Pediatric Trials Network

Pharmacokinetics of Antistaphylococcal Antibiotics in Infants

Phase 1 Trial

Funding Sponsor:
The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)

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STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP): Consolidated Guideline, and the applicable regulatory requirements from the United States Code of Federal Regulations (CFR), including 45 CFR 46 (human subjects protection, incorporating Subpart D Additional Protections for Children Involved as Subjects in Research), 21 CFR 312 (Investigational New Drug [IND]), 21 CFR 50 (Protection of Human Subjects, incorporating Subpart D Additional Safeguards for Children in Clinical Investigations), 21 CFR 11 (electronic records and signatures), 21 CFR 54 (financial disclosure), and 21 CFR 56 (institutional review board [IRB]).

All individuals responsible for the design and/or conduct of this study have completed human subjects' protection training and are qualified to be conducting this research.

SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read the protocol, including all appendices and the investigator brochure or product label, and I agree that it contains all necessary details for my staff and me to conduct this study as described. I personally will oversee the conduct of this study as outlined herein and will make a reasonable effort to complete the study within the time designated. I agree to make all reasonable efforts to adhere to the attached protocol. I understand and am aware of my responsibilities as an investigator as described in the applicable GCP regulations.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the sponsor or its representative. I will discuss this material with them to ensure that they are fully informed about the efficacy and safety parameters and the conduct of the study in general. I am aware that, before beginning this study, the institutional review board responsible for such matters must approve this protocol in the clinical facility where it will be conducted.

I agree to provide all participants with informed consent forms, as required by government and International Conference on Harmonisation regulations. I further agree to report to the sponsor or its representative any adverse events in accordance with the terms of this protocol and the U.S. Code of Federal Regulations, Title 21, part 312.64.

_____
Principal Investigator Name (print)

_____ _____
Signature Date

STUDY PRINCIPAL INVESTIGATOR / IND SPONSOR SIGNATURE

The signature below documents the review and approval of this protocol and the attachments (e.g., package inserts) and provides the necessary assurances that this clinical study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality and according to local legal and regulatory requirements and to the principles outlined in applicable U.S. federal regulations and ICH guidelines. It is the investigator's responsibility to ensure that, prior to initiating this study; this protocol is reviewed and approved by the appropriate local IRB. The composition and conduct of this committee must conform to the U.S. CFR, including provisions outlined in 45 CFR 46, subpart D.

Pediatric Trials Network Study Principal Investigator Name (print)

Signature

Date

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List of Abbreviations

AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
$AUC_{0–24}$	Area Under the Concentration-Time Curve 0–24 hours
AUC_{ss}	Area Under the Curve at Steady State
BPCA	Best Pharmaceuticals for Children Act
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CI	Confidence Interval
CL	Clearance
C_{max}	Maximum Concentration
CRF	Case Report Form
CSF	Cerebrospinal Fluid
CV%	Coefficient of Variance
DBS	Dried Blood Spot
DCC	Data Coordinating Center
DCF	Data Collection Form
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOT	End of Treatment
FDA	Food and Drug Administration
GA	Gestational Age
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
kg	Kilogram
LDH	Lactate Dehydrogenase
MedDRA ®	Medical Dictionary for Regulatory Activities
mg	Milligram
MRSA	Methicillin-resistant *Staphylococcus aureus*
µg	Microgram
MOP	Manual of Procedures
N	Number (typically refers to participants)
NICHD	National Institute of Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health

List of Abbreviations - *continued*

PNA	Postnatal Age
PK	Pharmacokinetics
SAE	Serious Adverse Event
SGOT	Serum Aspartate Aminotransferase
SGPT	Serum Alanine Aminotransferase
$t_{1/2}$	Half-life
TORO	Transfer of Regulatory Obligations
V	Volume
V_{ss}	Volume of Distribution at Steady State
WHO	World Health Organization

PROTOCOL SYNOPSIS

Protocol Title:	**Pharmacokinetics of Antistaphylococcal Antibiotics in Infants**
Phase:	I
Product:	Rifampin, ticarcillin-clavulanate, clindamycin
Objectives:	Primary: Describe the pharmacokinetics (PK) of rifampin, ticarcillin-clavulanate, and clindamycin in infants Secondary: Describe the safety profile of rifampin, ticarcillin-clavulanate, and clindamycin in infants
Study Design:	Multiple center, open-label, PK study
Study Population:	Hospitalized infants: 1. Rifampin: <121 days postnatal age (PNA) 2. Ticarcillin-clavulanate: <91 days PNA AND <30 weeks gestational age (GA) 3. Clindamycin: <121 days PNA AND <30 weeks GA
Number of Participants:	16–32 evaluable per study drug
Number of Sites:	Approximately 15 sites
Duration of Participant Participation:	Approximately 30 days
Dose Duration:	Study drug will be administered for 2–4 days
Estimated Start:	July 2012
Estimated Time to Complete Enrollment:	Approximately 24 months

