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Ampicillin Pharmacokinetics and Safety in Infants

Protocol:	NICHD-2012-AMP01
Development Phase:	PK Analysis – Phase 1 Retrospective Safety Analyses – Phase 4
Investigational Product:	Ampicillin
IND Number:	TBD
Date of Inclusion of First Participant:	N/A (no participant was enrolled under this protocol)
Date of Completion of Last Participant:	N/A (no participant was enrolled under this protocol)
Indication Studied:	N/A
Methodology:	Characterize the PK and safety of ampicillin from samples obtained in PK studies of ampicillin prescribed to infants per standard of care by their treating caregiver
IND Sponsor:	Daniel K Benjamin Jr., MD, PhD, MPH Kiser-Arena Distinguished Professor of Pediatrics, Duke University Duke Clinical Research Institute P.O. Box 17969 Durham, NC 27715 Redacted
Medical Monitor:	Redacted Chief Medical Officer The EMMES Corporation 401 N. Washington Street, Suite 700 Rockville, MD 20850 Redacted
Report Written by:	The EMMES Corporation / Best Pharmaceuticals for Children Act (BPCA) Data Coordinating Center (DCC)
Date of Final Report:	November 24, 2014

This study was conducted in compliance with Good Clinical Practices (GCP) as outlined in the International Conference on Harmonization (ICH), including the archiving of essential documents.

2 SYNOPSIS

NAME OF IND SPONSOR/COMPANY: Daniel K Benjamin Jr., MD, PhD, MPH; Duke Clinical Research Institute	
NAME OF FUNDING SPONSOR: *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (NICHD)	
NAME OF FINISHED PRODUCT: Ampicillin	**NAME OF ACTIVE INGREDIENT:** Ampicillin
TITLE OF STUDY: Ampicillin Pharmacokinetics and Safety in Infants	
PRINCIPAL INVESTIGATORS:	Daniel K Benjamin Jr., MD, PhD, MPH Duke Clinical Research Institute (DCRI)
PUBLICATION (REF): Tremoulet A, Le J, Poindexter B, Sullivan JE, Laughon M, Delmore P, Salgado A, Chong SI, Melloni C, Gao J, Benjamin DK, Capparelli EV, Cohen-Wolkowiez M. *Characterization of the Population Pharmacokinetics of Ampicillin in Neonates Using an Opportunistic Study Design*. Antimicrob. Agents Chemother. 2014;58(6):3013-3020.	
STUDY PERIOD FOR PK STUDY: February 16, 2012 – August 1, 2012	**PHASE OF DEVELOPMENT:** PK Analysis – Phase 1 Retrospective Safety Analyses – Phase 4
PRIMARY OBJECTIVES: 1. Characterize the PK of ampicillin from samples obtained in a PK study of ampicillin 2. Characterize safety of ampicillin prescribed to infants per standard of care (SOC) by their treating caregiver	
METHODOLOGY FOR PK STUDY: Sparse sampling, PK/PD modeling	
NUMBER OF PARTICIPANTS (ANALYZED): PK Analysis: • 75 participants (infants) enrolled under the NICHD-2011-POP01 study. Safety Analysis: • 75 participants (infants) enrolled under the NICHD-2011-POP01 study. • 199,785 infants who received ampicillin in the Pediatrix Medical Group Database (NICHD-2012-AMP01). • 68 participants (infants) who received ampicillin while enrolled in the Pediatric Pharmacology Research Unit (PPRU) study at Redacted (NICHD-2012-AMP01).	
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION: **PK and Safety (NICHD-2011-POP01)** **Inclusion Criteria** Infants with a postnatal age < 30 days who were receiving ampicillin per standard of care **Exclusion Criteria** Failure to obtain consent/assent (as indicated)	

Safety (NICHD-2012-AMP01)

Inclusion Criteria

Infants enrolled in the PPRU Antimicrobial PK in High Risk Infants study at Redacted and infants exposed to ampicillin discharged from a NICU managed by the Pediatrix Medical Group between 1997 and 2010.

Exclusion Criteria

None

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER:

Objective 1 - PK Analysis – Ampicillin is a generic therapeutic and was supplied by the site clinical pharmacy.

Objective 2 - Safety Analysis - This was a secondary data analysis study. The administration of ampicillin study drug was not a part of this protocol.

CRITERIA FOR EVALUATION:

Efficacy: Efficacy evaluation of the drugs under study was beyond the scope of this investigation.

PK: The PK analysis assessed clearance (CL) and volume (V) through compartmental population modeling (nonlinear mixed effects modeling in NONMEM). In addition, the proportion of simulated participants infants with a trough concentration above the minimum inhibitory concentration (MIC) of ≥8 mcg/mL (the MIC_90 per CLSI breakpoints) was evaluated as the surrogate PD endpoint in simulations.

Safety:

- NICHD-2011-POP01 study: Adverse Events (AEs) and Serious Adverse Events (SAEs) caused by study specimen collections were reported at each time point when the study specified biological sample collection occurred up until 1 hour after collection. Serious, unexpected, suspected adverse reactions of the understudied drug of interest were reported from the time of first biological sample collection until 3 days after the last biological sample was collected if participant was available. AE information was gained from direct monitoring of the study subject as well as from clinician observation, and self-reporting by the study subject or his/her guardian(s).
- Pediatrix Database: AE and severe AE (SvAE) were defined by pre-specified diagnosis and laboratory cut-offs.
- Retrospective chart review (PPRU at Red): AE and SvAE were collected through retrospective chart review. Frequency of AE and SvAE, microbiological data, physical examination findings and growth parameters (weight, height and head circumference) collected at the beginning and at the end of the study were reported.

STATISTICAL METHODS:

Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, minimum, and maximum are presented for continuous variables. Other descriptive statistics such as counts, proportions, and/or percentages are presented to summarize discrete variables (such as race, sex, etc.). Demographic and safety characteristics are summarized by gestational and postnatal age group. The percentage of days with each AE was determined for each dose and dosing interval. The median ampicillin exposure on days with and without each AE and SvAE was determined. The percentage of infant days with each AE and SvAE were determined for each decile of ampicillin exposure. These analyses were performed for all infants and by gestational and postnatal age group. These parameters were also determined with the analysis limited to infants with documented infection.

RESULTS:

PK/PD: A population PK analysis was performed on the plasma pharmacokinetic samples obtained in the NICHD-2011-POP01study. As this was an opportunistic PK study, the dosing of ampicillin was based on that chosen by the primary treating physician. The average total daily dosing of ampicillin prescribed for the participants in the study exceeded the most common dosing references (Neofax, Harriet Lane and Pediatric Drug Dosing). Neonates were stratified into 4 groups by gestational age (GA, ≤ 34 weeks or > 34 weeks) and postnatal age (PNA, ≤ 7 days or > 7 days).

Overall, a total of 160 ampicillin plasma concentrations in 75 participants were collected. Of these, a total of 142 ampicillin plasma concentrations in 73 participants were used to construct the population PK model. Data were fit to a 1-compartment model (ADVAN1 TRANS2) using NONMEM version 7.2 and the First Order Conditional Estimation method with ETA-EPS interaction (FOCE-I). Weight (WTKG) was assumed to be a significant covariate for CL (L/h) and V (L) and was included in the base model prior to assessment of other potential covariates. A univariable covariate screen was performed for potential associations with PK parameters. Both PMA- and GA/PNA-based models were constructed. Mechanistically, the final PMA-based model was better than GA/PNA-based model. The final population model is: $V = \theta_{(1)} * WTKG$ and $CL = \theta_{(2)} * WTKG * (0.6/SCR)^{\theta(3)} (PMA/37)^{\theta(4)}$; where $\theta_{(1)}$ =0. 399, $\theta_{(2)}$= 0.078, $\theta_{(3)}$ =0.428, and $\theta_{(4)}$ =1.34. The between-subject variability for CL was 23%, and the residual variability was 34%. Monte Carlo simulations (N=1920) were performed using the final model to determine the most clinically applicable optimal dosing regimen for achieving a trough concentration above the MIC of ≥8 mcg/mL in at least 90% of simulated participants. Dosing by GA/PNA and PMA (the latter using empirically-derived thresholds for PMA and PNA) achieved similar PD target attainment. The dosing regimen by GA/PNA was selected to offer the specificity needed to account for prematurity and developmental changes that occur with age, especially during the first 1 to 2 weeks of life, the dosing regimen was simplified to: 50 mg/kg every 12 hours for Group 1 (GA≤ 34 weeks and PNA≤ 7 days), 75 mg/kg every 12 hours for Group 2 (GA≤ 34 weeks and PNA 8-28 days) and 50 mg/kg every 8 hours for Groups 3 (GA> 34 weeks and PNA≤ 7 days) and 4 (GA>34 weeks and PNA 8-28 days).

Efficacy: No efficacy analyses were conducted as part of this study.

SAFETY:

No adverse events or reactions were reported from the NICHD-2011-POP01 study.

From the Pediatrix Database: Adverse events during ampicillin exposure were uncommon. Shorter dosing intervals were associated with more frequent hematologic AEs for infants of all age groups. Thrombocytopenia was the most common laboratory AE occurring on 3.4% of infant-days. Thrombocytopenia occurred more frequently at shorter dosing intervals. There was no obvious relationship between ampicillin dose or exposure and thrombocytopenia. Leukopenia AE and SvAE occurred at higher ampicillin exposures for the youngest infants (Group 1). There was no clear relationship between ampicillin exposure and leukopenia for older infants (Groups 2 & 3). Ampicillin exposure was not clearly related to neutropenia AE and SvAE.

AST elevation was very uncommon occurring on approximately 0.1% of infant-days. AST AE and SvAE appeared to occur sporadically with no obvious relationship to ampicillin dose or dosing interval. No infants with documented infection had an AST AE or SvAE during ampicillin exposure. Most AST AEs and SvAEs occurred in the youngest infants (Group 1).

Seizures occurred more frequently with higher doses of ampicillin and shorter dosing intervals and occurred on 0.3% of infant-days. The association of ampicillin exposure with seizure was unclear: infant-days on which a seizure occurred tended to have higher ampicillin exposures but infant-days with the highest exposures often did not have a seizure occur.

CONCLUSIONS:

PK: The final population model is:

V (L) = $\theta_{(1)}$ * WTKG

CL (L/h) = $\theta_{(2)}$ * WTKG * (0.6/SCR)$\theta_{(3)}$ (PMA/37)$\theta_{(4)}$

Here, $\theta_{(1)}$ =0. 399, $\theta_{(2)}$= 0.078, $\theta_{(3)}$ =0.428, and $\theta_{(4)}$ =1.34. This population pharmacokinetics study of ampicillin in participants demonstrated the importance of PMA, composed of PNA and GA, in drug CL.

The Monte Carlo simulation for the surrogate PD endpoint demonstrated that the higher dose of ampicillin currently being prescribed by most physicians, and demonstrated by the average daily dose of ampicillin ordered by the primary caregiver for the participants in NICHD-2011-POP01, achieved the surrogate PD endpoint of trough concentrations at steady state ≥8 mcg/mL in >97% of virtual subjects as compared to 90% of virtual subjects with the current dosing references. As the goal was to achieve a trough concentration above the MIC of ≥8 mcg/ml in at least 90% of the infants, we devised the following dosing regimen that simplifies the current dosing references:

Gestational age (weeks)	Postnatal age (days)	Maintenance dose (mg/kg)	Dosing interval (hours)
≤ 34	≤ 7	50	12
≤ 34	≥ 8 & ≤ 28	75	12
> 34	≤ 28	50	8

Safety: In the Pediatrix database, adverse events of special interest during exposure to ampicillin were uncommon. Seizure occurred more frequently at higher doses. Seizure, neutropenia, leukopenia and thrombocytopenia occurred more frequently at shorter dosing intervals. Shorter dosing interval had the greatest influence on the incidence of AEs. Safety events at the dosing regimen suggested above resulted in AEs and SvAEs occurring at rates equal to or less than other dosing regimens.

