Study Title: Ampicillin Pharmacokinetics and Safety in Infants
***Study Drug:** Ampicillin
IND Number: **113,645**

Report Date: April 20, 2014
Prepared by: Redacted

Redacted

Redacted

11-24-2014
Date

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

Redacted

Date

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0714

Redacted

Date

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

Danny Benjamin, MD, PhD, MPH
Study Principal Investigator
Duke Clinical Research Institute
2400 Pratt Street
Durham, NC 27715

Date

Study Title: Ampicillin Pharmacokinetics and Safety in Infants
***Study Drug:** Ampicillin
IND Number: **113,645**

Report Date: April 20, 2014
Prepared by: Redacted

Redacted
University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

Date

Redacted

Redacted
University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0714

November 20, 2014
Date

Redacted
University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

Date

Danny Benjamin, MD, PhD, MPH
Study Principal Investigator
Duke Clinical Research Institute
2400 Pratt Street
Durham, NC 27715

Date

Study Title: Ampicillin Pharmacokinetics and Safety in Infants
***Study Drug:** Ampicillin
IND Number: **113,645**

Report Date: April 20, 2014
Prepared by: Redacted

Redacted _____ Date

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

Redacted _____ Date

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0714

Redacted

11/24/2014
Date

Redacted

University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0831

_____ Date

Danny Benjamin, MD, PhD, MPH
Study Principal Investigator
Duke Clinical Research Institute
2400 Pratt Street
Durham, NC 27715

Study Title: Ampicillin Pharmacokinetics and Safety in Infants
***Study Drug:** Ampicillin
IND Number: **113,645**

Report Date: April 20, 2014
Prepared by: Redacted

Redacted
Date
University of California–San Diego
9500 Gilman Drive Redacted
La Jolla, CA 92093-0831

Redacted
Date
University of California–San Diego
9500 Gilman Drive, Redacted
La Jolla, CA 92093-0714

Redacted
Date
University of California–San Diego
9500 Gilman I Redacted
La Jolla, CA 92093-0831

Redacted
11/21/2014
Date
Danny Benjamin, MD, PhD, MPH
Study Principal Investigator
Duke Clinical Research Institute
2400 Pratt Street
Durham, NC 27715

TABLE OF CONTENTS

List of In-Text Tables

List of In-Text Figures

1. LIST OF FIGURES IN APPENDIX

Figure	Description
1	GA distribution
2	PNA distribution
3	PMA distribution
4	Box plot PNA by group
5	Box plot PMA by group
6	Box plot GA by group
7	WTKG vs. PNA
8	SCR all vs. PMA
9	SCR 1ST obs per subject vs. PMA
10	POPS AMP timed plasma sample concentration distribution
11	POPS AMP timed plasma sample collection time distribution
12	POPS AMP time concentration profile
13	POPS AMP time concentration profile – semi-log scale
14	POPS AMP time concentration profile – semi-log scale Group 1
15	POPS AMP time concentration profile – semi-log scale Group 2
16	POPS AMP time concentration profile – semi-log scale Group 3
17	POPS AMP time concentration profile – semi-log scale Group 4
18	DV-POPS AMP vs. PRED scatter – base*
19	DV-POPS AMP vs. IPRED scatter – base*
20	CWRES vs. TAFD scatter – base
21	CWRES vs. PRED scatter – base
22	LN (DV-POPS AMP/IPRED) vs. TAD scatter – base*
23	LN (DV-POPS AMP/IPRED) vs. IPRED scatter – base
24	LN (DV-POPS AMP/PRED) vs. TAD scatter – base*
25	DV-POPS AMP vs. PRED scatter – final*
26	DV-POPS AMP vs. IPRED scatter – final*
27	CWRES vs. TAFD scatter – final
28	CWRES vs. PRED scatter – final
29	LN (DV-POPS AMP/IPRED) vs. TIME scatter – final*
30	LN (DV-POPS AMP/IPRED) vs. IPRED scatter – final

Figure	Description
31	LN (DV-POPS AMP/PRED) vs. TIME scatter – final*
32	Visual predictive check – final*
33	Standardized visual predictive check plot by dose – final*
34	Standardized visual predictive check plot by time – final*
35	Standardized visual predictive check plot by post-menstrual age – final
36	Standardized visual predictive check plot by weight – final
37	Standardized visual predictive check plot by serum creatinine – final
38	POPS AMP CL (WTKG) distribution by group – final
39	POPS AMP CL (WTKG) vs. PNA scatter – final
40	POPS AMP CL (WTKG) vs. PMA scatter – final
41	POPS AMP CL (WTKG) vs. GA scatter – final
42	POPS AMP CL (WTKG) vs. PNA by GA group scatter – final
43	POPS AMP CL (WTKG) vs. GA by PNA group scatter – final
44	POPS AMP CL (WTKG) vs. GA and PNA 3-D scatter – final
45	POPS AMP CL (WTKG) vs. GA and PMA 3-D scatter – final
46	POPS AMP CL (WTKG) vs. SCR scatter – final
47	POPS AMP CL ETA distribution – final
48	POPS AMP CL ETA vs. PNA – final*
49	POPS AMP CL ETA vs. PMA – final*
50	POPS AMP CL ETA vs. GA – final
51	POPS AMP CL ETA vs. SCR – final
52	POPS AMP CL ETA vs. birth weight – final
53	POPS AMP CL ETA vs. WTKG – final
54	POPS AMP CL box plot by gender – final
55	POPS AMP CL box plot by race – final
56	POPS AMP CL box plot by ethnicity – final
57	POPS AMP V (WTKG) box plot by group – final
58	POPS AMP V (WTKG) vs. PNA scatter – final
59	POPS AMP V (WTKG) vs. GA scatter – final
60	POPS AMP V (WTKG) vs. PMA scatter – final
61	POPS AMP V (WTKG) vs. PNA by GA group scatter – final
62	POPS AMP V (WTKG) vs. GA by PNA group scatter – final*

Figure	Description
63	POPS AMP V (WTKG) vs. SCR scatter – final
64	POPS AMP HFLF box plot by group – final
65	POPS AMP HFLF vs. PNA scatter – final
66	POPS AMP HFLF vs. GA scatter – final
67	POPS AMP HFLF vs. PMA scatter – final*
68	POPS AMP HFLF vs. SCR scatter – final
69	Cmin box plot (by group) – final
70	CWRES distribution – final

* Also presented in this report.

2. LIST OF TABLES IN APPENDIX

Table	Title
1	Ampicillin demographics/baseline evaluations*
2	Ampicillin measured and predicted conc overall and by age group
3	Ampicillin measured and predicted conc by TAD
4	Ampicillin pop PK base model population parameter estimates and variance
5	Ampicillin pop PK final model population parameter estimates and variance*
6	Ampicillin posthoc individual parameters – by age group*
7	Ampicillin posthoc exposure thresholds
8	Ampicillin simulation exposure thresholds
9	Ampicillin pop PK model building*
10	Ampicillin model validation – BS performance
11	Ampicillin model validation – CI*
12	PK sample collection strategy*

* Also presented in this report.

3. LIST OF LISTINGS IN APPENDIX

Number	Listing Title
1	Subjects with timed PK samples – demographics
2	Timed PK samples – ampicillin concentrations/final model pred, IPRED
3	Post-hoc individual PK parameters
4	Model validation – BS results parameters and variances
5	Excluded samples

4. LIST OF ABBREVIATIONS

Abbreviation	Definition
BPCA_DCC	Best Pharmaceutical Children's Act Data Coordinating Center
BQL	Concentration below quantitative limit
$Cmin_{SS}$	Trough concentrations at steady-state
$Cmax_{SS}$	Peak concentrations at steady-state
CI	Confidence interval
CL	Clearance
CLSI	Clinical and Laboratory Standards Institute
CV%	Coefficient of variance
DOL	Days of life (PNA +1)
DV	Dependent Variable
ETA	Inter-subject variability
FOCE-I	First order conditional estimation with interaction
GA	Gestational age at birth (weeks)
HCT	Hematocrit
IPRED	Individual predicted concentration
LC/MS/MS	Liquid chromatography–mass spectrometry
LN	Natural log
LNIRES	Natural log of (measured concentration/individual predicted conc) LN (DV/IPRED)
MIC	Minimum inhibitory concentration
MOF	Minimum objective function
PD	Pharmacodynamics
PK	Pharmacokinetics
PMA	Postmenstrual age (weeks)
PNA	Postnatal age (days)
POPS	Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care (NICHD-POP01-2012)
PTN	Pediatric Trials Network
PRED	Population predicted concentration
SCR	Serum creatinine (mg/dL)
$T_{1/2}$	Half-life

Abbreviation	Definition
TAD	Time after dose
TAFD	Time after first dose
V	Volume of distribution
CWRES	Conditional weighted residuals
WTKG	Weight (kg)

5. POPULATION PHARMACOKINETIC ANALYSIS

5.1. Summary

A population pharmacokinetics (PK) analysis was performed on the PK samples. 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 infants in the study exceeded the most common dosing references (Neofax, Harriet Lane, and Pediatric Drug Dosing). Subjects were divided into 4 groups stratified by gestational age (GA; ≤34 weeks or >34 weeks) and postnatal age (PNA; ≤7 days or ≥8 days).