1 KEY ROLES

For questions regarding this protocol, contact:

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Staphylococcal Infections in Infants
Seventy percent of late-onset sepsis in the neonatal intensive care unit (NICU) is due to gram-positive organisms.[1] The majority of late-onset bacterial sepsis episodes are due to coagulase-negative *Staphylococcus*,[1,2] and *Staphylococcus aureus* is the second most commonly isolated pathogen.[2–7] *Staphylococcus aureus* is associated with overwhelming septic shock, severe necrotizing pneumonia,[2,8–11] and high risk of mortality (up to 40%).[12] The majority (95%) of coagulase-negative *Staphylococcus* isolates and 40% of *Staphylococcus aureus* isolates are methicillin-resistant (MRSA).[9,10] Infants with these infections have prolonged hospitalizations and an increased risk of neurodevelopmental impairment.[2,13–15] Rifampin, clindamycin, and ticarcillin-clavulanate all have activity against *Staphylococcus* species.

Rifampin: Rifampin is a semisynthetic derivative of rifamycin SV. Rifampin has a wide spectrum of antibacterial activity that includes methicillin-resistant staphylococcal species. Rifampin inhibits bacterial RNA polymerase but does not inhibit the mammalian enzyme.[16] Rifampin is not an option for monotherapy given the high likelihood for development of resistance but is often added to facilitate bacterial eradication among infants with persistent staphylococcal (including MRSA) bacteremia.[17]

Food and Drug Administration (FDA) label: Rifampin is approved for the treatment of tuberculosis and for the treatment of asymptomatic carriers of *Neisseria meningitidis* to eliminate meningococci from the nasopharynx.[16] Rifampin exhibits in vitro activity against *Staphylococcus aureus* (including MRSA) and *Staphylococcus epidermidis* (coagulase-negative *Staphylococcus*); however, the safety and effectiveness of rifampin in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled trials.

Understanding of the PK of rifampin in infants is limited (Table 1). The volume (V) of rifampin is larger, and clearance (CL) lower, than in older children or adults.[18] In 12 pediatric patients aged 3 months to 12 years (mean age 4.6 years),the V, clearance (CL), and half-life (t½) of rifampin were 0.63 L/kg, 0.16 L/kg/h, and 1.94 h, respectively.[19] In 9 pediatric patients aged 1 day to 18 years (mean age 5.6 years) who received a single dose of intravenous (IV) rifampin (20 mg/kg), the V, CL, and t½ of rifampicin were 1.1 L/kg, 0.29 L/kg/h, and 2.8 h, respectively. [20] Data from phase I trials in infants suggest that peak rifampin concentrations between 2 and 5 mg/L seem to be effective in the treatment of persistent staphylococcal bacteremia.[18] Rifampin clinical trials in infants are few. In 10 infants with persistent staphylococcal bacteremia, all subsequently cleared their infections in approximately 1 day after receiving IV rifampin 2.5–10 mg/kg.[21] A PK study of rifampin (5–10 mg/kg/day IV) in 21 infants (median gestational age 29 weeks [range 26–41] and median postnatal age 18 days [range 11–55 days]) found that the mean rifampin half-life was 4.9 +/- 1.7 hours, and drug CL increased with increasing postnatal age.[18] However, there was wide inter-patient variability in drug concentrations. This study was limited by lack of inclusion of older infants who may require higher rifampin doses. Differences in extracellular fluid volume, plasma protein levels, protein affinity to antimicrobials, rate, and mechanism of elimination may account for the PK discrepancies observed between infants and older populations.[18]

Table 1. Previous PK Studies of IV Rifampin in Children

Reference	Term Infants	Preterm Infants	Dose	Route	Age
1986[19]	12	0	74–450 mg/m^2 q8h	IV	3 mo – 12 yr Mean: 4.6 yr
1990[20]	9	0	20 mg/kg	IV	1 day – 18 yr Mean: 5.6 yr
1993[21]	0	10	2.5–10 mg/kg	IV	24–32 wks GA 12–77 days PNA
2006[18]	2	19	5–10 mg/kg q24h	IV	26–41 wks GA 11–55 days PNA

Ticarcillin-clavulanate: Ticarcillin is a semisynthetic penicillin with activity against a wide range of gram-negative and gram-positive organisms. Ticarcillin is potentially an excellent agent to use in infants because of its broad spectrum of activity and safety profile observed in older populations.[22] Similar to other beta-lactams, ticarcillin's main CL route is the renal system. Ticarcillin is used in combination with clavulanic acid, which is a beta-lactamase inhibitor; this combination is termed ticarcillin-clavulanate.