DATE OF THE REPORT: November 24, 2014

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4 GLOSSARY OF ABBREVIATIONS AND TERMS

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body Mass Index
BPCA	Best Pharmaceuticals for Children Act
CFR	Code of Federal Regulations
CL	Clearance
CRA	Clinical Research Associate
eCRF	Electronic Case Report Form
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DOL	Day of Life
ECMO	Extracorporeal Life Support
EDC	Electronic Data Capture
GA	Gestational Age
ICU	Intensive Care Unit
IV	Intravenous
Kg	Kilogram
mcg	Microgram
MIC	Minimum Inhibitory Concentration
ng	Nanogram
NICHD	Eunice Kennedy Shriver National Institute of Health and Human Development
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
PHI	Protected Health Information
PK	Pharmacokinetics
PD	Pharmacodynamics
PMA	Post menstrual age
PNA	Post natal age
PPRU	Pediatric Pharmacology Research Unit

Abbreviation	Definition
PTN	Pediatric Trials Network
SAE	Serious Adverse Event
SvAE	Severe Adverse Event
SCR	Serum Creatinine
SOC	Standard of Care (per prescribing physician)
V	Volume of Distribution
VLBW	Very Low Birth Weight
WBC	White Blood Cell
WTKG	Weight in Kilograms

5 ETHICS

5.1 Institutional Review Board (IRB)

Institutional Review Board (IRB) approval of the protocol and waiver of informed consent for the NICHD-2012-AMP01 study overall was obtained prior to receiving data from any of the involved studies. As this is a data analysis study, no participants were enrolled under this protocol.

For the NICHD-2011-POP01 study, Institutional Review Board (IRB) approval of the protocol and, any protocol amendments, informed consent/assents (as indicated) and all other forms of subject information related to the study, was obtained prior to authorization of any study procedures. Detailed IRB information is provided in Appendix 16.1.3. Participants were enrolled under this protocol.

5.2 Ethical Conduct of the Study

This study was conducted in accordance with the protocol, ethical principles originating from the Declaration of Helsinki and current GCP and in compliance with local regulatory requirements and 21 CFR 312.

5.3 Patient Information, Consent, and Assent

All participants included in the data analysis of this study were children (under the age of 21 years). No participants were enrolled under this protocol. No informed consent form was used for this data analysis protocol. A waiver of informed consent was obtained from the IRB. Please see Appendix 16.1.3.

Informed consent process was followed by the Investigators according to the original studies. The investigator or designated representative explained the nature of the study to the patient and the patient's parents or legal guardian, to the extent compatible with the patient's understanding, and answered all questions regarding this study. Prior to any study related screening procedures being performed on the patient, the informed consent (parental permission) statement was signed and dated by the patient's parent or legal guardian and by the person who administered the informed consent. A copy of the informed consent form (ICF) was given to the patient, or the patient's parents or legal guardian, and the original was placed in the patient's medical record. In addition, the Health Insurance Portability and Accountability Act (HIPAA) statement was reviewed, signed, and dated by the patient's parent or legal guardian. A sample ICF for the NICHD-2011-POP01 study is provided in Appendix 16.1.3.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

6.1 Principal Investigators

Objective #1 includes participants enrolled under the NICHD-2011-POP01 study, which was a multicenter study with 9 enrolling sites in the United States that enrolled participants who received ampicillin per standard of care (SOC). A list of principal investigators and their affiliations, roles in the study, and curriculum vitae are provided in Appendix 16.1.4. The signatures of the sponsor's responsible medical officer, medical monitor, and coordinating investigator for this study appear in Appendix 16.1.5.

6.2 Administrative Structure

The following is a list of organizations that were critical to the conduct of the study:

IND Sponsor and Principal Investigator	Daniel K Benjamin Jr., MD, PhD, MPH Professor, Duke University Pediatrics Duke Clinical Research Institute REDACTED Durham, NC 27715
Co-Investigator	Michael Cohen-Wolkowiez, MD, PhD Assistant Professor, Duke University Pediatrics Duke Clinical Research Institute REDACTED Durham, NC 27715
Funding Sponsor	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
Best Pharmaceuticals for Children Act (BPCA) Data Coordinating Center (DCC)	The EMMES Corporation 401 N. Washington Street, Suite 700 Rockville, MD 20850
Medical Monitor	Redacted Chief Medical Officer The EMMES Corporation 401 N. Washington Street, Suite 700 Rockville, MD 20850
Pharmacokinetic Analysis	Redacted Division of Pharmacology and Drug Discovery Schools of Medicine and Pharmacy and Pharmaceutical Sciences University of California San Diego, CA 92093 Redacted Division of Pharmacology and Drug Discovery School of Medicine University of California, San Diego 9500 Gilman Drive, Redacted La Jolla, CA 92093

Safety Analysis	Redacted Associate Professor, Department of Pediatrics Duke University Medical Center Duke Clinical Research Institute REDACTED Durham, NC 27715 Redacted Assistant Professor, Department of Pediatrics Department of Pediatrics Duke University Medical Center REDACTED Durham, NC, 27710

7 INTRODUCTION

7.1 Ampicillin Therapy for Bacterial Sepsis in Infants

Bacterial sepsis remains a leading cause of death in infants admitted to neonatal intensive care units (NICUs). The incidence of early onset sepsis (<4 postnatal days) in infants admitted to the NICU is 1.2% to 2% and for late onset sepsis (4-120 postnatal days) 14% to 36%. The incidence of late onset sepsis is higher in very low birth weight infants (VLBW, birth weight <1500 g) [1,2]. The most commonly administered medication in the NICU, with an exposure rate of 693 per 1000 NICU-discharged infants, is ampicillin [3]. Ampicillin is a beta-lactam antibiotic approved by the FDA to treat local and disseminated infections caused by susceptible organisms in patients of all age groups [4].

7.2 Ampicillin PK and Safety in Infants

The PK of ampicillin has been studied in children and adults [5-18], but data on dosing in the neonatal population are sparse. The first pharmacological studies of serum and cerebrospinal fluid concentrations after intramuscular injection of ampicillin in term and preterm (<2500 g) infants, were performed between 1967 and 1974. These studies included a combined total of 156 infants (gestational age not specified) and showed that the serum half-life of ampicillin decreases rapidly in the first two weeks of life as a result of increasing clearance. Doses studied ranged from 25-150 mg/kg, administered every 8-12 hours according to postnatal age [12-14]. A later study assessed the PK of ampicillin in 142 preterm infants with a gestational age (GA) ≥ 24 weeks. The study concluded that infants with a GA ≤ 28 weeks required a dosing interval of 18-24 hours and did not require initial dosing exceeding 50 mg/kg [15]. Current dosing regimens take into account the gestation and postmenstrual age related variation in renal drug clearance, and recommend lower doses and less frequent dosing in the most premature infants [11]. However, the available data in the literature is insufficient to support dosing of ampicillin in the most extreme premature infants (≤32 weeks gestational age at birth).

Although ampicillin is the most commonly administered medication in the NICU, its safety in the neonatal population is poorly described. Adverse events (AE) listed on the FDA label for ampicillin are not specific to the neonatal population. Ampicillin AEs are mostly due to sensitivity phenomena that are more common in people with a history of penicillin exposure. This is very uncommon in the neonatal population. In a recent study comparing clinical efficacy of ampicillin or penicillin in combination with gentamicin therapy for early onset sepsis in infants (including GA <26 weeks), both were well tolerated with no difference in AEs or laboratory abnormalities. However, the focus of this study was clinical effectiveness comparison, and not safety profiling [15]. Among the potential serious AEs in infants is the risk of ampicillin induced seizures [4]. As with other penicillin's, β-Lactam-induced neurotoxicity is of concern if cerebrospinal fluid concentrations of ampicillin are high. This has been reported in adult case reports, but the incidence of seizures in infants, treated with ampicillin, has not been evaluated [16, 17]. Another known side-effect of ampicillin most commonly seen after repeated dosing is inhibition of the coagulation cascade. The prevalence of this AE in the neonatal population, which is at risk of coagulopathy, is unknown [18]. Overall, given the importance of safety and improved long term outcomes in the neonatal population, a more detailed description of the safety of this commonly used antibiotic is warranted.

7.3 Opportunistic Studies in Children

Studies capitalizing on standard of care procedures, such as biological sample collection from infants already receiving drugs of interest, have produced meaningful PK data resulting in improved dosing recommendations in infants and children. These studies did not administer drugs to children, but rather collected samples from children who were already receiving drugs as standard of care as prescribed per the local caregiver. In addition, preliminary data obtained through opportunistic studies has served to design phase 1-3 trials in children as well as support applications for extramural research. Through the

infrastructure of the Pediatric Pharmacology Research Unit (PPRU), Wade et al. obtained timed and scavenged plasma samples to characterize the PK of fluconazole in premature infants [19]. This PK analysis led to the dose selection for a phase 3, randomized, placebo controlled trial of fluconazole prophylaxis in premature infants (clinicaltrials.gov NCT00734539). Opportunistic designs have also been successful to characterize metronidazole PK and develop piperacillin PK models in premature infants [20, 21].

7.4 Premature Infant PK Studies

The difficulties associated with infant phase 1 PK trials have forced investigators to rely upon the extrapolation of PK data obtained in older children and adults to estimate PK parameters and dosing recommendations in premature infants. However, this approach underestimates the complicated physiology of premature infants, which differs greatly from other populations. These differences include a larger extracellular fluid volume, immature renal and hepatic function, underdevelopment of metabolic enzymatic systems, and a unique blood-brain barrier, all of which can alter drug disposition significantly [17]. These infant-specific qualities result in discrepancies between adult and infant PK leading to the need for infant-derived PK assessments to better define dosing recommendations of commonly used drugs.

7.5 BPCA Drug Prioritization

NICHD developed the BPCA prioritization process to identify gaps in pediatric drugs, primarily off-patent drugs that need further study through clinical trials or other avenues of research. There are two main phases in the prioritization process. Phase 1 entails identifying therapeutic areas, which are general categories of conditions, diseases, setting of care, or populations with multiple pediatric needs to be addressed in Phase 2. Phase 2 involves determining more specific pediatric needs, including research associated with a particular drug, biologic, or device.

7.6 Data Sources

Despite the common use of ampicillin in infants admitted to the NICU, PK and safety studies to define optimal dosing are lacking. Challenges associated with clinical trials in infants limit the ability to conduct large PK and dosing trials in this population. Capitalizing on all available data sources to characterize the PK and safety of ampicillin used in infants is therefore essential. The first objective of this study was to characterize the PK of ampicillin administered to infants per standard of care as administered by their treating caregiver. PK analyses were performed on data collected for the NICHD-2011-POP01 protocol. The second objective of this study was to assess safety of ampicillin administered to infants per standard of care. Safety was primarily assessed using data from retrospective chart review of participants enrolled in a prior PPRU study and using a large epidemiological database from the Pediatrix Medical Group. Safety data collected from the NICHD-2011-POP01 study was analyzed as well.

7.6.1 NICHD-2011-POP01: Pharmacokinetics of Ampicillin in Infants Study

This prospective multi-center trial was conducted under protocol NICHD-2011-POP01 within the Pediatric Trials Network (PTN) to characterize the PK of ampicillin administered to infants per standard of care by their treating caregiver. This study was conducted under the same IND as NICHD-2012-AMP01 (IND 113,645).

This protocol (Appendix 16.4.1) enrolled participants under multiple drugs of interest, each of which are administered to children per standard of care. Only participants enrolled for ampicillin as the drug of interest are included in the analyses for this CSR. Also, only data that is related to plasma PK samples are included in the CSR submission. Sample collection records and protocol deviations related to the collection of other types of samples are not included in the CSR submission.

The first participant was enrolled for ampicillin in February, 2012, and the last participant completed in August, 2012. This study is the only source of PK data analyzed in this CSR.

7.6.2 Pediatric Pharmacology Research Unit (PPRU) Study Safety Review

In order to fill knowledge gaps in neonatal drug dosing, the NIH-sponsored pediatric pharmacology research unit (PPRU) conducted the Antimicrobial Pharmacokinetics (PK) in Premature Infants Trial [Jan. 2006 to Nov. 2010]. This multi-center, open-label, opportunistic study collected PK samples from premature infants <32 weeks gestational age who were receiving ampicillin per standard of care. Safety data was obtained from the subset of patients enrolled in the PPRU study at Redacted. The methods and results from this retrospective chart review are included in Appendix 16.4.2.