Overall, a total of 159 ampicillin plasma concentrations in 75 infants were collected. Of these, 142 ampicillin plasma concentrations in 73 infants were available 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). The base model was developed and used to screen for extreme outlier concentrations (>10-fold difference from predicted concentration); outliers were identified and some excluded from the model. Weight (WTKG) was assumed to be a significant covariate for clearance (CL) and volume of distribution (V) 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. The following potential covariates were included in this analysis: serum creatinine (SCR), day of life (DOL, which is PNA plus 1), GA, postmenstrual age (PMA), and sex. Missing WTKG and SCR values were imputed with the last recorded value carried forward. If a participant did not have SCR measured during the study period, SCR values were imputed based on POPS population median value. During the model-building process, potential covariates that reduced the objective function by more than 3.84 (p<~0.05) 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<~0.005) was required for retention of a covariate in the final model. Empiric Bayesian estimates of individual subject PK parameters were generated from the final model using the post-hoc subroutine. Monte Carlo simulations (N=1920) were performed using the final model to determine the most clinically applicable optimal dosing regimen for ampicillin divided by GA and PNA (Table 1). The goal was to achieve a trough concentration above the minimum inhibitory concentration (MIC)_90 of ≥8 mcg/ml in at least 90% of the participants.

Table 1: 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

The median and range at the time of first plasma PK sampling in infants with useable ampicillin concentration data are presented in Table 2.

Table 2: Demographics at the time of first plasma PK sampling

Variable	Median (range)
GA (weeks)	36 (24–41)

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

The univariable screen identified SCR and PMA as potential covariates for CL and none for V. In the final irreducible model, PMA and SCR were retained as significant for CL. The final population model is:

$V\ (L) = \theta_{(1)} * WTKG$
$CL\ (L/h) = \theta_{(2)} * WTKG * (0.6/SCR)^{\theta\ (3)} (PMA/37)^{\theta\ (4)}$

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%. The post-hoc Bayesian clearances increased and half-life ($t_{1/2}$) decreased with each advancing maturation group. The median (range) post-hoc PK parameters by group are presented in Table 3.

Table 3: Individual empiric Bayesian post-hoc parameter estimates*

Group	N	CL (L/h/kg)	V (L/kg)	Half-life (h)	$Cmin_{ss}$ (mcg/mL)	$Cmax_{ss}$ (mcg/mL)
1	21	0.055 (0.03–0.07)	0.40 (0.40–0.40)	5.0 (3.9–9.4)	77 (36–320)	318 (244–563)
2	7	0.070 (0.03–0.07)	0.40 (0.40–0.41)	4.0 (3.8–8.3)	33 (21–145)	266 (159–368)
3	27	0.086 (0.04–0.13)	0.40 (0.40–0.40)	3.2 (2.2–6.2)	48 (5–173)	274 (127–413)
4	18	0.11 (0.06–0.13)	0.40 (0.40–0.41)	2.4 (2.1–4.7)	28 (5–129)	246 (138–203)
Overall	**73**	**0.072 (0.03–0.13)**	**0.40 (0.40–0.41)**	**3.3 (2.1–9.4)**	**47 (5–320)**	**281 (127–563)**

*Median (range).

Predicted steady-state trough ($Cmin_{ss}$) ampicillin concentrations were highest among infants in Group 1, followed by Group 3, Group 2, and Group 4, respectively. The sample size for Group 2 was small relative to the other groups. 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 *Escheria coli* with an MIC_90 of 8 mcg/ml, based on Clinical and Laboratory Standards Institute (CLSI) breakpoints (1). *Streptococci* are also common pathogens in neonates but sensitive to ampicillin with MIC_90 <0.5 mcg/ml, and thus this analysis was designed to determine the dose needed to provide exposure above MIC of 2 mcg/ml and 8 mcg/ml for the 50%, 75%, and the entire dose interval for at least 90% of the participants. With standard-of-care ampicillin dosing, 100% of all infants had predicted trough concentrations at steady state >2 mcg/ml; 100% of infants in Groups 1 and 2 and 89% in Groups 3 and 4 had predicted trough concentrations > 8 mcg/ml. All of the infants 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 infants with trough concentrations < 8 mcg/ml. In contrast, 10% of infants in at least 1 group failed to

meet the surrogate pharmacodynamic (PD) target when dosing recommendations found in pediatric guidelines were used.

5.2. Introduction

Ampicillin is approved by the U.S. Food and Drug Administration for infections of the digestive, genitourinary, and respiratory tract systems. Ampicillin belongs to the beta-lactam antibiotic class that possesses antimicrobial activity against bacterial pathogens responsible for early-onset sepsis in young (<91 days) infants. The PK of ampicillin has been studied in children and adults, but data on dosing in the neonatal population are sparse (2–8). 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 (GA not specified) and showed that the serum half-life of ampicillin decreases rapidly in the first 2 weeks of life as a result of increasing clearance. Doses studied ranged from 25–150 mg/kg, administered every 8–12 hours according to PNA (4,5,7,). A later study assessed the PK of ampicillin in 142 preterm infants with a 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 (9). 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 (10). However, the data available in the literature are insufficient to support dosing of ampicillin in the most extreme premature infants (≤32 weeks GA at birth).

In infants, the ampicillin blood concentrations after administration of intramuscular ampicillin were measured in 34 infants (PNA from 3–108 days and PMA from 33–54 weeks) who received ampicillin (50 mg/kg/day divided every 6 hours); elimination $t_{1/2}$ ranged from 0.96–4.08 hours and was inversely related to PMA (8). A PK study of intravenous ampicillin in 28 children (1–12 years of age) receiving ampicillin and sulbactam for intra-abdominal infections and peri-orbital or facial cellulitis showed that the mean total CL, steady-state distribution volume, $t_{1/2}$, and C_{max} were 4.76 ml/min/kg, 0.32 liter/kg, 0.77h, and 200 mcg/ml, respectively (3).

Ampicillin demonstrates time-dependent PD. Clinical antimicrobial effects are observed when concentrations exceed the MIC for at least 30–40% of the dose interval in immunocompetent adults; for neutropenic subjects, levels should exceed the MIC for >75–100% of the dose interval. Neonates are relatively immunocompromised. The PK from this study can be used to assess dosing regimens that would meet a clinically relevant exposure to ampicillin.

6. OBJECTIVES, HYPOTHESES, ASSUMPTIONS

The purpose of this study is to evaluate the PK and safety profile of ampicillin administered to infants per standard of care by their treating caregiver. The specific PK aims of this trial were to characterize ampicillin multiple-dose PK in infants with suspected serious infection from the POPS study. We hypothesize that ampicillin CL will increase with indicators of maturation (i.e., PNA, PMA) and decrease with increasing SCR.

7. MATERIALS AND METHODS

7.1. Overview

POPS was a multi-center (N=9), prospective, PK, and safety study of ampicillin in preterm infants ≤28 days of age for the treatment of suspected serious infection. Infants received ampicillin per standard of care as administered by their treating caregiver; the prescribing of drugs to infants was not part of this protocol. The infants were stratified by GA (≤34 and>34 weeks) and PNA (≤7 and ≥8–28 days) to provide therapeutic exposure in the majority of infants. Safety was assessed in real time by the BPCA DCC's safety surveillance team and medical monitor, per the PTN-POPS protocol. Population PK was performed on 73 of the 75 infants who were enrolled. The infants in this trial received at least 1 day of therapy with ampicillin.

7.2. Data

7.2.1. Subjects

All infants with evaluable PK samples and who received ampicillin while enrolled in the POPS study were included in the PK analysis. Infants enrolled in the study were required to meet the following inclusion criteria: PNA <28 days at the time of first dose; participant must be receiving ampicillin as standard of care ; availability and willingness of the parent/legally authorized representative to provide written informed consent. For analysis, subjects were divided into 4 groups based on GA and PNA as presented in Table 4.

Table 4: POPS groups by GA and PNA

N	**21**	7	**27**	**18**
Group	1	2	3	4
Definition	GA ≤ 34 weeks	GA ≤ 34 weeks	GA > 34 weeks	GA > 34 weeks
	PNA ≤ 7 days	PNA ≥ 8 & ≤ 28 days	PNA ≤ 7 days	PNA ≥ 8 & ≤ 28 days

7.2.2. Ampicillin Dosing

Infants received ampicillin per standard of care as administered by their treating caregiver.

7.2.3. PK Sampling

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

The table below provides the optimal plasma sampling collection windows according to the dosing interval. These sample collection windows are relative to the end of the infusion and after the flush. The parent or guardian had the option as part of the informed consent process to give permission for just blood draws at the same time of standard-of-care laboratories or separate blood draws just for PK sampling. Given the opportunistic nature of this study, most infants only had 2 PK samples drawn out of the sample times presented in Table 5.

Table 5: Optimal plasma sampling collection windows

Sample #	6 hours	8 hours	12 hours
1	0	0	0
2	1–4	2–5	2–8
3	Pre	Pre	Pre
4 (elimination)	12–18	16–24	24–36

Pre = within 1 hour prior to administration of ampicillin.

7.2.4. Ampicillin Concentration Determination

Plasma samples were analyzed for total ampicillin concentrations by using a validated liquid chromatography method with tandem mass spectrometric detection (LC/MS/MS). Assay details are reported in the assay validation report.