FDA label: The safety and effectiveness of ticarcillin-clavulanate has not been established in infants <3 months of age. Ticarcillin-clavulanate is FDA-approved for patients older than 3 months of age and is indicated in the treatment of infections caused by susceptible strains of the designated microorganisms in the following conditions: septicemia (including bacteremia), lower respiratory infections, bone and joint infections, skin and skin structure infections, urinary tract infections, gynecologic infections, and intra-abdominal infections.

Understanding of the safety and PK of ticarcillin-clavulanate in preterm infants is extremely limited (Table 2). In a study evaluating the PK of single-dose ticarcillin in newborn infants, CL and half-life increased and decreased, respectively, with increasing GA at birth, birth weight, and chronological age.[23] For infants >2500 g at birth, CL increased from 6.0 mL/min to 18.9 mL/min from birth to the third week of life. Similarly, for infants 1500–2000 g, CL increased from 3.1 mL/min to 4.6 mL/min from birth to the third week of life. For term infants, half-life decreased from 2.5 hours to 1.5 hours from birth to the third week of life. For infants≤32 weeks gestation, half-life decreased from 3.7 hours to 1.1 hours from birth to the third week of life. The 54 participants were given 50 mg/kg of ticarcillin intramuscular (IM). Only 14 PK samples were obtained from infants <1500 g birth weight, and only 9 infants were <33 weeks GA at birth. A second study evaluated the PK of multiple-dose IM ticarcillin in infants.[24] Infants were enrolled into 3 groups: 1) 6 infants <2000 g and 0–7 days of age (75 mg/kg every 8 hours); 2) 22 infants >2000 g and 0–7 days of age (75 mg/kg every 6 hours); and 3) 8 infants >2000 g and 1–8 weeks of age (75–100 mg/kg every 6 hours). Plasma CL increased with increasing PNA and GA. The PK of ticarcillin-clavulanate was studied in 9 preterm (range 30–36 weeks GA) and 7 full-term infants.[25] The smallest infant had a birth weight of 1400 g. Infants were dosed as follows: preterm infants were given 83.3 mg/kg ticarcillin/3.3 mg/kg clavulanate every 8 hours, and term infants were given 100 mg/kg ticarcillin/4 mg/kg clavulanate every 8 hours by IV infusion. The doses were well tolerated. Drug accumulation was noted in 1 of the 2 smallest infants in the study. Another study in 11 low-birth-weight (<2200 g) infants evaluated the PK of 75 mg/kg of IV ticarcillin-clavulanate.[26] The median birth weight was 1250 g (range 683, 2110). Based on findings of sub-therapeutic exposures to both

ticarcillin and clavulanate, the authors recommended dosing of 50 mg/kg every 6 hours in this population. None of these PK studies in infants, however, included subjects born <29 weeks gestation.

Table 2. Previous PK Studies of IV Ticarcillin-clavulanate in Infants

Reference	Term Infants	Preterm Infants	Dose	Route	Age
1975[23]	0 0 31	9 21 0	Ticarcillin only: 50 mg/kg	IM IM IM	<33 weeks GA 33–35 GA >36 weeks GA
1978[24]	0 0 0	6 22 8	Ticarcillin only: 75 mg/kg q8h 75 mg/kg q6h 75–100 mg/kg q6h	IM IM IM	0–7 days PNA 0–7 days PNA 1–8 weeks PNA
1989[25]	7 0	0 9	Ticarcillin/clavulanate: 100/4 mg/kg 83.3/3.3 mg/kg	IV IV	36–43 weeks GA 30–36 weeks GA
1994[26]	0	11	Ticarcillin/clavulanate: 92 mg/kg q8h 3.65 mg/kg q8h	IV	29–32 weeks GA 4–117 days PNA