As specified in the NICHD-2012-AMP01 protocol (Appendix 16.1.1), PK data were collected for the PPRU study. However, these data are not included in this CSR submission.

7.6.3 Safety of Ampicillin Pediatrix Medical Group Database

This was a multi-center retrospective cohort conducted using the Pediatrix Medical Group administrative database. The Pediatrix Medical Group database is prospectively created from electronic health records of infants admitted to >300 NICUs in the United States (US). It is one of the largest repositories of data for neonatal medicine. The database includes approximately 20% of all NICU patients in the US and >90,000 NICU admissions annually. A detailed description of study procedure, analysis and results for this study is provided in Appendix 16.4.3.

7.7 Structure for this Clinical Study Report

As explained in Section 7.6, this report analyzes and discusses data derived from one prospective study (NICHD-2011-POP01) and two retrospective studies (PPRU study and Pediatrix epidemiological database). Sections 9, 10 and 11 of this report will cover the investigational plan, overall study population and PK analysis of data from the NICHD-2011-POP01 study. Section 12, which covers the safety analysis, will include safety data from all three studies including NICHD-2011-POP01, the PPRU study and the Pediatrix epidemiological database.

8 STUDY OBJECTIVES

1. Characterize the PK of ampicillin from samples obtained in a PK study of ampicillin
2. Characterize the safety of ampicillin prescribed to infants per standard of care by their treating caregiver

9 INVESTIGATIONAL PLAN: NICHD-2011-POP01

9.1 Overall Study Design and Plan Description

As discussed in Section 7.7, Sections 9-11 of this CSR discuss study design and analyses associated with PK data collected as part of the NICHD-2011-POP01 protocol (Appendix 16.4.1) in infants administered ampicillin per standard of care. This was an open label, multi-center, opportunistic PK study of drugs administered per routine medical care. Participants were not prescribed ampicillin as a part of this protocol. A legal guardian of the participant meeting inclusion criteria in the inpatient hospital setting was approached for consent for this study.

9.2 Discussion of Study Design

The NICHD-2011-POP01 ampicillin PK data was collected through an open label, opportunistic study design. No control groups were included in this study. The dosing, sampling, and demographic information recorded on the electronic Case Report Form (eCRF) was merged with the bioanalytical information to create a PK dataset for ampicillin. Because a sparse sampling scheme was employed in this study, population PK methodologies were used to analyze the PK data.

9.3 Study Population

A total of 64 participants were planned for enrollment in this study. As shown in Table 9-1, 16 participants were scheduled to be enrolled in each strata separated by post-natal age (PNA) and gestation age (GA).

Table 9-1 Planned sample size in each PNA and GA cohort.

PNA (Days)	≤ 7		8-28	
GA Groups (Weeks)	≤ 34	> 34	≤ 34	> 34
Group Number	1	3	2	4
Planned Sample Size	16	16	16	16

9.3.1 Inclusion Criteria

Children (< 21 years of age) who were receiving understudied drugs of interest per standard of care as prescribed by their treating caregiver were eligible for the NICHD-2011-POP01 protocol. Infants who were receiving ampicillin per standard of care and belonged to one of the enrollment groups in Table 9-1 were targeted for inclusion in the ampicillin analysis.

9.3.2 Exclusion Criteria

1. Failure to obtain consent/assent (as indicated).

9.3.3 Removal of Subjects from Assessment

1. Parents or legal guardians of participants who withdrew consent to participate in the study and requested no further follow-up were terminated from the study.
2. In cases of early / premature termination (defined as a participant with no samples obtained / drawn for the study and / or participant withdrawn from the study), a replacement participant could be enrolled.

Participants could be enrolled in the NICHD-2011-POP01 protocol under multiple drugs of interest, but they could not be enrolled multiple times for the same drug.

9.4 Treatments

Ampicillin was administered in accordance with local standard of care as prescribed by the treating caregiver. Participants continued standard therapy for existing acute or chronic medical conditions.

9.4.1 Identity of Investigational Products

Ampicillin is a generic therapeutic and was supplied by the site clinical pharmacy.

9.4.2 Blinding

This was an open-label study and no study treatments were blinded or masked.

9.4.3 Prior and Concomitant Therapy

Presence of the concomitant medication of interest – probenecid – within 24 hours before and/or after any dose of ampicillin closest to the time of biological sample collection was recorded.

9.4.4 Treatment Compliance

All participants were under the care of a primary physician. Therefore each dose of drug administration was given and monitored by clinical staff, then reviewed by study personnel. The prescription of drugs to participants was not part of this protocol.

9.5 Pharmacokinetic and Safety Variables

9.5.1 Pharmacokinetic Variables

The PK analysis assessed clearance (CL) and volume (V) through compartmental population modeling. As part of a secondary analysis, the proportion of simulated infants with a trough concentration above the minimum inhibitory concentration (MIC) of ≥8 mcg/mL in at least 90% of simulated participants was used for a surrogate PD endpoint in simulations. This breakpoint was used as it is the MIC per the Clinical and Laboratory Standards Institute (CLSI) needed to inhibit growth of 90% of *Escherichia coli* organisms, a common pathogenic infection in infants [22].

9.5.2 Safety Variables

AEs or serious adverse events (SAE) related to study procedures (collection of biological samples for research purposes) and serious, unexpected, suspected adverse reactions of ampicillin were the only safety variables in the NICHD-2011-POP01study.

AEs and SAEs caused by the study specimen collections were reported at each time point when the study specified biological sample collection occurred up until 1 hour after collection. AE information was gained from direct monitoring of the study subject as well as from clinician observation, and self-reporting by the study subject or his/her guardian(s).

Serious, unexpected, suspected adverse reactions of the understudied drug of interest were reported from the time of first biological sample collection until 3 days after the last biological sample was collected if infant was available.

9.5.3 Schedule of Assessments

After a participant was enrolled in the study the following information was recorded/collected on Day 1 through Day 90 or until a Termination Visit, whichever occurred first:

Sampling Information:

1. Exact date and time the sample was drawn
2. Exact date and time the sample was frozen/stored
3. Sample volume (estimated)

4. Sample type (i.e. plasma)
5. Sample acquisition site
6. Correlation to flush

Ampicillin Information:

1. Exact infusion start and end date and time of previous dose of drug of interest
2. Dose amount
3. Dose number
4. Dose frequency
5. Indication
6. Dosing weight
7. Actual weight
8. Route of administration
9. Indication

Ampicillin information for up to 8 doses prior to the sampling dose (last dose prior to first biological sample collection) was recorded.

Concomitant Medication Information (Probenecid):

1. Presence of the only concomitant medication of interest, probenecid, within 24 hours before and after the dose of drug of interest closest to the time of biological sample collection
2. Date and time probenecid administered
3. Total daily dose (per day)
4. Route of administration

Demographic Information:

1. Age
2. Gender
3. Race
4. Ethnicity
5. Current weight
6. Current height/length
7. Gestational age at birth
8. Birth weight

Clinical Information (if available):

1. Serum creatinine
2. Plasma albumin
3. Aspartate aminotransferase (AST)
4. Alanine aminotransferase (ALT)
5. Bilirubin (total and direct)

For serum creatinine, plasma albumin, AST, ALT, and bilirubin, the concentration value as well as the date of collection was obtained. The laboratory data defined above was collected only if drawn within 24 hours before or after the dose of ampicillin closest to the time of biological sample collection. If more than 1 result was available within the 24 hour time frame, the result closest to the time of the dose of ampicillin was recorded. If more than 1 biological sample was collected within a 24 hour period, one serum creatinine, plasma albumin, AST, ALT, and/or bilirubin value (closest to the time of the dose of ampicillin) was obtained. If biological samples were collected in a span of more than 1 day, above laboratory data was collected each day of biological sample collection and closest to the time of the ampicillin dose closest to the time of biological sample collection.

9.6 Biological Sample Analysis

Each participant could have had no more than 10 research-only blood samples associated with ampicillin.

A PK sampling scheme was employed such that no more than a pre-determined weight-based maximum volume of blood for samples drawn for research purposes was obtained from each subject within a 30 day period as follows: ≤1 kg, 5 mL; 2kg, 10 mL; 3 kg, 12 mL; 4 kg, 16 mL; 5 kg, 20 mL; 6 kg, 24 mL.

After enrollment, biological samples were collected using recommended sampling windows (Figure 9-1) according to the below dosing interval (see Table 9-2). Participating sites were encouraged to collect biological samples when clinical specimens were being obtained per standard of care. However, the option to consent for sample collection for research purposes was given to the parent/guardian of the participating infant.

Fresh plasma samples collected as part of this study were sent to the a central laboratory for drug concentration measurements (PK samples). Samples were labeled only with a unique accession number without protected health information (PHI) that could identify the study subject. Once samples were analyzed, data were sent to the BPCA DCC and entered into the study records.

Table 9-2 Recommended sampling windows and dosing intervals for intravenous drug administration

	Dosing Interval (Hours)							
Sample Name	**2**	**4**	**6**	**8**	**12**	**24**	**48**	**72**
Sample #1	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
Sample #2	0.15-0.5	0.5-2	1-4	2-5	2-8	3-12	3-24	3-48
Sample #3	Pre	Pre	Pre	Pre	Pre	Pre	Pre	Pre
Sample #4 (elimination)	4-8	8-12	12-18	16-24	24-36	48-72	96-144	144-216

[a]Time 0 = end of infusion; collect sample after flush* ends
Pre = within 1 hour prior to next administration of study drug; d = days

Figure 9-1 Sample collection associated with Table 9-2

* Graphic [<----------IV Dose administration ---------->][<---------- Flush---------->]
↑ ↑
Dose End Time PK Draw Time

A flow chart of study procedures is presented in Table 9-3

Table 9-3 Flow chart of study procedures.

Procedure	Day 0 Pre-Sample Collection	Day 1 Day of first sample collection	Day 1- up to Day 90/Termination Visit Day of any subsequent sample collection
Informed Consent/ Permission/Assent	X		
Infant Demographics		X	
Clinical Data[d]		X	X
Drug(s) of Interest Data	X[b]	X[a]	X[a]
Biological Sample Collection		X[a]	X[a,e]
Safety		X	X[c]

a: Procedure may be collected once on Day 1 and / or continued for up to 90 days for ampicillin according to drug frequency of administration and number of biological samples collected as per prescribing physician's standard of care treatment. Site will store biological samples until batch shipped to testing lab or storage lab.
b: Collect ampicillin information for up to 8 doses prior to the sampling dose (last dose prior to first biological sample collection).
c: Serious, unexpected, suspected adverse reactions related to ampicillin will be reported from the time of first biological sample collection until 3 days after the last biological sample is collected if infant is available.
d: May include lab collection (which could also be pre-sample collection)
e: If infant is accessible, collect 1 PK plasma sample at time of identification of serious, unexpected, suspected adverse reactions of the understudied drug of interest.

9.6.1 Drug Concentration Measurements

An assay to measure ampicillin concentrations in collected specimens was performed at a central lab, OpAns Laboratory, using a validated bio-analytical assay (See Appendix 16.4.4 for validation report).

9.7 Data Quality Assurance

An electronic case report form (eCRF) was used to record infant data in the Advantage EDC SM system. GlobalTrace[SM] was used as an electronic specimen inventory. The eCRF was used for the recording of all historical infant information and study data as specified by this protocol. The eCRF was completed by designated and trained study personnel. Certain data elements were collected on provided source documents, but were not entered into the Advantage EDC system.

Prior to the initiation of the study, the Pediatrics Trial Network selected sites that were fully capable of carrying out the study. The BPCA DCC trained the investigators, sub-investigators and their study coordinators in eCRF completion, study management tools, and regulatory compliance. The Duke Clinical Research Institute (DCRI) PI trained site staff on the protocol, performance of study procedures, and specimen collection methods. In addition to the investigators' meeting, the study personnel at each site were trained on the study procedures by a DCRI Clinical Research Association (CRA) as necessary.

EMMES monitored each site throughout the study. At each visit, data recorded in the EDC system were compared to source documentation to ensure accuracy. Quality assurance checks were performed to ensure that the investigators were complying with the protocol and all applicable regulations. The data for primary outcomes were monitored.