7.3. Equations Employed in This Study

Standard PK models and equations incorporated into NONMEM ADVAN1 and TRANS 2 subroutines were used in this analysis. The elimination $t_{1/2}$ and other PK parameters from the empiric Bayesian analysis were calculated as:

$$Ke=CL/V$$

$$T_{1/2}= 0.693*V/CL$$

$$Cmax_{ss}=((Dose/t_{in})/CL) * (1-e^{(-Ke*tin)}) * (1/1-e^{(-Ke*tau)})$$

(where t_{in} is infusion duration and tau is the dosing interval)

$$Cmin_{ss}= Cmax_{ss} * e^{(-Ke*T- tin)}$$

7.4. Description of Software

The concentration-time data were modeled using NONMEM version 7.2 (ICON; Ellicott City, MD, USA). Diagnostic plots were executed in PLT Tools 4.6.5 (PLTSoft; San Francisco, CA) and R Project 2.15.1 (University of California, Los Angeles, CA). The bootstrap was performed using WINGS for NONMEM version 7.2 (Auckland, NZ), and 1000 bootstrap sample datasets were generated. R Project 2.15.1 (University of California, Los Angeles, CA) and SPSS version 21 (IBM; Chicago, Illinois) were used to generate PK tables, figures, and listings.

7.5. PK End Points

The following PK parameters were estimated:

1. Plasma clearance (CL)
2. Volume of distribution (V)

The plasma concentration-time profiles of ampicillin are presented in tabular and graphical form by subject and age group level. The relationship between plasma concentrations and/or PK parameters with clinical characteristics (DOL [equivalent to PNA + 1], GA, PMA, SCR, and hematocrit [HCT]) and co-administered medications was evaluated. Missing WTKG and SCR values were imputed with the last recorded value carried forward. If a participant did not have SCR measured during the study period, SCR values were imputed based on PMA using the original data.

7.6. Model Building

A 1-compartment model with proportional residual error was chosen to describe ampicillin plasma PK. Diagnostic plots were used to assess the appropriateness of this structure for the base model. Once the

base model was identified, covariates were investigated for their influence on PK parameters such as CL and V.

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 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.

In a final step, the irreducible model was identified. Covariates that did not lead to a significant reduction in the minimum objective function (MOF) were eliminated from the model. This covariate assessment was repeated until the removal of any covariate increased the MOF by at least 7.88, which is equivalent to retaining covariates at the $p < \sim 0.005$ level. The critical value for significance was set at 0.005 to account for the multiplicity and the asymptotic approach of the test statistic (MOF change) to the chi-square distribution. In the final model, the variability for V shrunk to 0 and therefore was not estimated.

Covariates investigated for inclusion in the ampicillin PK model were GA, DOL, PMA, and SCR. Weight was included as a covariate for V and CL in the base model. The final model predictive performance (or validation) was evaluated by the bootstrap technique, visual predictive check, and standardized visual predictive check.

7.7. Monte Carlo Simulation

Monte Carlo simulations 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, Harriet Lane and Lexicomp's Pediatric Dosage Handbook. 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 – 75 mg 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 infants (11). 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. Median and 95% confidence interval (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%, 75%, and 100% of the dose interval (trough concentrations).

Additional Monte Carlo simulations were conducted, applying the final PK PMA-based model to infants from the Pediatrix database (N=132,966) meeting demographic and covariate characteristics within the range of those used to build the population PK model. The revised dosing regimens by GA/PNA and PMA (the latter using empirically derived breakpoints) were simulated and compared with different

pediatric dosing guidelines using the lowest recommended doses. The dosing references used in these simulations were: FDA (via Micromedex), Neofax, Harriet Lane, Lexicomp's Pediatric Dosage Handbook, and Red Book by the American Academy of Pediatrics. The probabilities of target attainment for 50%, 75%, and 100% of the dosing interval at MIC_90 of ≥2 and ≥8 mcg/mL were determined.

8. POPULATION PK RESULTS

8.1. Data Included

Data used in this analysis was provided by the EMMES Corporation and represent data sent to Duke University on December 21, 2012 (AMP_NMRAW_005). All ampicillin concentrations were incorporated into the NONMEM raw data input file. 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:

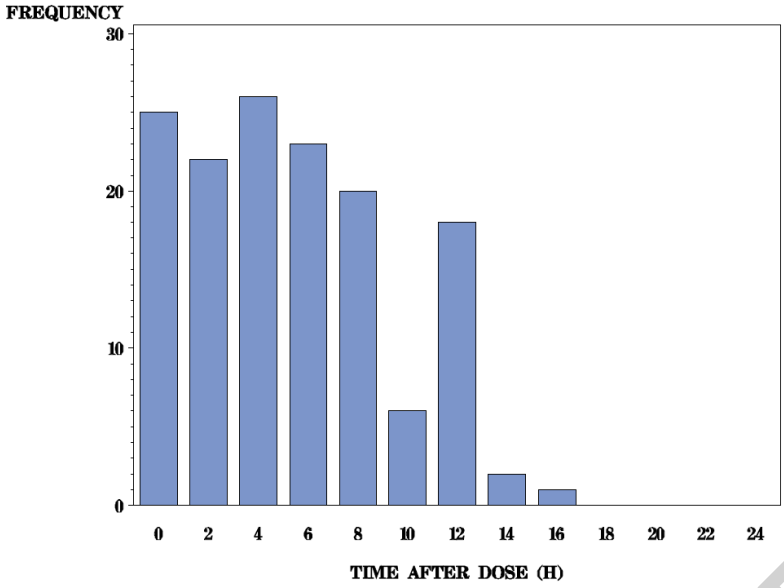
1. DV <0.05 that was below BQL
2. Missing time
3. Samples drawn during infusion or flush
4. Results poorly characterized by the model with high weighted residuals (WRES) and large differences between individual predicted concentrations (IPRED) and measured concentrations, suggesting sample contamination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

Of 75 infants, 2 were excluded for the following reasons: (1) 1 subject had only 1 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 infant) 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 hours, 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 sample drawn after intramuscular administration.

The final data were integrated in a dataset titled AMP_NMRAW_006, which was used in the population PK model-building. A total of 73 infants with 142 observed drug concentrations were included. The median (range) time of PK sampling in infants with useable ampicillin concentration data was 4.8 (0.2, 15.2) hours after the last dose, and the median (range) observed concentration was 123 (0.85, 464) mcg/ml.

The time after dose graph (Figure 1) demonstrates that we had relatively consistent sampling through the dosing interval with little information during the washout period of the drug.

Figure 1: Time after dose



Subjects: The number of infants in Groups 1–4 were 21, 7, 27, and 18, respectively, with a total of 73 infants. The overall median and range of key clinical characteristics of the study population are presented in
Table 6.

Table 6: Key clinical study characteristics of the study population

Group	N	GA (weeks)	PNA (days)	PMA (weeks)	Weight (kg)	Sex (% male)	SCR (mg/dL)
1	21	32 (24–34)	1 (0–7)	32 (25–34)	1.4 (0.5–2.6)	43	0.8 (0.6–2.5)
2	7	26 (25–32)	16 (9–21)	28 (27–35)	1.1 (0.7–1.9)	43	0.6 (0.5–1.2)
3	27	38 (34–41)	2 (0–7)	39 (34–42)	3.4 (2.0–5.4)	67	0.6 (0.2–1.3)
4	18	39 (35–41)	12.5 (8–25)	40 (36–43)	2.9 (1.9–4.5)	44	0.5 (0.2–0.8)
Overall	73	36 (24–41)	5 (0–25)	37 (25–43)	2.5 (0.5–5.4)	52	0.6 (0.2–2.5)

The typical POPS dose was determined by the median total daily dose (rounded to 25 mg increments) divided by the median dosing interval (Table 7).

Table 7: Ampicillin as prescribed by primary physician

Group	N	Daily dose (mg/kg/day)	Amount per dose (mg/kg)	Dosing interval (h)	Typical POPS dose
1	21	200 (161–303)	100 (81–109)	19% every 8 h 81% every 12 h	100 mg/kg q12h
2	7	185 (113–194)	93 (57–97)	100% every 12 h	100 mg/kg q12h
3	27	218 (100–307)	100 (43–102)	59% every 8 h 41% every 12 h	75 mg/kg q8h

Group	N	Daily dose (mg/kg/day)	Amount per dose (mg/kg)	Dosing interval (h)	Typical POPS dose
4	18	282 (184–350)	92 (46–100)	44% every 6 h 28% every 8 h 28% every 12 h	100 mg/kg q8h
Overall	73	200 (100–350)	98 (43–109)	11% every 6 h 34% every 8 h 55% every 12 h	100 mg/kg q12h