Clindamycin: Clindamycin is a semisynthetic antibiotic produced by a 7(S)-chloro-substitution of the 7(R)-hydroxyl group of the parent compound lincomycin that acts by inhibiting protein synthesis in much the same way as erythromycin. It was originally isolated from the soil fungus *Streptomyces lincolnensis* and first synthesized in 1967. It is rapidly absorbed when given by mouth and penetrates most tissues well, although cerebrospinal fluid (CSF) penetration is poor. Clindamycin has activity against MRSA and *Staphylococcus epidermidis* (coagulase-negative *Staphylococcus*), as well as a wide range gram-positive and gram-negative anaerobic organisms. Clindamycin is potentially an excellent agent to use in infants because of its safety profile and broad spectrum observed in older populations.[22]

FDA label: Clindamycin is approved for the treatment of adult patients with serious infections caused by susceptible anaerobic bacteria, infections due to susceptible strains of streptococci, pneumococci, and staphylococci, including serious respiratory tract infections, serious skin and soft tissue infections, and female pelvis and genital tract infections. This product contains benzyl alcohol as a preservative. Benzyl alcohol has been associated with a fatal "Gasping Syndrome" in premature infants. The potential for the toxic effect in the pediatric population from chemicals that may leach from the single dose premixed IV preparation in plastic has not been evaluated.

Published data on the safety and PK of clindamycin in preterm and term infants are extremely limited (Table 3). In a study evaluating the PK of 20 mg/kg/day of clindamycin divided q 6–8 hours in term and preterm infants, CL was directly related to PNA.[27] The half-life of clindamycin was 8.7 hours for infants <28 days of age and 3.6 hours for infants >4 weeks of life. Serum half-life in adults and older children are 3.0 and 2.5 hours, respectively.[28] CL was not presented by body weight but was found to be 1.92 L/hr for infants >3.5 kg and 0.31 L/hr for infants <3.5 kg. However, no infant in this study was <28 weeks gestation at birth, and only 1 infant was <1000 g birth weight. Only 2 term infants <14 days of age were enrolled in the study. Drug

accumulation was observed in the 1 infant <1000 g who was administered 15 mg/kg/day every 8 hours. No safety data were collected in this study. In a second study of 12 infants, no correlation between clindamycin CL and renal function, PNA, GA, or postmenstrual age was observed.[29] However, the assay used in this study could not distinguish between clindamycin or its metabolites. CL ranged from 37.8 to 135 mL/kg/hr. Again, no safety data were presented.

Table 3. Previous PK Studies of IV Clindamycin in Infants

Reference	Term Infants	Preterm Infants	Dose	Route	Age
1984[27]	5	0	5 mg/kg q6h	IV	2–27 days PNA
	0	16	6.67 mg/kg q8h	IV	28–40 weeks GA
	0	3	5 mg/kg q8h	IV	28–40 weeks GA
	19	0	5 mg/kg q6h	IV	4–51 weeks PNA
1986[29]	2	10	3.15-11 mg/kg q6h	IV	26–39 weeks GA

2.2 Scientific Rationale

Dosing for hepatically cleared therapeutics in preterm and term infants are likely to vary greatly from older children and adults due to immaturity of metabolic and renal pathways.

2.2.1 Potential Risks

It is the investigator's responsibility to ensure that, prior to initiating this study, this protocol is reviewed and approved by the appropriate local IRB. The composition and conduct of this committee must conform to the U.S. CFR, including provisions outlined in 45 CFR 46 subpart D.

Clindamycin and rifampin are protein-bound drugs. There are theoretical risks of increased bilirubin in infants with elevated bilirubin.

Risks of Blood Drawing

There are small risks to blood sampling, usually some pain/discomfort with the blood stick. We will make every effort to avoid additional (to standard of care) sticks for this study and will time clinical blood draws to coincide with timed samples, using existing intravenous lines when possible.

Risks of Rifampin

From the FDA label and review of the literature, side effects in patients administered rifampin include impacts to: gastrointestinal system (heartburn, anorexia, nausea, vomiting, jaundice, flatulence, cramps, and diarrhea); blood (low platelets occurred primarily with high-dose intermittent therapy; this effect is reversible if the drug is discontinued as soon as bruising occurs); central nervous system (headache, fever, drowsiness, fatigue, trouble walking, dizziness, inability to concentrate, mental confusion, behavioral changes, pains in arms or feet, and generalized numbness); renal system (elevations in kidney laboratory values [blood urea nitrogen {BUN} and serum uric acid] have been reported and are reversible when rifampin is discontinued and appropriate therapy instituted); and skin (skin reactions are mild and self-limiting and do not appear to be allergic reactions).