In addition to the quality assurance checks incorporated into the EDC system, additional logic checks were run to check for such items such as inconsistent study dates and outlying laboratory values. Any necessary corrections were made by site staff in the eCRF and documented via audit trail, prior to study data lock.

9.8 Statistical Methods and Determination of Sample Size

Only participants enrolled for ampicillin as part of the NICHD-2011-POP01 protocol are included in the analysis described in Sections 10, 11, and 12. Participants were enrolled in the GA and PNA stratums specified in Section 9.3.1. All participants with at least one available plasma sample drawn for ampicillin were eligible for the PK analysis. All enrolled participants with at least one sample drawn were eligible for the safety analysis.

9.8.1 Statistical and Analytical Plans

Table summaries are stratified by the GA and PNA groups used to stratify enrollment. For continuous variables, descriptive statistics include number of observations, mean, standard deviation, median, minimum and maximum value. Discrete variable summaries include counts and proportions. Except for PK analyses, analyses and data summaries for the NICHD-2011-POP01 protocol were produced using SAS® Software version 9.2 or later. A generic Statistical Analysis Plan (SAP) was prepared for CSRs submitted for drugs of interest analyzed as part of the NICHD-2011-POP01 protocol and can be found in Appendix 16.1.9.

9.8.1.1 Study Patient, Demographics, and Baseline Characteristics

9.8.1.1.1 Disposition of Participants and Withdrawals

All participants with at least 1 sample drawn for ampicillin were accounted for in this analysis. A summary of participant accounting and final study disposition is provided.

9.8.1.1.2 Inclusion and Exclusion criteria

Inclusion and exclusion criteria for each infant are summarized in Listing 16.2.2.

9.8.1.1.3 Protocol Violations and Deviations

Protocol deviations were reported by site and category of deviation. A detailed listing of protocol deviations by participant is included. Deviations related to samples other than fresh plasma samples are not included in these summaries. Deviations were collected from enrollment of the first infant at the site to completion of the last infant at the site and are included in the site specific protocol deviation summary.

9.8.1.1.4 Demographic and Baseline Characteristics and Data of Special Interest

Demographic and baseline variables include gender, ethnicity, race, gestational age, post natal age, height/length, and birth weight, Body Mass Index (BMI). Medical history such as diagnosis of cystic fibrosis, congenital heart disease and extracorporeal life support (ECMO) are also summarized.

9.8.1.2 Safety Analyses

An **adverse event** is any untoward medical occurrence in humans, whether or not considered drug related which occurs during the conduct of a clinical trial.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A reasonable possibility implies that there is evidence that the drug caused the event.

Adverse reaction is any adverse event caused by the drug.

A **serious adverse event** or **serious suspected adverse reaction** or **serious adverse reaction** as determined by the Investigator or the sponsor is any event that results in any of the following outcomes:

1. Death
2. Life-threatening AE (Life-threatening means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred.)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.

An unexpected adverse event is any adverse event, the specificity or severity of which is not consistent with the package insert or investigational plan or informed consent.

As all participants in this study had pre-existing medical conditions and were hospitalized, those pre-existing conditions were not considered as adverse events. AEs or serious adverse events (SAE) related to study procedures (collection of biological samples for research purposes) and serious, unexpected, suspected adverse reactions of understudied drug of interest were reportable in the data system.

9.8.1.2.1 Planned Guidelines for Assessing Intensity of an Adverse Event

AEs related to study procedures (collection of biological samples) were planned to be graded as follows:

- Mild: Participant is aware of symptoms or has minor findings, but tolerates them well and no or minimal intervention required
- Moderate: Participant experiences enough symptoms or findings to require intervention
- Severe: Participant experiences symptoms or findings that require significant intervention

9.8.1.2.2 Planned Guidelines for Determining Causality of an Adverse Event

The Investigator was to use the following question when assessing causality of an adverse event to study drug: Is there a reasonable possibility that the drug caused the event?

An affirmative answer would have designated the event as a suspected adverse reaction.

9.8.1.2.3 Adverse Event Collection Period

AEs and SAEs caused by the study specimen collections were to be reported at each time point when the study specified biological sample collection occurs up until 1 hour after collection. AE information was to be gained from direct monitoring of the study subject as well as from clinician observation, and self-reporting by the study subject or his/her guardian(s).

AEs related to study procedures was to be followed until resolution even if this extended beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Serious, unexpected, suspected adverse reactions of the understudied drug of interest were to be reported from the time of first biological sample collection until 3 days after the last biological sample is collected if subject was available.

9.8.1.3 Dosing, PK Sampling, and Laboratory values

Analysis of dosing includes summaries of dose route and strength for sampling doses associated with available PK plasma doses.

Analysis of sample collection includes the number of samples collected, number of samples in each sampling window, number collected after flush (when applicable), and a summary of time until freezing. Concentrations are summarized in tables by sampling window.

Clinical laboratory measurements are summarized. Only the closest lab measurement collected within 24 hours to the start of the sampling dose are included in the table summary. Baseline measurements were not available. All recorded values are included in listings.

9.8.1.4 Pharmacokinetic Analysis

PK models for ampicillin concentration data were explored by non-linear mixed effects modeling using NONMEM. Appropriate compartmental models were examined, and between-subject variability on model parameters was explored. Data were fit to a 1-compartment model (ADVAN1 TRANS2) with proportionate residual error using NONMEM version 7.2 and the First Order Conditional Estimation method with ETA-EPS interaction (FOCE-I). Proportional and additive residual error models were explored. Diagnostic plots were used to assess the appropriateness of this structure for the base model.

Missing weight (WTKG) and serum creatinine (SCR) values were imputed with the last recorded value carried forward. If an infant did not have serum creatinine (SCR) measured during the study period, SCR values were imputed based on NICHD-2011-POP01 population median value. Covariate analysis examined the correlation between model parameters with demographic factors. Weight (WTKG) was assumed to be a significant covariate for CL and V and was included in the base model prior to assessment of other potential covariates.

The investigation of the relationship between potential covariates and PK parameters proceeded by estimating the basic population PK model with the generation of the Bayesian individual PK parameters (e.g., CL and V). With these individual parameter estimates, their deviation from the typical population parameter values were also generated; individual subject ETAs (η). Next, graphical assessment of the relationships between PK parameters and potential covariates was performed by plotting ETAs versus potential clinically relevant covariates. Clinical variables were evaluated as potential covariates for PK parameters using a univariate screen in NONMEM followed by a multivariate assessment of the final population PK model. The following potential covariates were included in the univariable analysis: SCR, days of life (DOL, which is PNA plus 1), GA, post menstrual age (PMA), and sex. The construction of a final population PK model was done with all variables as indicated from the multivariable exploration if applicable, NONMEM univariate screen, and graphical exploration.

During the model building process, potential covariates that reduced the objective function by more than 3.84 ($p<\sim0.05$) were planned for inclusion in the subsequent multivariate analysis. A forward inclusion approach with backwards elimination was planned for the multivariate step, and a reduction of 7.88 ($p<\sim0.005$) was required for retention of a covariate in the final model.

Standard model diagnostic plots and procedures were used to evaluate model appropriateness. Empirical Bayesian estimates of individual subject PK parameters were generated from the final model. Model evaluation was performed by visual predictive check, standard visual predictive check, and bootstrapping procedures as needed. Monte Carlo simulations were performed using the final model to determine the most clinically applicable optimal dosing regimen for ampicillin divided by GA and PNA.

9.8.1.5 Analysis of Surrogate Pharmacodynamic Endpoint

A simulation-based analysis was designed to determine the dose needed to provide exposure at the trough concentration above the MIC of either 2 mcg/mL or 8 mcg/mL for at least 90% of the participants. The final compartmental population PK model was used in these simulations.

9.8.1.6 Interim Pharmacokinetic Analysis

No interim PK analysis was performed.

9.8.2 Determination of Sample Size

Due to the paucity of preliminary PK/PD data on the drugs under study, formal sample size calculations were not performed. Biological samples were scheduled to be processed for PK analysis after enrollment of approximately 64 infants for ampicillin.

9.9 Changes in the conduct of the Study or the Planned Analyses

Changes to the first version of the NICHD-2011-POP01 protocol are listed in Appendix 16.4.1. A second protocol amendment (protocol version 3.0) was finalized on November 28, 2012, after the completion of the last participant enrolled under ampicillin and is not included in the appendix.

10 STUDY PATIENTS – NICHD-2011-POP01 STUDY

10.1 Disposition of Participants

Participant accounting is summarized in Table 10-1 and in Section 14, Table 14.1.1.1. A total of 75 participants were consented for the study and had available PK samples. Participants were enrolled based on PNA and GA. One participant with a PNA of 30 days was enrolled into the study and is included in the 8-28 day group. As discussed in Section 11.4.1, this participant is not included in the PK analysis. The largest group, participants with a PNA ≤ 7 days and GA > 34 weeks, enrolled 27 participants. The smallest group, participants with a PNA of 8-28 days and GA ≤ 34 weeks, enrolled 8 participants.

Table 10-1 Participant disposition by PNA and GA group.

	PNA ≤ 7 Days		PNA = 8-28 Days		
	GA ≤ 34 weeks	*GA > 34 weeks*	*GA ≤ 34 weeks*	*GA >34 weeks*	*Total*
Number of Enrolled Participants	21	27	8	19	75
Number of Participants with At Least 1 Available Plasma Sample	21	27	8	19	75
Number of Participants with Research-Only Samples	6	14	3	5	28

All 75 enrolled participants were eligible for the safety analysis. Twenty-eight (37%) of the 75 participants had research-only samples collected and were eligible for the AE safety analysis associated with the collection of research-only samples.

As shown in Table 14.1.1.2, 42 (56%) of the 75 participants were enrolled at one site, Redacted . Four of the 9 sites enrolled 2 or fewer participants.

10.2 Protocol Deviations and Waivers

Tables 14.1.3.1-3 summarize participant specific protocol deviations. All participant specific deviations are presented in Listing 16.2.5. As seen in Table 14.1.3.1-2, 12 deviations were reported in 9 (12%) enrolled participants. Eight (67%) of the 12 deviations were related to informed consent and 1 (8%) was related to protocol procedure or assessment. Listing 16.2.5 shows that the deviation related to protocol procedure was due to a sample that was not spun in a refrigerated centrifuge. Deviations related to informed consent were due to one of the following reasons – signature by the wrong site personnel, delayed signature on the HIPAA consent form with respect to the ICF, and exclusion of HIPAA authorization forms for the substudy from the initial informed consent packet. No site specific deviations (such as sample shipping deviations) were reported for sites while enrolling ampicillin participants.

11 PHARMACOKINETIC EVALUATION

11.1 Data Sets Analyzed

A total of 75 participants were enrolled in this study under the drug of interest ampicillin. All enrolled participants had at least one available plasma PK sample. Of these 75 participants, 73 (97%) were included in the PK analysis. Reasons for removing these two participants from the analysis are provided in Section 11.4.1.

11.2 Summary of Demography, Dosing, and Data of Special Interest for Overall Study Population

11.2.1 Demographic and Other Baseline Characteristics

Summary of baseline demographics for the safety population are presented in Table 11-1 and Section 14.1.2. Out of 75 participants in the safety population, forty (53%) participants were male, 58 (77%) were of Caucasian descent and 14 (19%) were Hispanic or Latino. The median PNA was 6 days (range 0 to 30 days) and median weight was 2500 g (range 500 to 5400 g) for the total population.