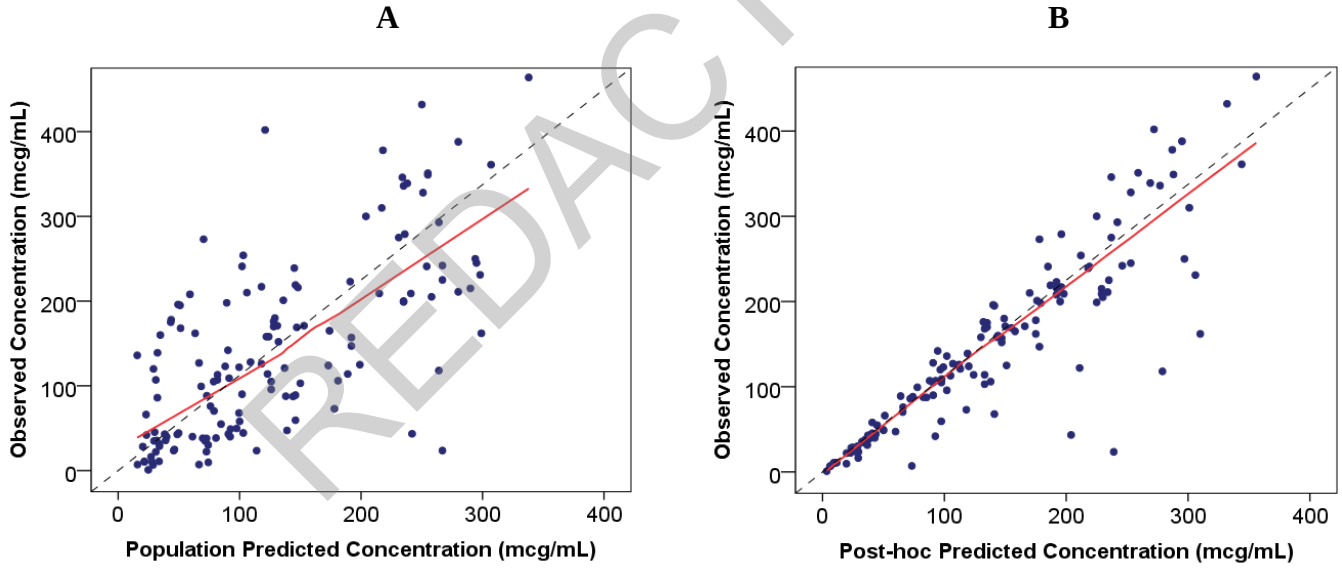
8.2. Basic Model Identification

A 1-compartment model with proportional error was found to describe the data adequately.

8.3. Goodness-of-Fit Assessments

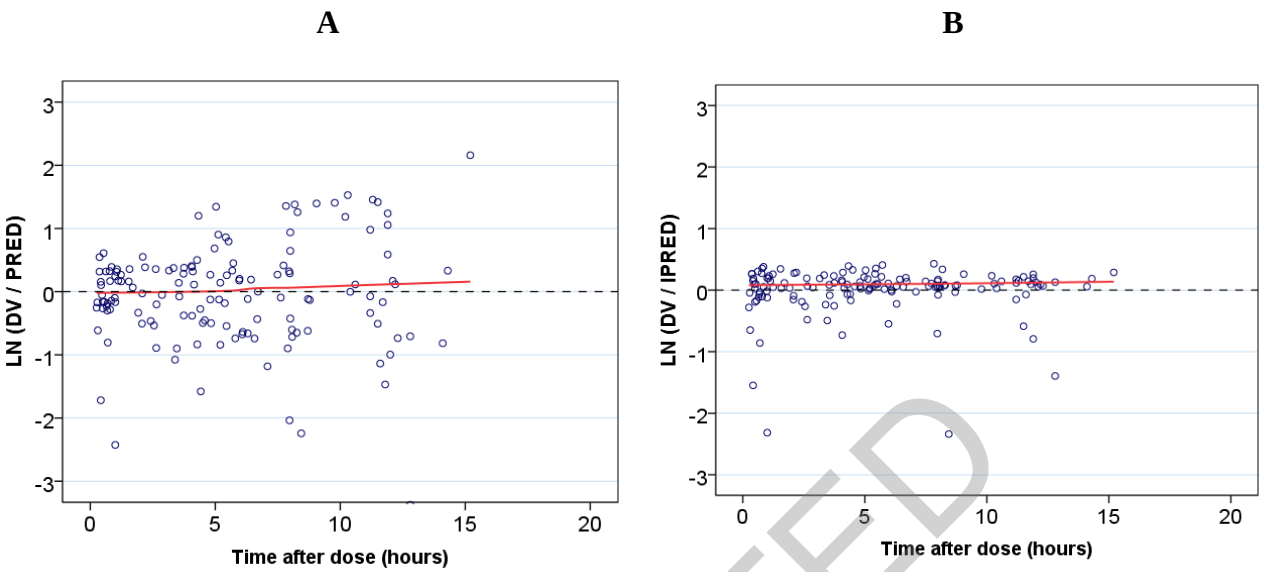
General model appropriateness was assessed by comparing observed concentrations with population predicted (PRED) and IPRED (Figure 2). The black segmented line represents the line of unity, and the red line 90% fit Loess.

Figure 2: Observed versus population (A) and individual (B) predictions, base model (AMP_101_006)



Plots of the weighted residual versus the predicted concentration were also assessed. The weighted fitted diagnostics used to assess the model were: LN (DV/PRED) (Figure 3) and LN (DV/IPRED), where the natural log (LN) of the ratio of the observed concentration (DV) is divided by the predicted concentrations (PRED or IPRED). This diagnostic is symmetrical about the horizontal line=0 for observed concentrations that are between 50% to double of predicted (represented with LN[DV/IPRED] values of 0.693 and -0.693, respectively). The black segmented horizontal straight line is the zero reference line, and the red line 90% fit Loess.

Figure 3: Diagnostic plots of population (A) and individual (B) predictions vs. time, base model (AMP_101_006)

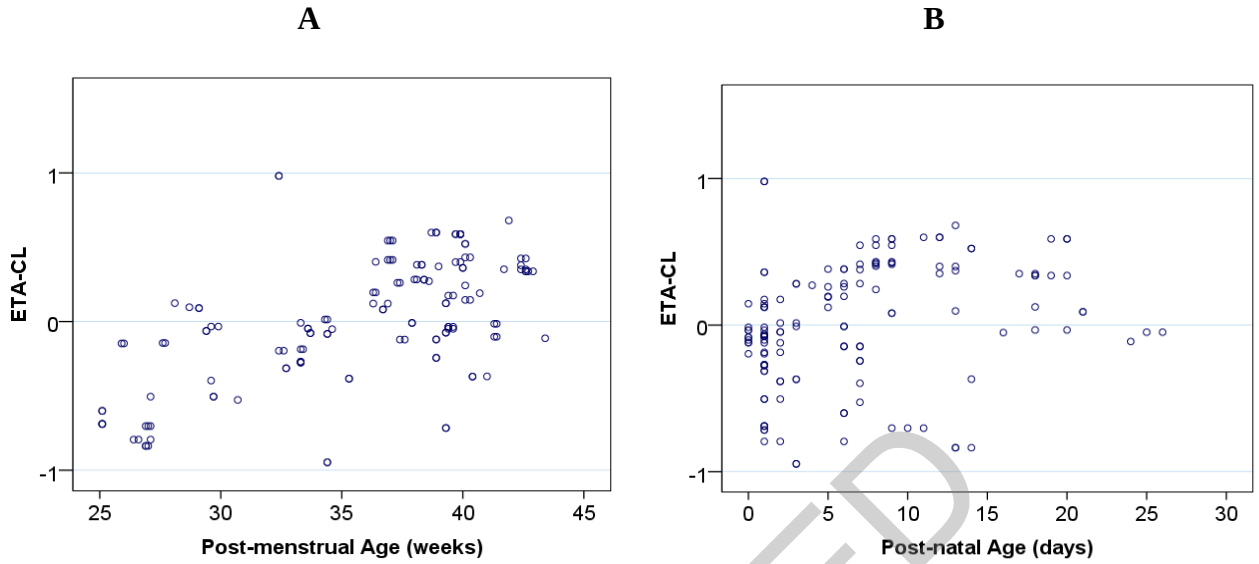


The base ampicillin population PK model had data evenly scattered about the zero reference line in the plot versus time after dose, indicating no bias in predictions at various times during the dose interval.

8.4. Covariate Selection

Multiple plots were used to determine which covariates would be assessed for inclusion in the final model. Figure 4 and Figure 5 were used to help in deciding on the potential covariates. ETA clearance, the individual deviation from the population-typical value for CL, and ETA volume of distribution, the individual deviation from the population-typical value for volume of distribution, were plotted against potential covariates. (Note that these ETA values are not exactly the difference between the subject individual value and the population value but are directly proportional to those differences). When these deviations were plotted versus a potential covariate, equal distribution around the horizontal line at zero across all values of the potential covariate indicated the lack of an association between the PK parameter and potential covariate. If the plot had a systematic upward or downward trend, that was taken as an indication that the potential covariate should be tested for inclusion in the PK model. For covariates that warranted assessment in the population PK model, the systematic pattern of ETA values versus the covariate was reduced after inclusion of the covariate into the population PK model.

Figure 4: ETA clearance vs. PMA and PNA



The plots of PMA (A) and PNA (B) versus ampicillin ETA clearance from the base model were highly suggestive of clearance changes associated with a maturational component (Figure 4).

Competing ampicillin models, their covariates, and their objective function are presented in Table 8. PK modeling was conducted to compare PMA- and GA/PNA-(or GA/DOL)-based models. Mechanistically, the final PMA-based model was better than GA/PNA-based model with or without SCR. A complete summary of all model-building steps can be found in the Appendix 16.4.4.2 (Table 9).

