides instructions regarding the conduct of the data management processes, describing in detail the following: the work to be performed, the responsible individuals/groups, applicable SOPs and Guidelines, and required documentation.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

Reviewing IRBs must be registered with the OHRP to conduct FDA-regulated studies. In the United States and in other countries, only institutions holding a current US Federalwide Assurance (FWA) issued by OHRP will be allowed to participate in this study.

This protocol, informed consent documents, relevant supporting information, and all types of volunteer recruitment or advertisement information will be submitted to the Institutional Review Board (IRB) for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB prior to implementing changes in the study.

The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once per year. The investigator must also keep the IRB informed of any significant AEs.

All IRB approved documents as well as relevant study correspondence should be copied and sent to the UAB Central Unit.

For this study, clinical sites will have the opportunity to participate in a Facilitated Central IRB process supported by the UAB Institutional Review Board and the UAB Central Unit. Participating sites will be provided with detailed instructions on required responsibilities for participating an OHRP compliant Facilitated IRB.

14.3 Informed Consent Process

The process of obtaining informed consent must be documented in the medical records, clinic chart, and/or research chart. The consent form must be signed and dated by the subject's parent(s)/legal guardian(s) before participation in the study. A copy of the signed consent form must be provided to the subject's parent(s)/legal guardian(s). Signed consent forms must remain in each study participants study file and must be available for verification by study monitors at any time.

The investigational nature and research objectives of this trial, the procedure, and its attendant risks and discomforts will be carefully explained to the subject's parent(s)/legal guardian(s). A signed informed consent document will be obtained from each subject's parent(s)/legal guardian(s) prior to entry into this study. At any time during participation in the protocol, if new information becomes available relating to risks, AEs, or toxicities, this information will be provided orally or in writing to all enrolled or prospective subject's parent(s)/legal guardian(s). Documentation will be provided to the IRB and, if necessary, the informed consent will be amended to reflect any relevant information.

An investigator shall seek such consent only under circumstances that provide the subject's parent(s)/legal guardian(s) sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject's parent(s)/legal guardian(s) shall be in language understandable to the subject's parent(s)/legal guardian(s).

Subject's parent(s)/legal guardian(s) will sign the informed consent document prior to any procedures being done specifically for the study. Subject's parent(s)/legal guardian(s) should have the opportunity to discuss the study with their family, friends or personal physician, or think about it prior to agreeing to participate. Subject's parent(s)/legal guardian(s) may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subject's parent(s)/legal guardian(s) for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

If required by the site's IRB, the parent or legal guardian will sign a waiver of assent for the minor due to the study participant's age. Assent age will be determined by the local IRB. Appropriate documentation will be required for subjects who are the age of assent, whether mature enough to read and capable of providing signed assent, or whether too young to read but capable of providing verbal assent, as determined by the local IRB in compliance with 45CFR46. Local IRBs will review and assign the risk level.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This study will be conducted solely in premature infants. The study will not exclude young children, females, or minorities.

14.5 Subject Confidentiality

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is prohibited. The results of the research study may be published, but study participant's names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the principal investigators at each site will keep records in locked cabinets or locked rooms and the results of tests will be coded to prevent association with volunteers' names. Data entered into computerized files will be accessible only by authorized personnel directly involved with the study and will be encoded. Data received by DMID will not include subject specific data but only encoded data. However, subject specific information will be available to the clinical monitors, to the FDA and to health authorities where provided by law. .

The study investigator is obliged to provide the UAB Central Unit and NIAID/DMID with complete test results and all data developed in this study. The NIAID/DMID or UAB may disclose this information to appropriate regulatory authorities or clinical practice management groups (such as Pediatric Infectious Disease Society) as deemed necessary.

Subject-specific information may be provided to other appropriate medical personnel only with the study participant's parent/legal guardian permission. To ensure compliance with current ICH guidelines, data generated by this study must be available for inspection upon request by representatives of national and local health authorities, the NIAID/DMID, UAB Central Unit personnel, and the IRB/IEC for each study site.

14.6 Study Discontinuation

Therapy is not being provided by the study in this sampling trial. Therefore, there is no procedure for subjects to continue therapy if the study is discontinued.

14.7 Future Use of Stored Specimens

Some of the specimens obtained from study participants during this study will be stored indefinitely in the UAB Central Laboratory at the University of Alabama at Birmingham and may be used in future CMV research. These specimens will be labeled with a code number and not with the study participant's name. At the time of consent for study participation, study participant's parent/legal guardian will have the opportunity to either agree to have their specimens used in future CMV research or decline to have their specimens used in future CMV research. The study participant's parent/legal guardian will indicate his/her preference by initialing the appropriate line or checking the appropriate box of the Consent Form in the section entitled, "Future Use of Specimens". Non-protocol designated, future testing of samples will be performed only on samples from study participants who have consented for future testing of samples. These specimens will only be utilized to better understand the natural history of CMV

or improve diagnosis. Residual specimens from subject who decline future use will be destroyed after the study, final analysis and report writing is complete.