Table 11-1 Summary of Demographic Characteristics

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks (N=21)	GA >34 Weeks (N=27)	GA ≤34 Weeks (N=8)	GA >34 Weeks (N=19)	Total (N=75)
Post-Natal Age at Day of First Plasma PK Sample (Days)					
N	21	27	8	19	75
Mean (SD)	2.7 (2.4)	2.9 (2.7)	14.8 (4.2)	14.4 (6.5)	7.0 (6.9)
Median (Min,Max)	2.0 (0.0,7.0)	2.0 (0.0,7.0)	14.5 (9.0, 21.0)	13.0 (8.0,30.0)	6.0 (0.0,30.0)
Gestational Age (Weeks)					
N	21	27	8	19	75
Mean (SD)	30.3 (3.4)	38.2 (2.0)	27.8 (3.4)	38.3 (1.7)	34.9 (5.0)
Median (Min,Max)	32.3 (24.3,34.0)	38.0 (34.4,41.4)	26.5 (25.0,33.9)	38.6 (35.3,41.3)	36.1 (24.3,41.4)
Gender					
Male	9 (42.9%)	18 (66.7%)	4 (50.0%)	9 (47.4%)	40 (53.3%)
Female	12 (57.1%)	9 (33.3%)	4 (50.0%)	10 (52.6%)	35 (46.7%)
Ethnicity					
Hispanic or Latino	3 (14.3%)	6 (22.2%)	2 (25.0%)	3 (15.8%)	14 (18.7%)
Not Hispanic or Latino	18 (85.7%)	19 (70.4%)	5 (62.5%)	15 (78.9%)	57 (76.0%)
Not reported	0	2 (7.4%)	1 (12.5%)	1 (5.3%)	4 (5.3%)
Race					
Black or African American	4 (19.0%)	3 (11.1%)	3 (37.5%)	2 (10.5%)	12 (16.0%)
White or Caucasian	16 (76.2%)	23 (85.2%)	4 (50.0%)	15 (78.9%)	58 (77.3%)
Not reported	0	0	0	1 (5.3%)	1 (1.3%)
Other	1 (4.8%)	0	1 (12.5%)	1 (5.3%)	3 (4.0%)

The summary of baseline medical history of participants is presented in Section 14.1.2. One participant was diagnosed with cystic fibrosis, and 2 participants were diagnosed with congenital heart disease.

11.2.2 Drug Dose

All 75 participants in this study had ampicillin administered per standard of care. Table 14.2.1 summarizes doses by PNA and GA group. Overall, the average number of recorded doses was 7.7 per participant. Thirty (40%) participants had a single sampling dose in which an available plasma PK sample was drawn after the dose. Thirty-seven (49%) participants had 2 sampling doses, and the remaining 8 (11%) participants had 3 sampling doses.

The maximum sampling dose amount for the 75 participants were distributed with a median of 99 mg/kg (range, 8-129 mg/kg). This amount was consistent across PNA and GA groups. Only one sampling dose was not administered through IV infusion. This sampling dose was administered via an intramuscular injection.

11.2.3 Data Related to the PK Analysis

No administrations of probenecid, the only concomitant medication of interest, were reported.

The distribution of lab values is summarized in Table 14.2.5. Only the closest lab measurements within 24 hours of a plasma sample are included in this summary. Participants had lab draws for serum creatinine (57 participants), total bilirubin (54 participants), direct bilirubin (28 participants), albumin (15 participants), AST (11 participants), and ALT (11 participants).

11.2.4 Sample Collection and Drug Concentration

11.2.4.1 PK Sampling

Table 14.2.2 summarizes PK sampling by PNA and GA group. One hundred sixty available plasma PK samples were collected from the 75 participants, which is an average of 2.1 per participant. Seventeen (23%) participants had more than two samples collected. All plasma samples were available and had recorded drug concentrations.

Ten (6%) plasma samples were collected within an hour before a dose, 4 (3%) were collected before the end of flush, and an additional 47 (29%) samples were not collected within a sampling window. The remaining samples were collected within one of the post-dose sampling windows defined in Table 9-2.

11.2.4.2 PK Drug Concentration

Table 14.2.3 summarizes the recorded drug concentration by PNA and GA group for each sampling window. The window with the highest median concentration was the window that includes samples taken immediately after the end of IV dose and flush. The median level in this window was 233.4 ng/mL. The highest recorded concentration was 777.1 ng/mL, which was recorded in the 1-4 hour window after a 72 mg/kg QID dose.

11.3 Measurement of Treatment Compliance

All participants were under the care of a primary treating caregiver; therefore each dose of drug administration was given and monitored by clinical staff, then reviewed by study personnel. The prescribing of drugs to children was not part of this protocol.

11.4 Summary of PK Analysis

11.4.1 Excluded Data

PK results that were excluded from the final NONMEM dataset were categorized into 4 groups and filtered from the analysis using the IGNORE function. The categories included:

1. Concentrations (DV) < 0.05 that were below BQL
2. Missing times
3. Samples drawn during infusion or flush
4. Results poorly characterized by the model with high WRES and large differences between IPRED and measured concentrations, suggesting sample contamination or other collection or dosing error.

Of 75 participants, two were excluded for the following reasons: (1) one subject had only one DV that was < 0.4 (BQL) and PNA at the time of the first PK sample was beyond the 28 day threshold, specifically 30 days; and (2) another subject received a recorded dose that was unreasonably low (i.e., 10 mg for 1290 gram participant) with a high concentration. Fourteen (9%) of 156 DV samples from the 73 participants were excluded: 6 were DV below BQL that were thought to be unreliable given the time after dose, 5 had levels drawn after 24 hrs which given the dosing interval were deemed to not be reliable, 1 had a sample drawn during infusion or flush, 1 had unusually high DV, and 1 had a sample drawn after intramuscular administration. A total of 73 participants with 142 observed drug concentrations were included in the PK analysis.

Of the 142 measured DV samples, 68 (48%) SCR values from 40 subjects were imputed using the closest SCR value available. A carry-forward or back-fill approach was used depending on which date was closest. If a subject did not have a SCR value for any time point, then the SCR was estimated using the population median value. Of the 142 measured DV samples, 62 (44%) WT values from 39 subjects were imputed using the closest value available. A carry-forward approach was primarily used to estimate WT from a preceding date.

11.4.2 Population PK Analysis

11.4.2.1 Demographics for PK Analysis Population

The median and range of demographic, baseline, and dosing variables at the time of first plasma pharmacokinetic sampling in participants with useable ampicillin concentration data are presented in Table 11-2.

Table 11-2 Demographic, baseline, and dosing summary for participants included in the population PK analysis.

Variable	Median (Range)
GA (weeks)	36 (24-41)
PNA (days)	5 (0-25)
PMA (weeks)	37 (25-43)
Weight (kg)	2.5 (0.5-5.4)
M/F	52% / 48%
SCR (mg/dL)	0.6 (0.2-2.5)
Total daily dose (mg/kg/day)	200 (50 - 350)

The total daily dose was calculated from the recorded doses of ampicillin administered to each participant. This is the maximum recorded daily dose.

11.4.3 Population PK Covariate Selection and Model Summary

One-compartmental population PK models were fit as discussed in Section 9.8.1.4. As summarized in Table 11-3, the univariable screen identified SCR and PMA as potential covariates for CL and none for V.

Table 11-3 Summary of significant steps in the ampicillin model-building process

Model	Population Model	OFV	ΔOFV
V	$V = \theta V * (WTKG)$	1284	-
CL base model	$CL = \theta CL * (WTKG)$	1284	-
GA, DOL, SCR	$CL = \theta CL * (WTKG) * EXP(\theta 2_{SCR} * (SCR - 0.6) * (GA/36)^{\theta 2\text{-}GA}) * (\theta 2_{DOL} * DOL/7)$	1364	+80.48
GA, DOL	$CL = \theta CL * (WTKG) * (GA/36)^{\theta 2\text{-}GA} * (DOL/7)^{\theta 2\text{-}DOL}$	1239	+44.83
Birth weight	$CL = \theta CL * (WTKG) * (BW/2500)^{\theta(2)\text{-}BW}$	1284	0
DOL	$CL = \theta CL * (WTKG) * (DOL/7)^{\theta(2)\text{-}DOL}$	1278	-6.108
GA	$CL = \theta CL * (WTKG) * (GA/36)^{\theta(2)\text{-}GA}$	1257	-26.81
PMA	$CL = \theta CL * (WTKG) * (PMA/37)^{\theta(2)\text{-}PMA}$	1251	-33.34
SCR	$CL = \theta CL * (WTKG) * (0.6/SCR)^{\theta(2)\text{-}SCR}$	1249	-34.85
SCR, PMA (Final)	$CL = \theta CL * (WTKG) * (0.6/SCR)^{\theta(2)\text{-}SCR} * (PMA/37)^{\theta(2)\text{-}PMA}$	1229	-55.19

The final model used the conditional estimation method with interaction (FOCE-I). The explicit formulas for the typical values of V and CL were:

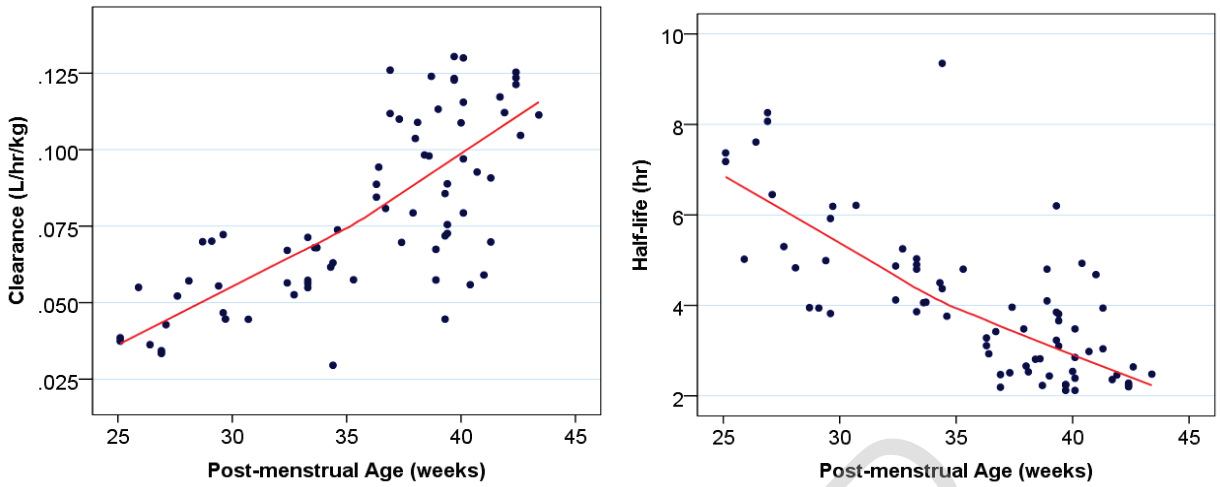
$V\ (L) = \theta_{(1)} * WTKG$

$CL\ (L/h) = \theta_{(2)} * WTKG * (0.6/SCR)^{\theta(3)}\ (PMA/37)^{\theta(4)}$

where $\theta_{(1)} = 0.399$, $\theta_{(2)} = 0.078$, $\theta_{(3)} = 0.428$, and $\theta_{(4)} = 1.34$. WTKG is weight (kg), SCR is serum creatinine (mg/dL) and PMA is post-menstrual age (weeks). The between-subject variability for CL was 23%, and the residual variability was 34%. The estimated values for the population PK parameters, covariate and variances, along with the standard error of these estimates and bootstrap medians and the 95% confidence intervals for these values, are listed in Table 11-4. The ETA shrinkage value for CL was 21% while the EPS shrinkage value for CL was 13%.

Individual subject post-hoc CL estimates appeared to increase with GA and PNA, as reflected by increasing CL with each group (i.e., Group 1 had the lowest CL and Group 4, the highest). Half-life decreased with increasing both components of PMA, GA and PNA, as would be expected with the increasing CL when V is constant (Figure 11-1).

Figure 11-1 Clearance (A) and half-life (B) vs. PMA.