Table 8: 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	$CL = \theta CL * (WTKG) * (BW/2500)^{\theta(2),BW}$	1284	0
DOL	$CL = \theta CL * (WTKG) * (DOL/7)^{\theta(2),DOL}$	1278	-6.108
GA	$CL = \theta CL * (WTKG) * (GA/36)^{\theta(2),GA}$	1257	-26.81
PMA	$CL = \theta CL * (WTKG) * (PMA/37)^{\theta(2),PMA}$	1251	-33.34
SCR	$CL = \theta CL * (WTKG) * (0.6/SCR)^{\theta(2),SCR}$	1249	-34.85
SCR, PMA (Final)	$CL = \theta CL * (WTKG) * (0.6/SCR)^{\theta(2),SCR} * (PMA/37)^{\theta(2),PMA}$	1229	-55.19

8.5. The Final Irreducible Model

The final model (Table 9) used the conditional estimation method with interaction (FOCE-I). The explicit code for the typical values of V and CL was:

$V\ (L) = \theta_{(1)} * WTKG$
$CL\ (L/h) = \theta_{(2)} * WTKG * (0.6/SCR)^{\theta\,(3)} (PMA/37)^{\theta\,(4)}$

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 postmenstrual 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% CIs for these values, are listed below. The ETA shrinkage value for CL was 21% while the EPS shrinkage value for CL was 13%.

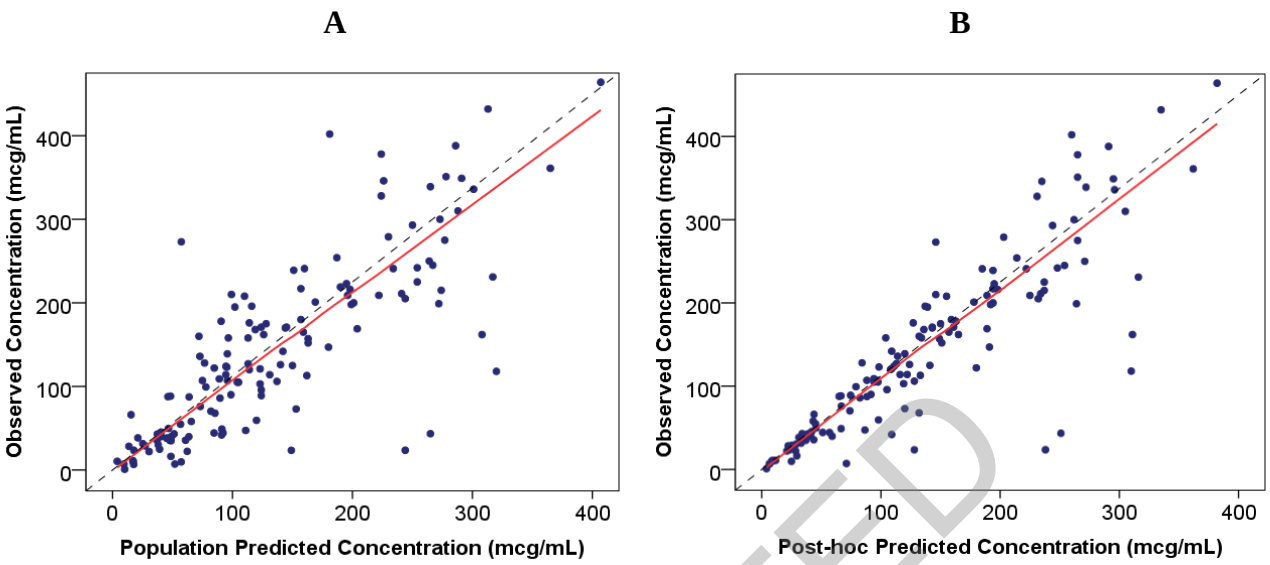
In addition to the current weight-based (linear weight) scaling, we also evaluated allometric scaling with a fixed component of 0.75 for CL and 1.0 for V. The MOF for the model got worse (increased by 2.494), while the covariate effect (THETA estimate) for SCR did not change significantly and the impact of the PMA effect was reduced. We also assessed an allometric model allowing the exponents to be determined by the data. This required 2 extra THETAS and made a small insignificant change in the MOF (dropped 0.267) relative to the final model based on weight. In addition, the estimates for the fitted exponents were close to 1.0, at 0.94 and 1.02, with 95% CIs that included 1.0.

Table 9: Ampicillin final PK model, AMP_102_006, 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
Inter-individual variance (CV%)						
CL	ω^2_{CL}	22.8	0.07	12.1	21.9	28.7
Residual variance (CV%)	σ^2	33.9	0.08	26.6	33.5	41.4

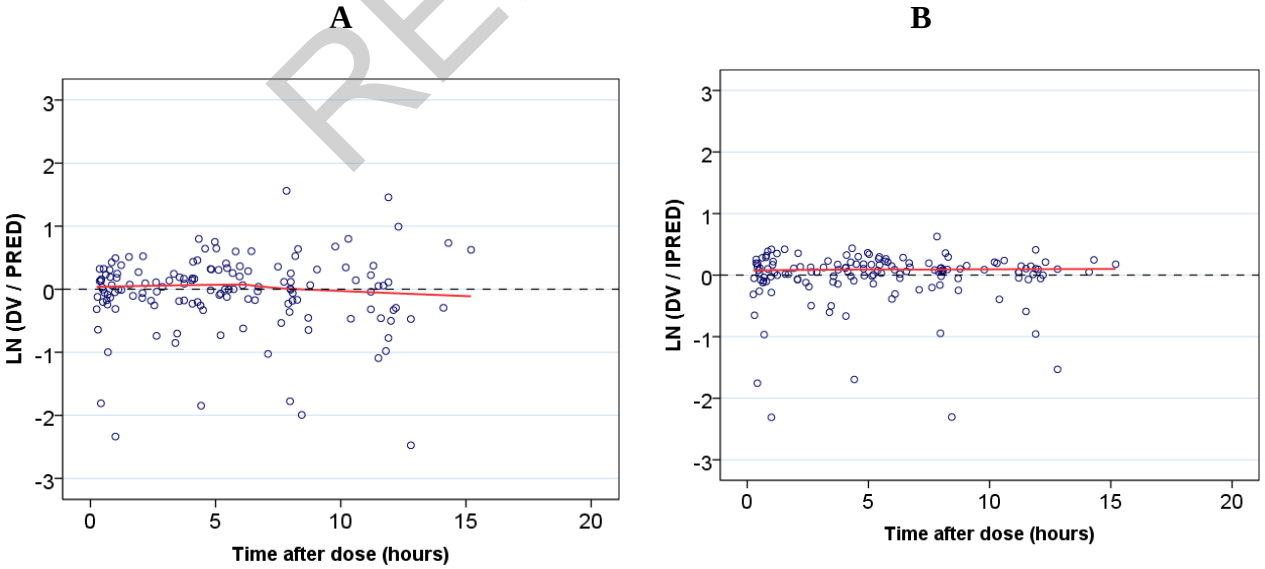
Predicted versus observed concentrations of the final model are presented in Figure 5.

Figure 5: Observed versus population (A) and individual (B) predictions, final model (AMP_102_006)



Plots of the weighted residual versus the time and individual predicted concentrations are presented in Figure 6. The segmented line represents the line of unity, and the red line 90% fit Loess. In a few cases, the predicted concentration was much higher than the observed. This occurred specifically in records where the sample was taken <1 hour after the dose was completed. It is possible that, in these samples, an error in sampling time would lead to this discrepancy.

Figure 6: Weighted residuals of population (A) and individual (B) predictions vs. time, final model (AMP_102_006).



The black segmented horizontal straight line is the zero reference line, and the red line 90% fit Loess. For the final model, predictions were randomly scattered across the zero reference line, and no trends were observed with increasing time. The plots also show improvement in the model fit of observed vs. population predicted concentrations after incorporation of the SCR and PMA covariates, and indicate that the model is without significant bias.

The median empiric Bayesian post-hoc parameter estimate for CL and V were 0.072 L/hr/kg and 0.40 L/kg, respectively (Table 3). 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). Individual subject post-hoc V estimates comparing THETAs were similar between the groups. 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 7). Differences in CL and V were observed at GA and PNA thresholds of 34 weeks and 7 days, respectively (

Figure 7). Furthermore, the most striking change in renal function due to an increase in renal blood flow occurs within the first week of life; thus, the PNA threshold of 7 days was used in the PK analysis and subsequent Monte Carlo simulations to determine the optimal dosing regimen (12,13).