Rifampin has the potential to act as a strong inducer of the metabolism of drugs that are CYP450 substrates, and such concomitantly administered drugs should be monitored for potential lack of efficacy during concurrent treatment with rifampin and for 1 month after discontinuation of rifampin therapy.

Rifampin should not be used as monotherapy. Empirical anti-staphylococcal therapy of an appropriate anti-staphylococcal antibiotic should be used in combination with rifampin.

Risks of Ticarcillin-clavulanate
From the FDA label and review of the literature, side effects of ticarcillin-clavulanate, as with other penicillins, include the following adverse reactions: hypersensitivity (skin rash, pruritus, urticaria, arthralgia, myalgia, drug fever, chills, chest discomfort, erythema multiforme, toxic epidermal necrolysis, Stevens-Johnson syndrome, and anaphylactic reactions); central nervous system (headache, giddiness, neuromuscular hyperirritability, or convulsive seizures); gastrointestinal disturbances (disturbances of taste and smell, stomatitis, flatulence, nausea, vomiting and diarrhea, epigastric pain, and pseudomembranous colitis have been reported; onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment); hemic and lymphatic systems (thrombocytopenia, leukopenia, neutropenia, eosinophilia, reduction of hemoglobin or hematocrit, and prolongation of prothrombin time and bleeding time); abnormalities of hepatic function tests (elevation of serum aspartate aminotransferase [SGOT], serum alanine aminotransferase [SGPT], serum alkaline phosphatase, serum lactate dehydrogenase [LDH], serum bilirubin; there have been reports of transient hepatitis and cholestatic jaundice, as with some other penicillins and some cephalosporins); renal and urinary effects (hemorrhagic cystitis, elevation of serum creatinine and/or BUN, hypernatremia, reduction in serum potassium and uric acid); and local reactions (pain, burning, swelling, and induration at the infusion site and thrombophlebitis with intravenous administration).

Available safety data for pediatric patients treated with ticarcillin-clavulanate demonstrate a similar adverse event profile to that observed in adult patients.

Ticarcillin-clavulanate should not be used as monotherapy when methicillin-resistant *Staphylococcus aureus* is suspected.

Risks of Clindamycin
From the FDA label and review of the literature, the following are adverse reactions of clindamycin: antibiotic-associated colitis, pseudomembranous colitis, abdominal pain, nausea, and vomiting; hypersensitivity reactions (maculopapular rash and urticaria have been observed during drug therapy; generalized mild-to-moderate morbilliform-like skin rashes are the most frequently reported of all adverse reactions; rare instances of erythema multiforme, some resembling Stevens-Johnson syndrome, have been associated with clindamycin; a few cases of anaphylactoid reactions have been reported); skin and mucous membranes (pruritus, vaginitis, and rare instances of exfoliative dermatitis have been reported); liver (jaundice and abnormalities in liver function tests have been observed during clindamycin therapy); renal system (although no direct relationship of clindamycin to renal damage has been established, renal dysfunction as evidenced by azotemia, oliguria, and/or proteinuria has been observed in rare instances); hematopoietic (transient neutropenia [leukopenia] and eosinophilia have been reported; reports of agranulocytosis and thrombocytopenia have been made; no direct etiologic relationship to concurrent clindamycin therapy could be made in any of these instances); local reactions (pain, induration, and sterile abscess have been reported after intramuscular injection

and thrombophlebitis after intravenous infusion); musculoskeletal (rare instances of polyarthritis have been reported); and cardiovascular (rare instances of cardiopulmonary arrest and hypotension have been reported following too-rapid intravenous administration).

3 OBJECTIVE

The objective of this study is to determine the PK of rifampin, ticarcillin-clavulanate, and clindamycin in infants.

3.1 Study Outcome Measures

3.1.1 Primary Outcome Measures

1. Area under the curve 0–24 hours (AUC_{0-24})
2. Area under the curve at steady state (AUC_{ss})
3. Maximum concentration (C_{max})
4. Clearance (CL)
5. Volume of distribution at steady state (V_{ss})
6. Half life ($t_{1/2}$)

3.1.2 Secondary Outcome Measures

Safety profile of rifampin, ticarcillin-clavulanate, and clindamycin in infants.

4 STUDY DESIGN

Study design: Multiple center, open-label, multiple-dose PK study.