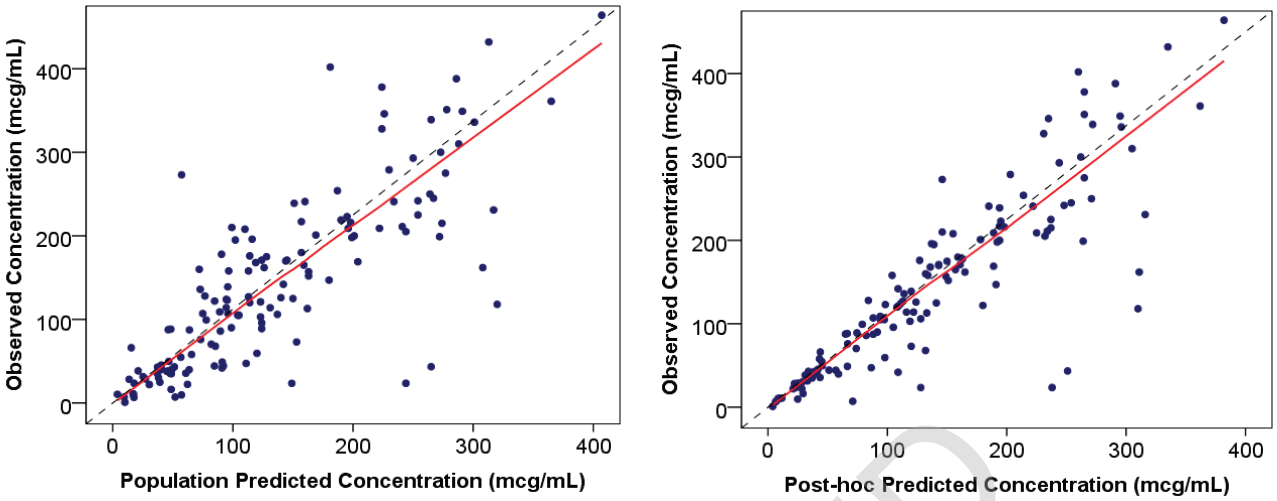
A B

Table 11-4 Final PK model parameters.

				Bootstrap CI		
Parameter	**Symbol**	**Point Estimate**	**%RSE**	**2.5%**	**Median**	**97.5%**
CL	$\theta_{(2)}$	0.078	4.37	0.071	0.077	0.084
V	$\theta_{(1)}$	0.399	6.34	0.350	0.398	0.452
CL, SCR	$\theta_{(2),SCR}$	0.428	21.40	0.235	0.433	0.639
CL, PMA	$\theta_{(2),PMA}$	1.34	23.73	0.651	1.31	1.96
CL Inter-individual variability (CV%)	ω^2_{CL}	22.8	0.07	12.1	21.9	28.7
Residual variability (CV%)	σ^2	33.9	0.08	26.6	33.5	41.4

The goodness of fit plots demonstrated that the model generally fit the observed concentrations both for the population and the individual (Figure 11-2).

Figure 11-2 Observed versus Population (A) and Individual (B) Predictions, Final Model



A B

11.4.4 Pharmacodynamic (PD) Analysis

The most pathogenic infections treated with ampicillin in neonates, who are relatively immunodeficient, are *Listeria monocytogenes* with an MIC 90 of 2 mcg/mL and *Escherichia coli* with an MIC 90 of 8 mcg/mL. *Streptococci* are also common pathogens in neonates but sensitive to ampicillin with MIC <0.5 mcg/mL. Thus, this analysis was designed to determine the dose needed to provide exposure above a trough concentration for MIC of 2 mcg/mL and 8 mcg/mL in 90% of simulated participants.

Monte Carlo simulations (N=1920) were performed using the final population PK model to determine the distribution of steady-state ampicillin concentrations from the "typical" dose selected by clinicians for each age group in this study. The typical age group dose was determined to be the average total daily dose of the group divided by the median dose interval, rounded to the nearest 25mg/kg. For Groups 1 and 2 this was 100mg/kg every 12 hours, for Group 3 it was 75mg/kg every 8 hours and for Group 4 this was 100mg/kg every 8 hours.

In addition, simulations were performed using the dose recommendation from three references that are commonly used for neonatal doses: Neofax [11], Harriet Lane [23] and Pediatric Dosage Handbook [24]. Based on the relatively high concentrations seen with the typical current study dose used, a lower dosing strategy was also evaluated. This revised dosing was: Group 1 – 50 mg/kg every 12 hours, Group 2 – 75mg every 12 hours, Group 3 – 50 mg every 8 hours and Group 4 – 75mg every 8 hours. Simulations were performed to encompass the full range of gestational and postnatal ages across all four groups. 1920 virtual subjects, 480 in each age group, were included at the following gestational ages: 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 39 and 40 weeks and at the following postnatal ages: 1, 3, 7, 10 14, 21 and 28 days. Bodyweight and serum creatinine for each cohort were from a prior trial in premature participants [25]. An additional SCR variability of 30% (beyond fixed effects of GA and PNA) was included during the NONMEM simulation by including a random effect (ETA) on SCR with a variance (OMEGA) value of 0.09 (30% CV). Median, and 95% CI values were generated for the steady-state concentration time profiles of each age group using the various dosing strategies. In addition, the frequency of predicted concentrations greater than 2 and 8 mcg/mL were determined for 50% of the dose interval, 75% of the dose interval and 100% of the dose interval (trough concentrations). All participants had predicted concentrations >2 and 8 mcg/mL for 50% and 75% of the dose interval.

With standard of care ampicillin dosing, 100% of all participants had predicted trough concentrations at steady state > 2 mcg/mL; 100% of participants in Groups 1 and 2 and 89% in Group 3 and 4 had predicted trough concentrations ≥ 8 mcg/mL. All of the participants in Groups 3 and 4 who were below the 8 mcg/mL target were dosed every 12 hours as compared to every 8 hours. Because of variability in the primary caregiver's dose selection we evaluated standardized dosing using Monte Carlo simulations. Based on the Monte Carlo simulations all 4 groups (with an average daily dose of 100 mg/kg every 12h in Groups 1 and 2; 75 mg/kg every 8h in Group 3; 100 mg/kg every 8h Group 4) had <3% of virtual participants with trough concentrations < 8 mcg/mL. In contrast, 10% of participants in at least 1 group failed to meet the surrogate PD target when dosing recommendations found in pediatric guidelines were used.

Additional Monte Carlo simulations were conducted, applying the final pharmacokinetic PMA-based model to the Pediatrix Database (N=132,966). The revised dosing regimens by GA/PNA and PMA (the latter using empirically-derived breakpoints) were simulated and compared to different pediatric dosing guidelines using the lowest recommended doses. The probabilities of target attainment for 50%, 75% and 100% of the dosing interval at MIC_90 of ≥2 and ≥8 mcg/mL were 95 to 100% based on dosing by GA/PNA or PMA. To achieve 75% of the dosing interval at the MIC_90 of ≥8 mcg/mL, ampicillin dosing recommendations from Neofax produced the lowest target attainment at 95%; however, >90% of patients achieved the surrogate efficacy target. In addition, while the target attainment was 100% based on current FDA recommendation of 25 mg/kg every 4 hours (i.e., 150-200 mg/kg/day divided q3-4 hours without delineation by GA, PNA, or PMA), the high frequency of dose administration may deter its clinical application. In all groups defined by GA and PNA, the proposed dosing regimen produced 100% target attainment for 75% of the dosing interval and >95% for 100% of the dosing interval at MIC ≥ 8 mcg/mL.

Since the final PMA-based model was mechanistically better than GA/PNA-based model with or without SCR and dosing by GA/PNA and PMA (the latter using empirically-derived thresholds for PMA and PNA) achieved similar PD target attainment, dosing regimen by GA/PNA was selected to offer the specificity needed to account for prematurity and developmental changes that occur with age, especially during the first 1 to 2 weeks of life. With the goal of achieving a trough concentration above the MIC of ≥8 mcg/mL in at least 90% of simulated infants, we were able to simplify the dosing regimens (Table 11-5) from several references and devise a simplified dosing regimen for ampicillin.

Table 11-5 Optimal dosing regimen based on PK analysis

Gestational age (weeks)	Postnatal age (days)	Maintenance dose (mg/kg)	Dosing interval (hours)
≤ 34	≤ 7	50	12
≤ 34	≥ 8 & ≤ 28	75	12
> 34	≤ 28	50	8

11.4.5 PK and Surrogate PD Discussion

Ampicillin is a commonly used drug in infants. However, the lack of pharmacokinetic studies in premature infants and lack of uniformity of dosing has led to a variety of doses being used based on factors including GA, PNA, weight and PMA. As this was an opportunistic study, the study did not control for dosing. Dosing ranged from 100-350 mg/kg/day and generally exceeded the recommended dosing in the most commonly pediatric dosing handbooks (Neofax, Harriet Lane and Pediatric Dosage Handbook). The high dose of prescribed ampicillin appears to stem from concerns for meningitis in the infants being treated.

The present study evaluated the population PK of ampicillin in 73 infants as young as 24 weeks gestation and up to 28 days postnatal age. This population PK model allowed us to characterize the CL and V of ampicillin in these infants but we were limited in looking at intra-variability because we had an average of only two samples per subject. A 1-compartment model appropriately described the data and was precise as evidenced by population CL and V point estimates nearly identical to the median bootstrap values and narrow 95% confidence intervals. A maturational change in ampicillin clearance was included in the final model through the PMA and SCR covariates. Given the low exponent value of 0.42 for SCR, it was not as important as the PMA (which is composed of PNA and GA) with an exponent value of 1.3.

The Monte Carlo simulation demonstrated that the higher dose of ampicillin currently being prescribed by most physicians, demonstrated by the average daily dose of ampicillin ordered by the primary caregiver for the infants in POPS, achieved the surrogate PD endpoint of trough concentrations at steady state ≥8 mcg/mL in >97% of virtual subjects as compared to 90% of virtual subjects with the current dosing references.

11.5 PK and Surrogate PD Conclusions

This population pharmacokinetics study of ampicillin in infants demonstrated the importance of PMA, composed of PNA and GA, in drug CL. The current dose used by most practitioners in infants appears to provide a higher dose than pharmacologically necessary. Given the goal of achieving a trough concentration above the MIC of ≥ in at least 90% of simulated infants, we were able to simplify the dosing regimens from several references and devise a simplified dosing regimen for ampicillin based on the 4 groups used in this study: 50 mg/kg every 12 hours for Group 1, 75 mg/kg every 12 hours for Group 2 and 50 mg/kg every 8 hours for Groups 3 and 4. Furthermore, although some references suggest every 6 hour dosing for some PNA and GA groups, adjusting the total dose would allow for every 8 hour dosing, simplifying the frequency of ampicillin administration.

12 SAFETY EVALUATION

Safety was primarily assessed using data from retrospective chart review of participants enrolled in a prior PPRU study and using a large epidemiological database from the Pediatrix Medical Group. Safety data collected from the NICHD-2011-POP01 study were analyzed as well.

12.1 NICHD-2011-POP01

No adverse events or reactions were reported in this study.

12.2 PPRU Safety Analysis

In this study of 68 infants exposed to ampicillin, 46 (68%) experience one of the pre-specified diagnoses. Laboratory abnormalities were less common, with 19 infants (28%) having leukocytosis, the most common laboratory abnormality.

12.3 Pediatrix Safety Analysis

Seizures occurred more often at doses ≥250 mg/kg/dose and at dosing intervals ≤6 hours. AST elevation occurred more often at higher ampicillin exposures. Leukopenia, neutropenia and thrombocytopenia occurred more often at shorter dosing intervals ≤6 hours and thrombocytopenia occurred more often at higher ampicillin exposures.

12.4 Safety Conclusions

Based on this safety analysis from three separate data sources, ampicillin was associated with few AEs. AEs were uncommon in the Pediatrix database. Some, but not all, AEs were related to higher doses, shorter dosing intervals and higher ampicillin exposures.

13 DISCUSSION AND OVERALL CONCLUSIONS

13.1 Discussion

Ampicillin is a commonly used drug in infants. However, the lack of pharmacokinetic studies in premature infants and lack of uniformity of dosing has led to a variety of doses being used based on factors including GA, PNA, weight and PMA. As the PK study was an opportunistic study, the study did not control for dosing. Dosing ranged from 100-350 mg/kg/day and generally exceeded the recommended dosing in the most commonly pediatric dosing handbooks (Neofax, Harriet Lane and Pediatric Dosage Handbook). The high dose of prescribed ampicillin appears to stem from concerns for meningitis in the infants being treated.

The present PK study evaluated the population PK of ampicillin in 73 infants as young as 24 weeks gestation and up to 28 days postnatal age. This population PK model allowed us to characterize the CL and V of ampicillin in these infants but we were limited in looking at intra-variability because we had an average of only two samples per subject. A 1-compartment model appropriately described the data and was precise as evidenced by population CL and V point estimates nearly identical to the median bootstrap values and narrow 95% confidence intervals. A maturational change in ampicillin clearance was included in the final model through the PMA and SCR covariates. Given the low exponent value of 0.42 for SCR, it was not as important as the PMA (which is composed of PNA and GA) with an exponent value of 1.3.