A repository for residual samples will be established according to OHRP guidelines ensuring that codes or other personally identifying links will not be distributed to future researchers.

The specimens will be stored indefinitely in the UAB Central Laboratory at the University of Alabama at Birmingham. CMV specimens from study participants will be labeled and coded without study participant's identifiers. If the study participant's parent/legal guardian has indicated in the signed consent form that he/she does not agree to allow the future use of specimens for future CMV research, then his/her child's specimens will be destroyed at the completion of the study.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Electronic case report forms (eCRFs) will be developed by the UAB Statistical and Data Coordinating Center. The eCRFs will be provided electronically by the UAB Central Unit. Original data will be recorded on source documents (e.g., medical records, research progress notes, Source Document Worksheets documenting research related procedures). The DMID 11-0067 DMP provides a description of how the system will function. Source Document Worksheets that mirror each data field on the eCRF will be available for use by sites as a tool to record and maintain data for each study participant enrolled in the study when other source documents are not used to collect original data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL. Specific guidance to investigators and study staff on making corrections to source documents and eCRFs will be provided in the MOP for this study.

Data recorded on the eCRF that differ from source documents must be explained on the Comments eCRF and in the subject's source documents.

15.1 Data Management Responsibilities

All eCRFS must be reviewed by the investigator's research team, under the supervision of the investigator, who will ensure that they are accurate and complete. All data must be supported by source documents, which will remain available for review by regulatory personnel and monitors. Adverse events must be graded, assessed for intensity and causality, and reviewed by the site investigator or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The UAB Central Unit and Statistical and Data Coordinating will be responsible for data management, quality review, analysis of the study data, and writing of the clinical study report. These tasks are detailed in the DMID 11-0067 DMP.

15.2 Data Capture Methods

Clinical and laboratory data will be entered into a 21 CRF Part 11 compliant electronic Data Entry System (eDES) provided by the UAB Statistical and Data Coordinating Center. The data system includes password protection and internal quality checks, such as automated range checks, to identify data that appear to be inconsistent, incomplete, or inaccurate.

15.3 Types of Data

Data for this study will include clinical laboratory, pharmacokinetic, and virologic results, and clinical and outcome measures (e.g., PK, weight, and virology).

15.4 Timing/Reports

There are no planned interim analyses or safety reviews for this trial. Stopping rules are detailed in Section 9.5. Following enrollment and PK assessments on the first four study subjects, safety and pharmacokinetic data will be summarized and reviewed by the Sponsor (e.g., the DMID medical officer and the DMID medical monitor), Protocol Chair, the UAB Central Unit, and an independent pharmacology monitor. The frequency of future data reviews will be determined at that time.

15.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for at least 2 years following completion of the study. No study records shall be destroyed without prior authorization from the UAB Central Unit and NIAID/DMID. These documents should be retained for a longer period, however, if required by local regulations. It is the responsibility of the sponsor to notifiy the UAB Central Unit, which will notify the investigators, when these documents no longer need to be retained.

15.6 Protocol Deviations

Each investigator must adhere to the protocol as detailed in this document and agree that any changes to the protocol must be approved by the UAB Central Unit and NIAID/DMID prior to seeking approval from the IRB/IEC. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria.

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the study

participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6 GCP sections:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1 and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 1 business day of identification of the protocol deviation that increases subject risk. Deviations that do not increase subject risk can be reported within 5 business days of knowledge of the event. All deviations must be promptly reported to the UAB Central Unit. UAB will report all deviations to DMID in accordance with DMID's instructions.

All deviations from the protocol must be addressed in the source documents. A completed copy of the DMID protocol deviation form must be maintained in the regulatory file (Project Notebook or designated location) as well as in the subject's source documents. A log of protocol deviation will be maintained in the Project Notebook. Protocol deviations must be sent to the local IRB per the IRB's guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

16 PUBLICATION POLICY

Following completion of this study, the investigators are expected to publish the results in a scientific journal. All research reports and other publications resulting from the work completed in this protocol shall:

- Acknowledge the support of the National Institutes of Health whenever publicizing the results from this clinical trial in any media by including an acknowledgement substantially as follows:
    - Be submitted to the Project Director in the form of advance copies for review and comment prior to the publication to ensure appropriate coordination of the research results.
    - Be furnished in a list of publications resulting from the research as part of the annual progress report submitted to the principal investigator.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

Unless exempted, this trial will be registered prior to enrollment of study subjects. It is the responsibility of the study's PI (e.g., Dr. Kimberlin) to register the non-exempted trials and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS (FOLLOWING ACQUISITION OF INFORMED CONSENT)

Table 1. Schematic of Study Design for Gan Premie
NOTE: Required Studies in Black Font, Optional Studies in Blue Font