Figure 7: Clearance (A) and half-life (B) vs. PMA

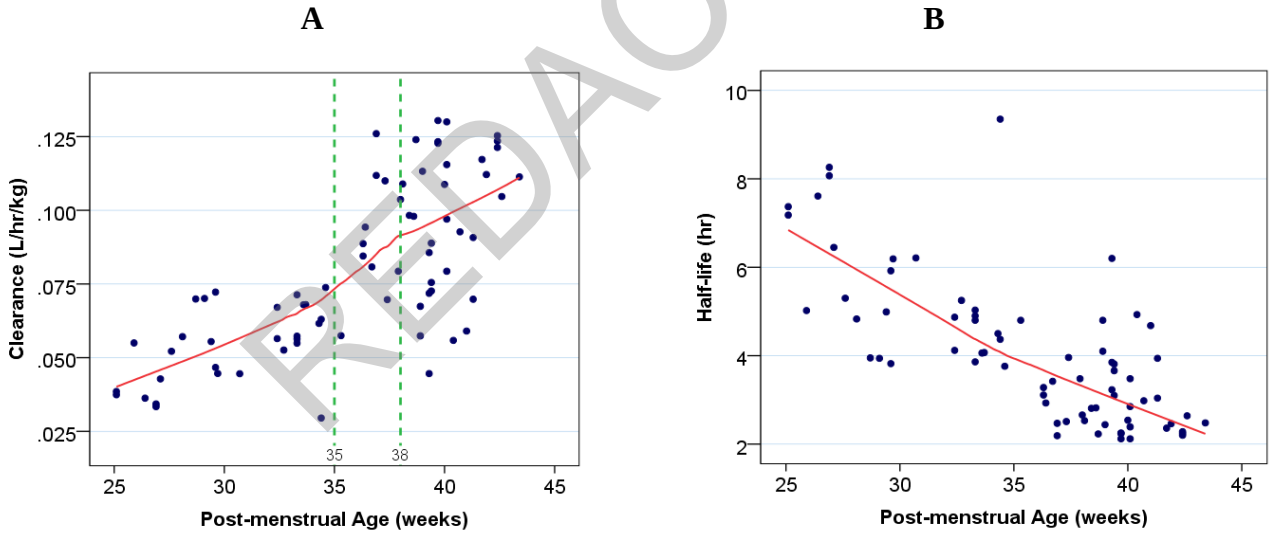
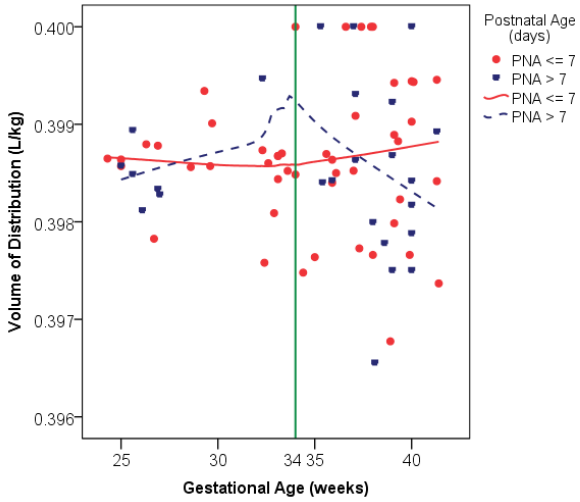
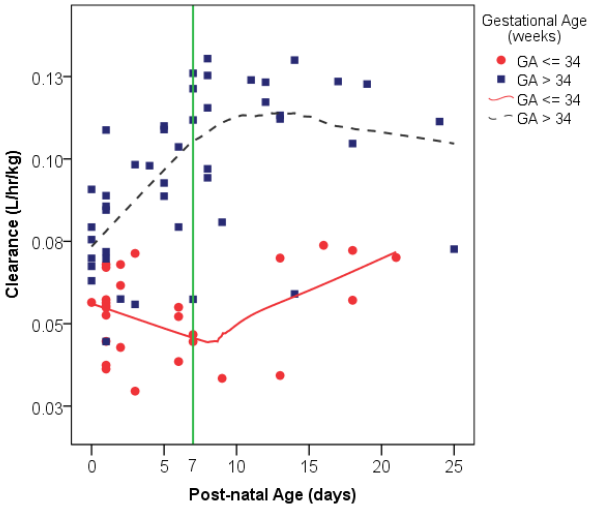


Figure 8: Clearance (A) and volume (B) vs. GA and PNA



A **B**



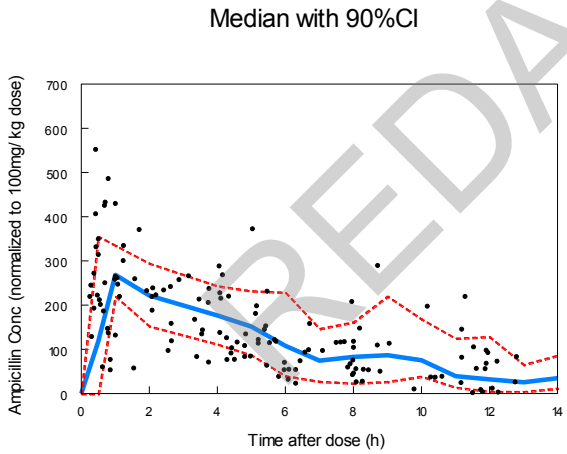
Solid red and segmented blue lines represent 90% fit Loess.

No apparent relationships were seen among sex, race, birth weight, days of life, and ampicillin CL, V, or $t_{1/2}$.

8.6. Bootstrap, Visual Predictive Check, and Standardized Visual Predictive Check Model Evaluation

The model was evaluated using a 1000-set bootstrap analysis in the program WINGS for NONMEM; 100% of bootstrap datasets converged to ≥3 significant digits. The median of bootstrap fixed effects parameter estimates were within 1.5% of population estimates from the original data set for all parameters. The visual predictive check (Figure 9) indicated that the model adequately described the data; 29% of the observations fell outside of the 90% prediction interval. This somewhat higher than frequency outside the target may be the result of dosing interval differences (while the doses were normalized to 100 mg/kg, the dose intervals were not) and binning, which was done in 1-hour increments or less to 12 hours and every 2 hours thereafter.

Figure 9: Visual predictive check

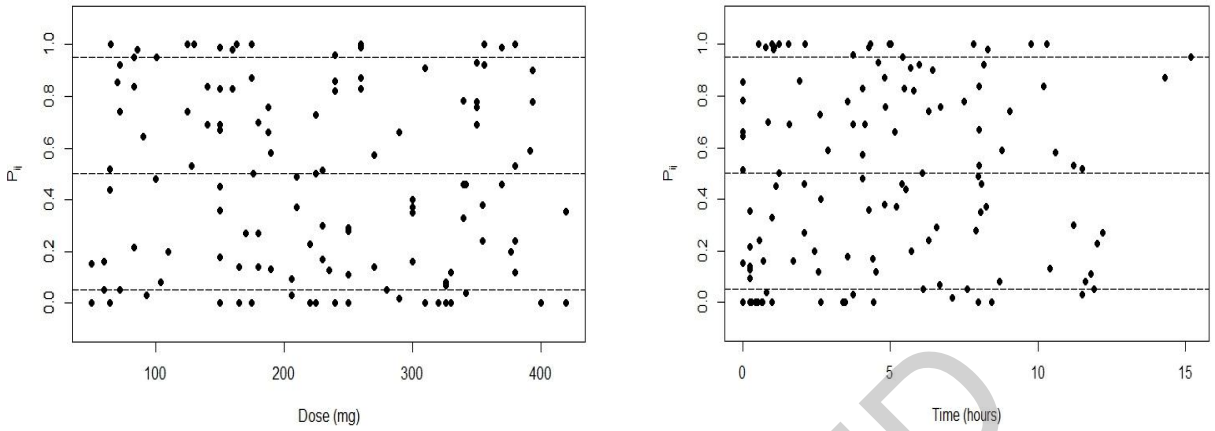


Solid black circles: observed concentrations; solid blue line: predicted median concentration; dashed red lines: 90% confidence intervals.

The standardized visual predictive check (Figure 10) provides the percentile of each observed concentration(s) for each participant in the marginal distribution of the final model-simulated end points (*Pij*) as a function of dose and time using that participant's dose, dosing schedule, and categorized influential covariates (including PMA, SCR, and WTKG). Percentile values were uniformly distributed between 0 and 1over dose and time, indicating good predictive performance.

Figure 10: Standardized visual predictive check by dose (A) and time (B)

A **B**



Circles represent calculated Pij for each observation versus dose; dashed line, model-predicted 5th, 50th, and 95th percentiles of model-predicted Pij.

8.7. Pharmacodynamic Targets and Monte Carlo Simulations

Using Monte Carlo simulations (N=1920 of virtual subjects), the performance of a dosing regimen based on the average daily dose prescribed in each group versus dosing regimens currently recommended in pediatric dosing guidelines (Neofax, Pediatric Dosage Handbook, and The Harriet Lane Handbook) were evaluated using the PMA PK model at 50%, 75%, and 100% of the dosing interval for an MIC_90 based on CLSI breakpoints of ≥2 and MIC ≥8 (Table 10). 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 subjects with an MIC < 8 mcg/ml (Figure 11). In comparison, the common dosing references had at least 1 group with 10% of virtual subjects with an MIC <8 mcg/ml.