The Monte Carlo simulation demonstrated that the higher dose of ampicillin currently being prescribed by most physicians, demonstrated by the average daily dose of ampicillin ordered by the primary caregiver for the infants in POPS, achieved the surrogate PD endpoint of trough concentrations at steady state >8 mcg/ml in >97% of virtual subjects as compared to 90% of virtual subjects with the current dosing references. As the goal was to achieve a trough concentration above the MIC of ≥8 mcg/ml in at least 90% of the infants, we devised a dosing regimen that simplifies the current dosing references.

13.2 Overall Conclusions

The population pharmacokinetics study of ampicillin in infants demonstrated the importance of PMA, composed of PNA and GA, in drug CL. The current dose used by most practitioners in infants appears to provide a higher dose than pharmacologically necessary. We were able to simplify the dosing regimens from several references and devise a simplified dosing regimen for ampicillin based on the 4 groups used in this study: 50 mg/kg every 12 hours for Group 1, 75 mg/kg every 12 hours for Group 2 and 50 mg/kg every 8 hours for Groups 3 and 4 (Table 11-5). This achieved a trough concentration above the MIC of ≥8 mcg/ml in at least 90% of the infants. Furthermore, although some references suggest every 6 hour dosing for some PNA and GA groups, adjusting the total dose would allow for every 8 hour dosing, simplifying the frequency of ampicillin administration.

The safety analysis shows that ampicillin is generally safe. Leukopenia, neutropenia and thrombocytopenia increased in incidence with shorter dosing intervals. Seizures were more common with higher doses and shorter dosing intervals.

14 TABLES REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 Study Disposition, Baseline Participant Characteristics, and Protocol Deviation Summary Tables (NICHD-2011-POP01)

14.1.1.1 Participant Accounting and Final Study Disposition by Age Group All Enrolled Ampicillin Participants

14.1.1.2 Participant Enrollment by Site and Age Group All Enrolled Ampicillin Participants

14.1.2 Demographic, Baseline, and Medical History Characteristics by Age Group All Enrolled Ampicillin Participants

14.1.3.1 Summary of Participant Specific Protocol Deviations by Site All Enrolled Ampicillin Participants

14.1.3.2 Number of Participants with Protocol Deviations by Site All Enrolled Ampicillin Participants

14.1.3.3 Reasons Indicated for Participant Specific Protocol Deviations Across All Sites All Enrolled Ampicillin Participants

14.2 Dosing and PK Collection Summary Tables (NICHD-2011-POP01)

14.2.1 Fresh Plasma Sampling Dose Summary by Age Group All Enrolled Ampicillin Participants

14.2.2 PK Sample Summary by Age Group All Enrolled Ampicillin Participants

14.2.3 Summary of the PK Concentration (ng/mL) by Sampling Window and Age Group All Enrolled Ampicillin Participants

14.2.4 Concomitant Medications by Age Group Safety Ampicillin Population

14.2.5 Clinical Laboratory Test Summary by Age Group All Enrolled Ampicillin Participants

14.3 Safety Tables (NICHD-2011-POP01)

14.3.1.1 Summary of Adverse Events by Age Group Safety Ampicillin Population

Table 14.1.1.1
Participant Accounting and Final Study Disposition by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks (N=21)	**GA >34 Weeks (N=27)**	**GA ≤34 Weeks (N=8)**	**GA >34 Weeks (N=19)**	**Total (N=75)**
Number of Enrolled Participants	21	27	8	19	75
Number of Participants with At Least 1 Available Plasma Sample	21	27	8	19	75
Number of Participants with Research-Only Samples	6	14	3	5	28
Number of Participants Enrolled Under Multiple DOIs at Time of Data Lock	1	2	3	1	7
Number of Participants Enrolled Under Each DOI					
Clindamycin	1	1	3	1	6
Metoclopramide	0	1	0	0	1

Note: One participant was enrolled with 30 days PNA.

Table 14.1.1.2
Participant Enrollment by Site and Age Group
All Enrolled Ampicillin Participants

Site	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		Total
	GA ≤34 Weeks (N=21)	GA >34 Weeks (N=27)	GA ≤34 Weeks (N=8)	GA >34 Weeks (N=19)	(N=75)
Redacted	0	0	0	1 (100.0%)	1 (100.0%)
Redacted	0	0	0	1 (100.0%)	1 (100.0%)
Redacted	1 (50.0%)	0	0	1 (50.0%)	2 (100.0%)
Redacted	14 (33.3%)	16 (38.1%)	4 (9.5%)	8 (19.0%)	42 (100.0%)
Redacted	0	0	0	2 (100.0%)	2 (100.0%)
Redacted	0	4 (50.0%)	0	4 (50.0%)	8 (100.0%)
Redacted	3 (33.3%)	2 (22.2%)	3 (33.3%)	1 (11.1%)	9 (100.0%)
Redacted	2 (40.0%)	1 (20.0%)	1 (20.0%)	1 (20.0%)	5 (100.0%)
Redacted	1 (20.0%)	4 (80.0%)	0	0	5 (100.0%)

Note: N's in the column header represent number of Ampicillin enrolled participants in each age group for all sites.
The denominator is the total number of participants at the site.

Table 14.1.2
Demographic, Baseline, and Medical History Characteristics by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks (N=21)	GA >34 Weeks (N=27)	GA ≤34 Weeks (N=8)	GA >34 Weeks (N=19)	Total (N=75)
Post-Natal Age at Day of First Plasma PK Sample (Days)					
N	21	27	8	19	75
Mean (SD)	2.7 (2.4)	2.9 (2.7)	14.8 (4.2)	14.4 (6.5)	7.0 (6.9)
Median (Min-Max)	2.0 (0.0 - 7.0)	2.0 (0.0 - 7.0)	14.5 (9.0 - 21.0)	13.0 (8.0 - 30.0)	6.0 (0.0 - 30.0)
Gestational Age (Weeks)					
N	21	27	8	19	75
Mean (SD)	30.3 (3.4)	38.2 (2.0)	27.8 (3.4)	38.3 (1.7)	34.9 (5.0)
Median (Min-Max)	32.3 (24.3 - 34.0)	38.0 (34.4 - 41.4)	26.5 (25.0 - 33.9)	38.6 (35.3 - 41.3)	36.1 (24.3 - 41.4)
Gender					
Male	9 (42.9%)	18 (66.7%)	4 (50.0%)	9 (47.4%)	40 (53.3%)
Female	12 (57.1%)	9 (33.3%)	4 (50.0%)	10 (52.6%)	35 (46.7%)
Ethnicity					
Hispanic or Latino	3 (14.3%)	6 (22.2%)	2 (25.0%)	3 (15.8%)	14 (18.7%)
Not Hispanic or Latino	18 (85.7%)	19 (70.4%)	5 (62.5%)	15 (78.9%)	57 (76.0%)
Not reported	0	2 (7.4%)	1 (12.5%)	1 (5.3%)	4 (5.3%)
Race					
Black or African American	4 (19.0%)	3 (11.1%)	3 (37.5%)	2 (10.5%)	12 (16.0%)
White or Caucasian	16 (76.2%)	23 (85.2%)	4 (50.0%)	15 (78.9%)	58 (77.3%)
Not reported	0	0	0	1 (5.3%)	1 (1.3%)
Other	1 (4.8%)	0	1 (12.5%)	1 (5.3%)	3 (4.0%)
More Than 1 Race	0	1 (3.7%)	0	0	1 (1.3%)

Table 14.1.2
Demographic, Baseline, and Medical History Characteristics by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks (N=21)	GA >34 Weeks (N=27)	GA ≤34 Weeks (N=8)	GA >34 Weeks (N=19)	Total (N=75)
Weight at first sample collection (gm)					
N	21	27	8	19	75
Mean (SD)	1407 (580.1)	3208 (739.2)	1088 (399.7)	3181 (768.5)	2471 (1139)
Median (Min-Max)	1460 (460.0 - 2600)	3385 (2001 - 5420)	1110 (660.0 - 1874)	3000 (1900 - 4450)	2500 (460.0 - 5420)
Height/length at time of first sample collection (cm)					
N	21	27	8	19	75
Mean (SD)	38.7 (6.3)	49.6 (5.0)	35.2 (3.9)	48.7 (3.6)	44.8 (7.5)
Median (Min-Max)	40.0 (23.5 - 48.0)	49.5 (39.0 - 67.2)	35.5 (30.0 - 41.0)	48.0 (41.5 - 55.0)	47.0 (23.5 - 67.2)
Diagnosis of cystic fibrosis					
Yes	0	1 (3.7%)	0	0	1 (1.3%)
No	21 (100.0%)	26 (96.3%)	8 (100.0%)	19 (100.0%)	74 (98.7%)
Diagnosis of congenital heart disease					
Yes	0	0	1 (12.5%)	1 (5.3%)	2 (2.7%)
No	21 (100.0%)	27 (100.0%)	7 (87.5%)	18 (94.7%)	73 (97.3%)
Extracorporeal Life Support					
No	21 (100.0%)	27 (100.0%)	8 (100.0%)	19 (100.0%)	75 (100.0%)

Table 14.1.3.1
Summary of Participant Specific Protocol Deviations by Site
All Enrolled Ampicillin Participants

Site	Informed Consent /Assent	Protocol Procedure /Assessment	Other	Total
Total	**8**	**1**	**3**	**12**
Redacted	0	0	1	1
Redacted	2	0	0	2
Redacted	0	1	2	3
Redacted	6	0	0	6

Note: Includes all deviations for participants enrolled under Ampicillin.

Table 14.1.3.2
Number of Participants with Protocol Deviations by Site
All Enrolled Ampicillin Participants

Site	Informed Consent	Protocol Procedure/Assessment	Other	Total Participants with Deviations
Total	**5 (6.7%)**	**1 (1.3%)**	**3 (4.0%)**	**9 (12.0%)**
Redacted	0	0	1 (50.0%)	1 (50.0%)
Redacted	2 (25.0%)	0	0	2 (25.0%)
Redacted	0	1 (11.1%)	2 (22.2%)	3 (33.3%)
Redacted	3 (60.0%)	0	0	3 (60.0%)

Note: Denominator is the number of participants enrolled at each site or total. Includes all deviations for participants enrolled under Ampicillin.

Table 14.1.3.3.1
Reasons Indicated for Participant Specific Protocol Deviations Across All Sites
All Enrolled Ampicillin Participants

All Sites (N=75)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**6**	**9**	**4**	**4**	**10**	**13**
Informed consent/assent	2	2	2	2	5	8
Informed consent/assent	3	6	2	2	5	8
Protocol procedure/assessment	1	1	2	2	2	2
Other	3	3	0	0	3	3

Note: N=Number of participants. Includes all deviations for participants enrolled under Ampicillin.

Table 14.1.3.3.2
Reasons Indicated for Participant Specific Protocol Deviations by Site
All Enrolled Ampicillin Participants

Redacted (N=42)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**0**	**0**	**1**	**1**	**1**	**1**
Protocol procedure/assessment	1	1	1	1	1	1

Redacted (N=2)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**1**	**1**	**0**	**0**	**1**	**1**
Other	1	1	0	0	1	1

Redacted (N=8)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**0**	**0**	**2**	**2**	**2**	**2**
Informed consent/assent	2	2	2	2	2	2

Redacted (N=9)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**2**	**2**	**1**	**1**	**3**	**3**
Protocol procedure/assessment	0	0	1	1	1	1
Other	2	2	0	0	2	2

Note: N=Number of participants at site. Includes all deviations for participants enrolled under Ampicillin.

Table 14.1.3.3.2
Reasons Indicated for Participant Specific Protocol Deviations by Site
All Enrolled Ampicillin Participants

Redacted (N=5)	Research staff/clinic error		Other		Total	
Deviation Category	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**	**# of Participants**	**# of Deviations**
Total	**3**	**6**	**0**	**0**	**3**	**6**
Informed consent/assent	3	6	0	0	3	6

Note: N=Number of participants at site. Includes all deviations for participants enrolled under Ampicillin.