Randomization: None

Study intervention: Each participant will receive study drug over 2–4 days.

Duration of participant participation: Approximately 30 days: 2–4 days of study drug plus up to 30 days of safety monitoring.

PK sampling: PK concentrations in plasma will be measured at a central lab using a validated bioanalytical assay. Plasma samples will be drawn according to specific schedules for each drug.

5 Study Population

5.1 Selection of the Study Population

Rifampin: 16 to 32 evaluable participants, at least 4 in each group
Group 1: GA <32 weeks, PNA <14 days
Group 2: GA <32 weeks, PNA ≥14 days–120 days
Group 3: GA ≥32 weeks, PNA <14 days
Group 4: GA ≥32 weeks, PNA ≥14 days–120 days

Ticarcillin-clavulanate: 16 to 32 evaluable participants, at least 4 in each group
Group 1: GA <30 weeks, PNA <14 days
Group 2: GA <30 weeks, PNA ≥14 days–45 days
Group 3: GA <30 weeks, PNA >45 days–90 days

Clindamycin: 16 to 32 evaluable participants, at least 4 in each group
Group 1: GA <30 weeks, PNA <14 days
Group 2: GA <30 weeks, PNA ≥14 days–45 days
Group 3: GA <30 weeks, PNA >45 days–120 days

5.2 Inclusion/Exclusion Criteria

Inclusion Criteria

1. Informed consent from legal guardian
2. GA/PNA
    i. Rifampin: <121 days PNA
    ii. Ticarcillin-clavulanate: <91 days PNA AND <30 weeks GA
    iii. Clindamycin: <121 days PNA AND <30 weeks GA
3. Sufficient intravascular access
4. Suspected systemic infection

OR

Receiving 1 of the study drugs per local standard of care

Exclusion Criteria

2. Allergic reactions:
    i. Rifampin: history of allergic reactions to rifampin
    ii. Ticarcillin-clavulanate: history of allergic reaction to any penicillin, cephalosporin, or clavulanate
    iii. Clindamycin: history of allergic reaction to clindamycin
2. Urine output <0.5 mL/hr/kg over the prior 24 hours
3. Serum creatinine >1.7 mg/dl
4. Any condition that in the judgment of the investigator precludes participation because it could affect participant safety

6 STUDY PROCEDURES

6.1 Summary of Procedures

Table 4. Schedule of Study Procedures and Assessments

	Screen[1]	Therapy[1]	Post Therapy (days)	
Time (Day)	-72 h – Day 0	Day 1–2/4	Day 3/5–9/11	EOT or Early Termination
Visit		1	2	3
Informed consent/assent	X			
Demographics	X			
Physical examination	X			
Medical history	X			
Serum creatinine	X			
Chemistry labs	X	X	X[3]	X
Hematology labs	X	X	X[3]	X
Microbiology labs	X	X	X[3]	
Study drug administration		X		
PK sampling[2]		X		
Adverse events		X	X	X
Concomitant medications		X	X	

[1]Day 0 refers to time point prior to start of study drug but may be the same calendar date as day 1.

[2] Includes urine PK collection.

[3]Labs need only to be collected up to 72 hours post last dose of study drug. EOT = end of treatment.

6.2 Screening

Infants in the GA and PNA range will be screened for eligibility by the sites.

6.3 Enrollment

Research staff will obtain written informed consent from the parent/guardian.

Screening/Pre-Dose Assessment
After the parent or legally authorized representative has signed the IRB-approved informed consent form and after it has been determined that the participant satisfies all inclusion and no exclusion criteria, the following evaluations will be recorded in the case report form (CRF):

1. Participant demographics
2. Medical history (from chart)
3. Concomitant medications
4. Physical examination, including weight
5. Laboratory determinations

6.4 Follow-up

Assessments/Procedures (Day 1–4)
The following assessments will be conducted each day while the participant is on study:

1. Date and time of each study drug dose will be recorded
2. Concomitant medications
3. PK sampling
4. Laboratory and microbiologic determinations
5. Adverse events

Table 5 below provides the optimal plasma sampling collection windows for the study drug according to dosing interval. PK samples can be collected after dose 3, 4, 5, or 6. Every effort should be made to collect biological samples within these windows; however, samples obtained outside of the sampling windows are acceptable. Sample collection windows are relative to the end of the flush. PK samples should not be drawn during infusions or during the flush. The start and stop times of the flush will be recorded and will be ≤15 minutes. All study drugs will be administered over 30 minutes. Elimination samples will only be obtained around the last dose of study drug.