Table 10: Dosing schemes evaluated (14) [a]

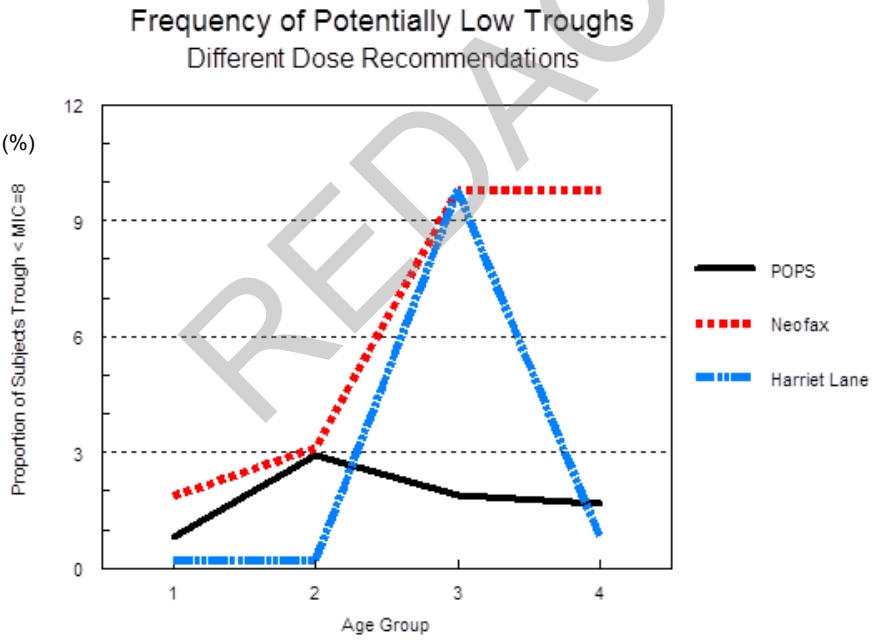
Weight	GA	PNA	PMA	Maintenance dose	Dosing interval
kg	*weeks*	*days*	*weeks*	*mg/kg*	*hours*
Simplified dosing regimen by GA and PNA					
NA	≤34	≤7	NA	50	12
NA	≤34	≥8 & ≤28	NA	75	12
NA	>34	≤28	NA	50	8
Dosing regimen by PMA using empirically derived breakpoints					
NA	NA	≤28	≤35	50	12
NA	NA	≤28	>35 & ≤38	75	12
NA	NA	≤28	>38	50	8
FDA (via Micromedex)					
NA	NA	NA	NA	25	4
The Harriet Lane Handbook					
<2	NA	<7	NA	25–50	12
≥2	NA	<7	NA	25–50	8
<1.2	NA	≥7	NA	25–50	12
1.2-2	NA	≥7	NA	25–50	8
≥2	NA	≥7	NA	25–50	6
Neofax					
NA	NA	≤28	≤29	25–50	12
NA	NA	>28	≤29	25–50	8
NA	NA	≤14	30-36	25–50	12
NA	NA	>14	30-36	25–50	8
NA	NA	≤7	37-44	25–50	12
NA	NA	>7	37-44	25–50	8
NA	NA	ALL	≥45	25–50	6
Lexicomp's Pediatric Dosage Handbook					
≤2	≤34	≤7	NA	50	12

Weight	GA	PNA	PMA	Maintenance dose	Dosing interval
kg	*weeks*	*days*	*weeks*	*mg/kg*	*hours*
>2	>34	≤7	NA	50	8
<1.2	<29	>7	NA	50	12
1.2-2	29-34	>7	NA	50	8
≥2	≥34	>7	NA	50	6
American Academy of Pediatrics (via Red Book 2012)					
≤2	NA	≤7	NA	50	12
>2	NA	>7	NA	50	8
≤2	NA	≤7	NA	50	8
>2	NA	>7	NA	50	6

[a] Administered intravenously or intramuscularly.

Figure 11 presents the proportion of infants meeting the surrogate PD based on POPS dosing versus common dosing regimens with the dosing handbooks Harriet Lane and Neofax. The curve for the pediatric dosage handbook was similar to that of Harriet Lane.

Figure 11: Proportion of virtual infants (N=1920) failing the surrogate PD target



To achieve a trough concentration above the MIC of ≥8 mcg/ml in at least 90% of the infants, we were able to devise a simplified dosing regimen by GA and PNA 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 1 and Table 10 under "Simplified Dosing Regimen by GA and PNA"). In addition, 2 PMA breakpoints at 35 and 38 weeks were determined empirically to discriminate

developmental changes in CL (Figure 7A). The dosing regimen by PMA was derived using these breakpoints (Table 10 under "Dosing Regimen by PMA using Empirically Derived Breakpoints"). The PMA-based dosing strategy was similar to the proposed dosing regimen by GA and PNA.

Only 1 of 5 current dosing strategies integrates PMA. Ampicillin dosing by Neofax incorporates both PMA and PNA, in which PMA embodies GA (Table 10). This method duplicates the use of PNA because PNA is already a component of PMA (i.e., PMA ≅ GA + PNA). In addition, other current dosing strategies (except for FDA recommendations) incorporate at least 2 covariates, including PNA and weight. In these scenarios, weight appears to serve as a surrogate for GA because it correlates to prematurity status especially if <1000 g. Consequently, the use of at least 2 covariates, with or without PMA, is a prudent strategy to optimize dosing of ampicillin in infants. For the PMA-based dosing strategy, PNA was the additional covariate used to account for developmental changes. The proposed dosing regimen incorporated GA and PNA (Table 10).

Applying the final PK PMA-based model to the Pediatrix database (N=132,966 with demographic information in Table 11), the dosing regimens by GA/PNA and PMA were simulated and compared to current pediatric dosing guidelines using the lowest recommended doses (Table 10). The probabilities of target attainment for 50%, 75%, and 100% of the dosing interval at MIC_90 of ≥2 and ≥8 mcg/mL were 95–100% based on dosing by GA/PNA or PMA (Table 12). 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 recommendations 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. The steady-state maximum and minimum concentrations achieved by the proposed PNA/GA-based and FDA dosing strategies are described in Table 13.

Table 11: Demographic characteristics of infants from the Pediatrix dataset (N=132,966)

	Gestational age ≤34 weeks		Gestational age >34 weeks		Total
	PNA ≤7 days	PNA 8–28 days	PNA ≤7 days	PNA 8–28 days	
Group	1	2	3	4	
N	61,748	833	69,782	603	132,966
Gestational age (weeks)					
Mean (SD)	31.0 (2.7)	29.4 (3.2)	37.6 (1.8)	37.8 (1.6)	34.4 (4.0)
Median (5th, 95th percentile)	32.0 (26.0,34.0)	30.0 (24.0,34.0)	38.0 (35.0,40.0)	38.0 (35.0,40.0)	35.0 (27.0,40.0)
Postnatal age (days)					
Mean (SD)	0.3 (1)	14.8 (5.3)	0.5 (0.9)	13.5 (4.9)	0.5 (1.8)
Median (5th, 95th percentile)	0 (0,1.0)	14.0 (8.0, 24.0)	0 (0,2.0)	12.0 (8.0, 23.0)	0 (0,2.0)
Postmenstrual age (weeks)					
Mean (SD)	31.0 (2.7)	31.5 (3.1)	37.6 (1.8)	39.8 (1.8)	34.5 (4.0)

	Gestational age ≤34 weeks		Gestational age >34 weeks		Total
	PNA ≤7 days	PNA 8–28 days	PNA ≤7 days	PNA 8–28 days	
Group	1	2	3	4	
N	61,748	833	69,782	603	132,966
Median (5th, 95th percentile)	32.0 (26.0,34.0)	31.9 (26.0,35.9)	38.0 (35.0,40.3)	40.0 (36.6,42.4)	35.0 (27.0,40.0)
Weight (kg)					
Mean (SD)	1.6 (0.6)	1.4 (0.6)	3.0 (0.6)	3.1 (0.7)	2.4 (0.9)
Median (5th, 95th percentile)	1.6 (0.7,2.5)	1.4 (0.7,2.4)	3.0 (2.0,4.0)	3.1 (2.0,4.1)	2.4 (0.9,3.8)
Serum creatinine (mg/dL)					
Mean (SD)	0.85 (0.23)	0.69 (0.32)	0.78 (0.25)	0.50 (0.26)	0.81 (0.25)
Median (5th, 95th percentile)	0.80 (0.50,1.20)	0.60 (0.30,1.20)	0.80 (0.40,1.20)	0.45 (0.23,0.90)	0.80 (0.40,1.20)

PNA = postnatal age; SD = standard deviation.

Table 12: Probability of target attainment from Monte Carlo simulations applying the final pharmacokinetic model to the Pediatrix dataset (N=132,966)

Group	Minimum inhibitory concentration ≥2 mcg/mL			Minimum inhibitory concentration ≥8 mcg/mL		
	50% T>MIC	75% T>MIC	100% T>MIC	50% T>MIC	75% T>MIC	100% T>MIC
Proposed dosing by GA and PNA						
1	100	100	100	100	100	99.9
2	100	100	100	100	100	95.7
3	100	100	100	100	100	100
4	100	100	100	100	100	100
Dosing by PMA						
1	100	100	100	100	100	99.9
2	100	100	100	100	100	96.5
3	100	100	100	100	99.9	98.3
4	100	100	100	100	99.8	96.2
FDA						
1	100	100	100	100	100	100
2	100	100	100	100	100	100
3	100	100	100	100	100	100
4	100	100	100	100	100	100
Harriet Lane						
1	100	100	100	100	99.8	98.4
2	100	100	100	100	100	98.2
3	100	100	100	100	99.9	97.7
4	100	100	100	100	99.8	99.7
Neofax						
1	100	100	100	100	99.7	98.1
2	100	100	100	100	98.7	96.9
3	100	100	100	100	94.9	90.2
4	100	100	100	100	99.3	90.2
Pediatric Dosing Handbook						

Group	Minimum inhibitory concentration ≥2 mcg/mL			Minimum inhibitory concentration ≥8 mcg/mL		
	50% T>MIC	75% T>MIC	100% T>MIC	50% T>MIC	75% T>MIC	100% T>MIC
1	100	100	100	100	100	98.1
2	100	100	100	100	100	96.9
3	100	100	100	100	100	90.2
4	100	100	100	100	100	90.2
American Academy of Pediatrics						
1	100	100	100	100	100	99.8
2	100	100	100	100	100	100
3	100	100	100	100	100	99.9
4	100	100	100	100	100	100

T>MIC = time during the dosing interval that is above minimum inhibitory concentration.