Table 14.2.1
Fresh Plasma Sampling Dose Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Dose Summary					
Number of Sampling Doses	34	51	12	31	128
Average Number of Recorded Doses Per Participant	5.9	7.6	8.6	9.3	7.7
Number of Sampling Doses Per Participant					
1	9 (42.9%)	7 (25.9%)	5 (62.5%)	9 (47.4%)	30 (40.0%)
2	11 (52.4%)	16 (59.3%)	2 (25.0%)	8 (42.1%)	37 (49.3%)
3	1 (4.8%)	4 (14.8%)	1 (12.5%)	2 (10.5%)	8 (10.7%)
Dose Amount: All Sampling Doses (mg/kg)					
N	34	51	12	31	128
Mean (SD)	98.6 (12.6)	92.7 (14.9)	79.3 (25.4)	86.0 (15.2)	91.4 (16.6)
Median (Min-Max)	99.7 (50.0 - 128.6)	100.0 (43.1 - 106.1)	86.1 (7.8 - 100.0)	92.1 (46.2 - 100.8)	99.0 (7.8 - 128.6)
Dose Amount: Maximum Sampling Dose Per Participant (mg/kg)					
N	21	27	8	19	75
Mean (SD)	98.7 (14.9)	92.2 (15.9)	78.6 (31.7)	85.8 (15.1)	90.9 (18.5)
Median (Min-Max)	100.0 (50.0 - 128.6)	100.0 (46.1 - 106.1)	92.3 (7.8 - 100.0)	87.5 (46.2 - 100.8)	99.3 (7.8 - 128.6)
Dosing Frequency: All Sampling Doses					
QID (6 hours)	0	0	0	14 (45.2%)	14 (10.9%)
TID (8 hours)	4 (11.8%)	27 (52.9%)	0	11 (35.5%)	42 (32.8%)
BID (12 hours)	29 (85.3%)	24 (47.1%)	11 (91.7%)	6 (19.4%)	70 (54.7%)
QD (24 hours)	1 (2.9%)	0	1 (8.3%)	0	2 (1.6%)

Table 14.2.1
Fresh Plasma Sampling Dose Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Administration Route: All Sampling Doses					
IV - Intravenous	34 (100.0%)	51 (100.0%)	12 (100.0%)	30 (96.8%)	127 (99.2%)
IM - Intramuscular	0	0	0	1 (3.2%)	1 (0.8%)

Table 14.2.2
PK Sample Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Number PK Samples					
Number of Available Fresh Plasma PK Samples	46	60	16	38	160
Number of Non-Plasma PK Samples	32	98	20	28	178
Number of Available Fresh Plasma PK Samples Per Participant					
1	2 (9.5%)	2 (7.4%)	3 (37.5%)	6 (31.6%)	13 (17.3%)
2	15 (71.4%)	19 (70.4%)	2 (25.0%)	9 (47.4%)	45 (60.0%)
3	2 (9.5%)	4 (14.8%)	3 (37.5%)	2 (10.5%)	11 (14.7%)
4	2 (9.5%)	2 (7.4%)	0	2 (10.5%)	6 (8.0%)
Number of Available Fresh Plasma PK Samples in Each Sampling Window					
After IV Dose/Flush	14 (30.4%)	14 (23.3%)	3 (18.8%)	7 (18.4%)	38 (23.8%)
1-4 Hours	0	0	0	6 (15.8%)	6 (3.8%)
2-5 Hours	2 (4.3%)	10 (16.7%)	0	2 (5.3%)	14 (8.8%)
2-8 Hours	12 (26.1%)	8 (13.3%)	5 (31.3%)	5 (13.2%)	30 (18.8%)
3-12 Hours	1 (2.2%)	0	1 (6.3%)	0	2 (1.3%)
Elimination: 24-36 Hours	4 (8.7%)	4 (6.7%)	0	1 (2.6%)	9 (5.6%)
Window 3: Pre-Dose	3 (6.5%)	5 (8.3%)	1 (6.3%)	1 (2.6%)	10 (6.3%)
Drawn Before End of Flush	1 (2.2%)	2 (3.3%)	0	1 (2.6%)	4 (2.5%)
Out-of-Window	9 (19.6%)	17 (28.3%)	6 (37.5%)	15 (39.5%)	47 (29.4%)

Note: Sampling windows are presented in the CSR.

Table 14.2.2
PK Sample Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Time Until Frozen for Evaluable Fresh Plasma PK Samples (minutes)					
N	46	60	16	38	160
Mean (SD)	35.1 (13.7)	31.5 (11.3)	35.4 (20.2)	42.7 (38.0)	35.6 (22.2)
Median (Min-Max)	33.0 (15.0 - 77.0)	27.0 (15.0 - 60.0)	30.0 (16.0 - 80.0)	31.0 (15.0 - 236.0)	30.0 (15.0 - 236.0)
Samples with Freeze Time > 60 Minutes	2 (4.3%)	0	2 (12.5%)	6 (15.8%)	10 (6.3%)

Note: Sampling windows are presented in the CSR.

Table 14.2.3
Summary of the PK Concentration (ng/mL) by Sampling Window and Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
After IV Dose/Flush					
N	14	14	3	7	38
Mean (SD)	273.4 (130.6)	241.0 (101.5)	123.7 (76.7)	210.3 (53.0)	238.1 (109.8)
Median (Min-Max)	304.9 (43.4 - 463.6)	233.4 (23.6 - 387.9)	125.7 (46.1 - 199.4)	214.5 (125.3 - 278.8)	233.4 (23.6 - 463.6)
1-4 Hours					
N	0	0	0	6	6
Mean (SD)				204.7 (283.0)	204.7 (283.0)
Median (Min-Max)				118.8 (40.0 - 777.1)	118.8 (40.0 - 777.1)
2-5 Hours					
N	2	10	0	2	14
Mean (SD)	244.1 (130.1)	151.2 (42.4)		122.9 (47.9)	160.4 (63.9)
Median (Min-Max)	244.1 (152.2 - 336.1)	161.7 (99.3 - 238.8)		122.9 (89.0 - 156.8)	157.4 (89.0 - 336.1)
2-8 Hours					
N	12	8	5	5	30
Mean (SD)	175.4 (63.8)	92.0 (39.4)	177.0 (138.8)	62.6 (20.3)	134.6 (83.8)
Median (Min-Max)	189.3 (23.5 - 254.1)	84.4 (54.8 - 176.5)	142.2 (35.6 - 402.5)	58.0 (40.0 - 90.1)	111.1 (23.5 - 402.5)
3-12 Hours					
N	1	0	1	0	2
Mean (SD)	194.7		17.4		106.0 (125.4)
Median (Min-Max)	194.7		17.4		106.0 (17.4 - 194.7)

Table 14.2.3
Summary of the PK Concentration (ng/mL) by Sampling Window and Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Elimination: 24-36 Hours					
N	4	4	0	1	9
Mean (SD)	8.7 (7.8)	6.7 (12.6)		0.2	6.8 (9.5)
Median (Min-Max)	8.9 (0.4 - 16.5)	0.4 (0.3 - 25.6)		0.2	0.5 (0.2 - 25.6)
Pre-Dose					
N	3	5	1	1	10
Mean (SD)	76.1 (37.1)	25.2 (15.9)	16.3	7.1	37.8 (33.9)
Median (Min-Max)	86.0 (35.0 - 107.3)	31.5 (6.6 - 42.7)	16.3	7.1	33.2 (6.6 - 107.3)
Drawn Before End of Flush					
N	1	2	0	1	4
Mean (SD)	104.6	196.4 (48.6)		199.6	174.2 (54.3)
Median (Min-Max)	104.6	196.4 (162.0 - 230.8)		199.6	180.8 (104.6 - 230.8)
Out-of-Window					
N	9	17	6	15	47
Mean (SD)	98.8 (71.1)	60.8 (68.6)	100.6 (78.7)	48.5 (51.4)	69.2 (66.8)
Median (Min-Max)	119.7 (0.2 - 208.2)	28.8 (0.1 - 273.0)	102.3 (4.3 - 177.8)	38.1 (0.4 - 209.7)	43.0 (0.1 - 273.0)

Table 14.2.4
Concomitant Medications by Age Group
Safety Ampicillin Population

No concomitant medications were reported.

Table 14.2.5
Clinical Laboratory Test Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
Number of Participants with Lab Measures					
Serum Creatinine	21 (100.0%)	19 (70.4%)	4 (50.0%)	13 (68.4%)	57 (76.0%)
Albumin	3 (14.3%)	5 (18.5%)	1 (12.5%)	6 (31.6%)	15 (20.0%)
AST	3 (14.3%)	3 (11.1%)	1 (12.5%)	4 (21.1%)	11 (14.7%)
ALT	3 (14.3%)	3 (11.1%)	1 (12.5%)	4 (21.1%)	11 (14.7%)
Direct Bilirubin	13 (61.9%)	7 (25.9%)	2 (25.0%)	6 (31.6%)	28 (37.3%)
Total Bilirubin	21 (100.0%)	22 (81.5%)	3 (37.5%)	8 (42.1%)	54 (72.0%)
Serum Creatine (mg/dL)					
N	24	24	7	15	70
Mean (SD)	0.9 (0.4)	0.5 (0.2)	0.9 (0.3)	0.4 (0.2)	0.7 (0.3)
Median (Min-Max)	0.8 (0.5 - 2.5)	0.5 (0.2 - 1.0)	0.8 (0.5 - 1.2)	0.3 (0.2 - 0.8)	0.6 (0.2 - 2.5)
Albumin (g/dL)					
N	3	5	1	7	16
Mean (SD)	2.3 (0.3)	3.2 (0.6)	3.6	2.8 (0.5)	2.9 (0.6)
Median (Min-Max)	2.2 (2.0 - 2.6)	3.4 (2.3 - 3.8)	3.6	2.9 (2.0 - 3.6)	2.9 (2.0 - 3.8)
AST (U/L)					
N	3	3	1	5	12
Mean (SD)	30.7 (12.6)	21.7 (3.1)	24.0	59.6 (58.1)	39.9 (39.7)
Median (Min-Max)	29.0 (19.0 - 44.0)	21.0 (19.0 - 25.0)	24.0	44.0 (13.0 - 161.0)	27.0 (13.0 - 161.0)

Note: Only includes the closest clinical laboratory test to start of each sampling dose associated with available fresh plasma PK samples. Labs collected within 24 hours of dose start.

Table 14.2.5
Clinical Laboratory Test Summary by Age Group
All Enrolled Ampicillin Participants

	Post-Natal Age ≤7 Days		Post-Natal Age 8-28 Days		
	GA ≤34 Weeks	GA >34 Weeks	GA ≤34 Weeks	GA >34 Weeks	Total
ALT (U/L)					
N	3	3	1	5	12
Mean (SD)	7.0 (1.7)	8.0 (2.0)	11.0	18.8 (8.0)	12.5 (7.5)
Median (Min-Max)	8.0 (5.0 - 8.0)	8.0 (6.0 - 10.0)	11.0	19.0 (8.0 - 30.0)	9.0 (5.0 - 30.0)
Direct Bilirubin (mg/dL)					
N	15	8	2	7	32
Mean (SD)	0.4 (0.3)	0.5 (0.6)	0.2 (0.3)	0.4 (0.3)	0.4 (0.4)
Median (Min-Max)	0.4 (0.0 - 0.9)	0.3 (0.1 - 2.1)	0.2 (0.0 - 0.4)	0.4 (0.0 - 0.7)	0.4 (0.0 - 2.1)
Total Bilirubin (mg/dL)					
N	26	25	3	9	63
Mean (SD)	5.7 (1.6)	7.7 (3.7)	3.1 (1.2)	5.0 (5.2)	6.2 (3.4)
Median (Min-Max)	6.0 (1.5 - 8.1)	7.3 (3.3 - 17.6)	2.8 (2.1 - 4.4)	2.6 (0.9 - 16.3)	6.0 (0.9 - 17.6)

Note: Only includes the closest clinical laboratory test to start of each sampling dose associated with available fresh plasma PK samples. Labs collected within 24 hours of dose start.

Table 14.3.1.1
Summary of Adverse Events by Age Group
Safety Ampicillin Population

No AEs or SAEs were reported.

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16 APPENDICES

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