Table 5. Optimal Plasma Sampling Collection Windows for Study Drug by Dosing Interval

	Dosing interval (hours)			
Sample #	**6**	**8**	**12**	**24**
1	0–15 min*	0–15 min*	0–15 min*	0–15 min*
2	30–60 min	30–60 min	30–60 min	30–60 min
3	1–2 hr	1–2 hr	1–2 hr	1–3 hr
4	2–3 hr	2–3 hr	2–4 hr	3–6 hr
5	3–4 hr	3–4 hr	5–8 hr	6–12 hr
6	4–5 hr	4–6 hr	8–10 hr	12–18 hr
7	15 min prior to next dose	15 min prior to next dose	15 min prior to next dose	15 min prior to next dose
8 (elimination)	12–18 hr	16–24 hr	24–36 hr	48–72 hr

*End of flush.

Priority PK samples: At least 3 timed PK plasma samples at different time points should be collected per subject. Ideally 5 timed PK plasma samples should be collected per subject. Priority samples include # 2, 5, and 7 for each drug (see Table 5). For example, if an infant is receiving a drug every 8 hours, after the third dose the study coordinator collects sample #1, 2, and 6 and after the fourth dose the study coordinator collects sample #3, 4, 5, and 7.

Flush times: Flush times will be recorded and will occur ≤15 minutes from the end of study drug infusion.

Minimizing blood loss: Plasma samples will be collected in 200 µL blood aliquots. To minimize the amount of blood sampling, a limited PK sampling scheme will be employed such that no more than 8 timed PK samples (1.6 mL of blood) are obtained from each participant for PK analysis.

Dried Blood Spots (DBS) PK Sampling: Collect 2 DBS samples at the same time as 2 of the timed plasma PK samples. If not enough blood is available for both samples, collect only timed plasma PK samples. DBS should be collected from the collected whole blood before spinning the samples for the plasma PK samples.

Scavenge PK Sampling: A maximum of 10 scavenged plasma samples will be collected per participant.

CSF PK Measurements: Collect 100-200 µL of additional CSF into a cryovial tube from infants when CSF is obtained as part of clinical care from the time of first dose of study drug until 12 hours after the last dose. Alternatively, scavenged CSF samples (samples left over in the clinical microbiology lab) may be used.

Urine Samples: During one dosing interval when the timed multiple dose blood samples are obtained (after 3rd, 4th, or 5th), collect urine from the beginning of infusion to the next scheduled infusion.

6.5 Final Study Visit

Follow-up Assessment (Day 11)
For 7 days following the last dose of study drug, participants will be followed for the development of adverse reactions. These will be recorded on the CRF.

6.6 Laboratory Evaluations

6.6.1 Clinical Laboratory Evaluations

Laboratory Determinations

1. Any hematology values obtained within 72 hours prior to the first dose of study drug, while the participant is on study drug, and 72 hours after the last dose of study drug should be recorded. These include: hematocrit, white blood cell count, platelet count,

and differential. If multiple values for a laboratory are obtained in the 72 hours prior to first dose, record the value closest to enrollment.

2. Any serum chemistry values obtained within 72 hours prior to the first dose of study drug, while the participant is on therapy, and 72 hours after the last dose of study drug should be recorded. These include: BUN, calcium, potassium, sodium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, albumin, magnesium, chloride, and bicarbonate. If multiple values for a laboratory are obtained in the 72 hours prior to first dose, record the value closest to enrollment. The participant must have a baseline serum creatinine within 72 hours prior to first dose of study drug.

Microbiological Determinations

We will record the results of all blood cultures that are obtained within 72 hours before the first dose of study drug and up to 72 hours after the last dose of study drug. If CSF or urine (obtained by catheterization or suprapubic tap) is collected as part of the sepsis work-up, these results will be recorded on the CRF.

Special instructions for the collection, labeling, preparation, handling, and storage of specimens will be clearly detailed in the Manual of Procedures (MOP).

7 STUDY PRODUCT DESCRIPTION

7.1 Dosage and Study Drug Information

7.1.1 Rationale for Dose Selection

Rifampin: Infants receiving rifampin 5–10 mg/kg/day demonstrate wide inter-patient variability in rifampin concentrations, with some patients demonstrating low peak and trough plasma concentrations.[18] A higher dose might provide higher therapeutic exposure without adverse effects. In addition, high inter-patient variability may lead to the need for rifampin therapeutic drug monitoring.