Table 13: Steady-state concentrations applying the final pharmacokinetic model to the Pediatrix dataset (N=132,966): proposed dosing regimen by GA/PNA and FDA

Characteristics	Value
Proposed dosing by GA and PNA	
Maximum steady-state drug concentration (mcg/mL)	
Mean (SD)	232.7 (20.1)
Median (5^{th}, 95^{th} percentile)	231.4 (204.4, 265.9)
Minimum steady-state drug concentration (mcg/mL)	
Mean (SD)	56.7 (18.9)
Median (5^{th}, 95^{th} percentile)	56.1 (28.0, 89.1)
Dosing by FDA at 200 mg/kg/day	
Regimen 33.3 mg/kg every 4 hours	
Maximum steady-state drug concentration (mcg/mL)	
Mean (SD)	175.5 (32.0)
Median (5^{th}, 95^{th} percentile)	170.1 (132.4, 238.0)
Minimum steady-state drug concentration (mcg/mL)	
Mean (SD)	102.8 (31.9)
Median (5^{th}, 95^{th} percentile)	97.4 (60.0, 165.1)
Regimen 25 mg/kg every 3 hours	
Maximum steady-state drug concentration (mcg/mL)	
Mean (SD)	163.8 (32.3)
Median (5^{th}, 95^{th} percentile)	158.4 (120.1, 226.8)
Minimum steady-state drug concentration (mcg/mL)	
Mean (SD)	111.7 (32.3)
Median (5^{th}, 95^{th} percentile)	106.4 (68.2, 174.6)

In close examination of the 57 of 132,966 infants who did not achieve 75% of the dosing interval above the MIC_90 of 8 mcg/mL, the predicted minimum steady-state ampicillin concentration was significantly lower using the PMA- vs. the PNA/GA-based dosing strategies (Table 14; mean 2.9 vs. 16.8 mcg/mL, respectively, $p < 0.001$ using the paired t-test).

Table 14: Characteristics of infants from the Pediatrix dataset who did not achieve pharmacodynamic target: proposed dosing regimen by GA/PNA versus PMA[a]

Characteristics (n=57)	Value
Gestational age (weeks)	
Mean (SD)	37.9 (0.4)
Median (5^{th}, 95^{th} percentile)	38.0 (37.0,38.0)
Postnatal age (days)	
Mean (SD)	0.7 (2)

Characteristics (n=57)	Value
Median (5th, 95th percentile)	0 (0,5.0)
Postmenstrual age (weeks)	
Mean (SD)	37.9 (0.12)
Median (5th, 95th percentile)	38.0 (37.6,38.0)
Weight (kg)	
Mean (SD)	3.2 (0.5)
Median (5th, 95th percentile)	3.1 (2.4,4.0)
Serum creatinine (mg/dL)	
Mean (SD)	0.20 (0.12)
Median (5th, 95th percentile)	0.20 (0.20,0.20)
Proposed Dosing by GA and PNA	
Maximum steady-state drug concentration (mcg/mL)	
Mean (SD)	188 (0.23)
Median (5th, 95th percentile)	188 (187,188)
Minimum steady-state drug concentration (mcg/mL)	
Mean (SD)	16.8 (0.19)
Median (5th, 95th percentile)	16.8 (16.8,17.5)
Dosing by PMA	
Maximum steady-state drug concentration (mcg/mL)	
Mean (SD)	118.2 (0.08)
Median (5th, 95th percentile)	118.2 (118.2, 118.5)
Minimum steady-state drug concentration (mcg/mL)	
Mean (SD)	2.9 (0.04)
Median (5th, 95th percentile)	2.9 (2.9,3.0)

[a] PD target was 75% time above MIC at MIC = 8 mcg/mL.

9. DISCUSSION

Ampicillin is a commonly used drug in infants. However, the lack of PK studies in premature infants and lack of uniformity of dosing have 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 used 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 2 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 end point 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 by GA and PNA that simplifies the current dosing references.

10. CONCLUSIONS

This population PK 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 is pharmacologically necessary. With the goal of achieving trough concentrations above the MIC of ≥8 mcg/ml in at least 90% of simulated subjects, dosing by GA/PNA and PMA are similar. 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 1). Furthermore, although some references suggest every 6 hour dosing for some GA and PNA groups, adjusting the total dose would allow for every 8 hour dosing, simplifying the frequency of ampicillin administration.

11. REFERENCES

1. Clinical and Laboratory Standards Instititue (CLSI), Performance Standards for Antimicrobial Disk Susceptibility Tests- Eleventh Edition. Pennsylvania: Clinical and Laboratory Standards Instititue; 2012.
2. Ginsburg CM, McCracken GH, Jr., Thomas ML, Clahsen J. Comparative pharmacokinetics of amoxicillin and ampicillin in infants and children. Pediatrics. 1979;64(5):627-31. Epub 1979/11/01. PubMed PMID: 492836.
3. Nahata MC, Vashi VI, Swanson RN, Messig MA, Chung M. Pharmacokinetics of ampicillin and sulbactam in pediatric patients. Antimicrob Agents Chemother. 1999;43(5):1225-9. Epub 1999/05/01. PubMed PMID: 10223940.
4. Axline SG, Yaffe SJ, Simon HJ. Clinical pharmacology of antimicrobials in premature infants. II. Ampicillin, methicillin, oxacillin, neomycin, and colistin. Pediatrics. 1967;39(1):97-107. Epub 1967/01/01. PubMed PMID: 6016648.
5. Boe RW, Williams CP, Bennett JV, Oliver TK, Jr. Serum levels of methicillin and ampicillin in newborn and premature infants in relation to postnatal age. Pediatrics. 1967;39(2):194-201. Epub 1967/02/01. PubMed PMID: 6017957.
6. Chmel H, Emmanuel G, Lie T, Anderson L, Ireland J. A prospective, double-blind, randomized study comparing the efficacy and safety of low-dose ciprofloxacin with ampicillin in the treatment of bronchitis. Diagnostic microbiology and infectious disease. 1990;13(2):149-51. Epub 1990/03/01. PubMed PMID: 2196150.
7. Kaplan JM, McCracken GH, Jr., Horton LJ, Thomas ML, Davis N. Pharmacologic studies in neonates given large dosages of ampicillin. The Journal of pediatrics. 1974;84(4):571-7. Epub 1974/04/01. PubMed PMID: 4600568.
8. Driessen OMJ, Sorgedrager N, Michel MF, Kerrebijn KF, Herman J. Pharmacokinetic aspects of therapy with ampicillin and kanamycin in newborn infants. Europ J Clin Pharmacol. 1978;13:449-57.
9. Metsvaht T, Ilmoja ML, Parm U, Maipuu L, Merila M, Lutsar I. Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatr. 2010;99(5):665-72. Epub 2010/01/26. doi: 10.1111/j.1651-2227.2010.01687.x. PubMed PMID: 20096030.
10. T R. Neofax 2011. Physicians Desk Reference I, editor2011.
11. Smith PB, Cohen-Wolkowiez M, Castro LM, Poindexter B, Bidegain M, Weitkamp JH, et al. Population pharmacokinetics of meropenem in plasma and cerebrospinal fluid of infants with suspected or complicated intra-abdominal infections. The Pediatric infectious disease journal. 2011;30(10):844-9. Epub 2011/08/11. doi: 10.1097/INF.0b013e31822e8b0b. PubMed PMID: 21829139; PubMed Central PMCID: PMC3173561.
12. van den Anker JN, Schwab M, Kearns GL. 2011. Developmental pharmacokinetics. Handb. Exp. Pharmacol. 205:51–75.

13. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. 2003. Developmental pharmacology—drug disposition, action, and therapy in infants and children. N. Engl. J. Med. 349:1157–1167.
14. Riddle WR, DonLevy SC, Lafleur BJ, Rosenbloom ST, Shenai JP. Equations describing percentiles for birth weight, head circumference, and length of preterm infants. Journal of perinatology : official journal of the California Perinatal Association. 2006;26(9):556-61. Epub 2006/08/04. doi: 10.1038/sj.jp.7211572. PubMed PMID: 16885988.

12. APPENDICES

1. Control Stream and Output for NONMEM Final Models
2. Datasets for Model Development and Validation
3. Define.pdf
4. Additional Figures
5. Additional Tables
6. Listings