	Period 1 (Study Assessment Day 1 through Study Assessment Day 3)	Period 2 (Study Assessment Day 4 through Study Assessment Day 10)[a]	Period 3 (Study Assessment Day 11 through Study Assessment Day 17)[a]	Period 4 (Study Assessment Day 18 through Study Assessment Day 24)[a]	Period 5 (Study Assessment Day 25 through Study Assessment Day 31)[a]	Period 6 (Study Assessment Day 32 through Study Assessment Day 38)[a]	Period 7 (Study Assessment Day 39 through Study Assessment Day 42)[a]
Baseline demographics[b]	X[c]						
Hematology labs[d]	X[c,e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]
Chemistry labs[f]	X[c,e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]
Creatinine	X[g]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]
Ganciclovir pharmacokinetics	X[h]			X[i,j]			
Virology blood specimens for CMV viral load[k]	X[l]	X[l]	X[l]	X[l]	X[l]	X[l,m]	X[l,n]
CMV detection from noninvasive urine specimen	X[o]	X[o]	X[o]	X[o]	X[o]	X[o]	X[o]
Ganciclovir dosing information	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]	X[p]
PRBC transfusion assessment	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]	X[q]

Assessment for AEs or SAEs		X	X	X	X	X	X
Total volume of blood required for study procedures	1.75 mL	0.5 mL	0.5 mL	0.5 mL or 1.5 mL[i]	0.5 mL	0.5 mL	0.5 mL

a) Data and specimens in this column to be captured if subject still receiving ganciclovir as clinical treatment
b) Gestational age at delivery, date of birth, day of life at initiation of IV ganciclovir therapy, gender, race, ethnicity, birth weight, weight at enrollment, length in centimeters at enrollment, reason ganciclovir being administered, ganciclovir dose and dosing frequency, manufacturer of the ganciclovir lot utilized at the treating facility, date ganciclovir therapy was initiated
c) Window: Birth through Study Assessment Day 3)
d) WBC with differential, hemoglobin, platelet count. Optional laboratory draws are not required by the protocol, and so no documentation on a source document will be required.
e) If being drawn for clinical reasons; any laboratory value obtained during the window will suffice, with an effort being made to capture the most abnormal value. Optional laboratory draws are not required by the protocol, and so no documentation on a source document will be required.
f) AST, ALT. Optional laboratory draws are not required by the protocol, and so no documentation on a source document will be required.
g) To be drawn on the calendar day that ganciclovir PKs are being drawn. Study related laboratory draws will be delayed or deferred if the investigator believes that obtaining them might increase subject risk. Additionally, a study-related laboratory specimen that is unevaluable due to clotting or laboratory related issues will not be repeated. If a study related laboratory draw is delayed or deferred, study staff will document this on a source document.
h) Ganciclovir concentrations will be obtained with one of the Study Assessment Doses 3, 4, 5, 6, 7, or 8 (Window: Study Assessment Day 1 through Study Assessment Day 5) at the following time points: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h; required amount of whole blood for plasma ganciclovir determination at each time point is 200 μL (0.2 mL). Study related laboratory draws will be delayed or deferred if the investigator believes that obtaining them might increase subject risk. Additionally, a study-related laboratory specimen that is unevaluable due to clotting or laboratory related issues will not be repeated. If a study related laboratory draw is delayed or deferred, study staff will document this on a source document.
i) If desired by the treating physicians, ganciclovir concentrations may be obtained around one dose during the window at the following time points: 0h (immediately prior to IV ganciclovir dose; window: -15 min), 1h (immediately after the end of the IV ganciclovir dose; window: +15 min), 2-3h, 5-7h, and 10-12h; required amount of whole blood for plasma ganciclovir determination at each time point is 200 μL (0.2 mL). Optional laboratory draws are not required by the protocol, and so no documentation on a source document will be required.
j) If subject weighs < 575 grams, the second set of PK draws cannot be performed (would exceed blood draw parameters of 7 mL/kg over 42 days)
k) To be obtained only once per study period; ideally, at least 72 hours should separate viral loads being obtained in different study periods
l) Required amount of whole blood for CMV PCR is 0.5 mL. Study related laboratory draws will be delayed or deferred if the investigator believes that obtaining them might increase subject risk. Additionally, a study-related laboratory specimen that is unevaluable due to clotting or laboratory related issues will not be repeated. If a study related laboratory draw is delayed or deferred, study staff will document this on a source document.
m) If subject weighs < 600 grams, the virology specimen for CMV viral load will not be performed (would exceed blood draw parameters of 7 mL/kg over 42 days)
n) If subject weighs < 650 grams, the virology specimen for CMV viral load will not be performed (would exceed blood draw parameters of 7 mL/kg over 42 days)
o) Urine collection will be non-invasive (e.g., bagged, indwelling foley catheter, excess clinical specimen, etc.). If urine is obtainable, it will be frozen and sent to the UAB Central Laboratory for processing. If an attempt to

obtain urine for CMV detection is unsuccessful, study staff will document the failed attempt on a source document.

p) mg/dose, dosing interval, subject weight

q) Once during each Study Period the study team will record the number of PRBC transfusions received by the study subject since enrollment (for the Period 1 documentation) or since the last recording (for documentation for Periods 2-7). A PRBC transfusion is defined as ≥ 10 mL/kg of PRBCs within a 24 hour period.