Record all doses of Rifampin, Ticarcillin-clavulante, or Clindamycin for up to 6 SOC doses prior to and including the first on-study dose after the informed consent is signed and any subsequent SOC doses through last dose. The last dose of study drug dosing is defined as the fourth dose for rifampin, and the sixth dose of clindamycin or ticarcillin-clavulanate after enrollment, even if the clinicians continue the study drug.

Table 5. Rifampin Dosing

Cohort	GA	PNA	Dose
1	<32 weeks	<14 days	10 mg/kg Q 24 hours x 4 doses
2	<32 weeks	≥14 days–120 days	15 mg/kg Q 24 hours x 4 doses
3	≥32 weeks	<14 days	15 mg/kg Q 24 hours x 4 doses
4	≥32 weeks	≥14 days–120 days	20 mg/kg Q 24 hours x 4 doses

Ticarcillin-clavulanate (based on ticarcillin component): Older infants receiving ticarcillin-clavulanate 50–100 mg (based on ticarcillin component) every 8 hours demonstrated wide variability in ticarcillin concentrations, with some patients demonstrating low peak and trough plasma concentrations. Premature infants will likely need a longer interval because of a lower CL.

Table 6. Ticarcillin Dosing

Cohort	GA	PNA	Dose
1	<30 weeks	<14 days	75 mg/kg Q12 hours x 6 doses
2	<30 weeks	≥14 days–45 days	75 mg/kg Q 8 hours x 6 doses
3	<30 weeks	>45 days–90 days	75 mg/kg Q 6 hours x 6 doses

All dosing regimens are similar to alternative published regimens.

Clindamycin: In adults, clindamycin is extensively metabolized by the liver cytochrome P450 system and subsequently eliminated by the kidneys.[30] Clindamycin inhibits cytochrome P450 2C9, which suggests this enzyme may be responsible for its metabolism. In a clindamycin PK study of newborns, term infants during the first 3 days of life had reduced clindamycin elimination with a weight-adjusted CL that was approximately one quarter of adult CL and an elimination half-life 3 times that of the adult value.[31] Infants born at 28–30 weeks gestation had further reduced CL to a third of that seen in term infants, with an associated half-life 3 times longer than term infants.[31] This large difference between term and preterm infants suggests rapid maturation of clindamycin elimination pathways. Thus, we propose to decrease the dosing interval.

Table 7. Clindamycin Dosing

Cohort	GA	PNA	Dose
1	<30 weeks	<14 days	10 mg/kg Q 12 hours x 6 doses
2	<30 weeks	≥14 days–45 days	10 mg/kg Q 8 hours x 6 doses
3	<30 weeks	>45 days–120 days	10 mg/kg Q 6 hours x 6 doses

7.1.2 Rifampin, ticarcillin-clavulanate, and clindamycin local standard of care dosing

If the site Principal Investigator deems it appropriate for the infant to receive the local standard of care dose, this SOC dose may be selected and administered as the study dose throughout the on-study dosing period.

7.1.3 Formulation, Packaging, and Labeling

All study drugs will be standard intravenous formulations. This protocol will not specify the brand of product. Each product will be “off the shelf” as determined by the site. Detailed information will be part of the MOP.

7.1.4 Product Storage and Stability

Detailed information will be part of the MOP.

7.1.5 Preparation and Administration of Study Intervention/Investigational Product

The pharmacist at each site will prepare and dispense the study drug. IV study drug will be dispensed by the pharmacy in appropriately sized syringes. If the infant receives study drug as part of clinical care, the dose and frequency will be recorded.

Detailed information will be part of the MOP.

7.2 Concomitant Medications/Treatments

All drugs and/or treatments are permitted while on study and will be recorded on the CRF.

8 ASSESSMENT OF SAFETY

8.1 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Safety will be assessed from the time of informed consent through 72 hours after last study dose (adverse events) and 7 days after last study dose (serious adverse events, serious suspected adverse reactions, serious adverse reactions, suspected adverse reaction, adverse reactions). The last dose of study drug dosing is defined as the 4th dose of rifampin and the 6th dose of clindamycin or ticarcillin-clavulanate after enrollment, even if the clinicians continue the study drug. Safety will be assessed by frequency and incidence of adverse events (AEs) and serious AEs (SAEs). The BPCA data monitoring committee (DMC) convened by NICHD will review data and safety information from study participants on a quarterly basis.

8.1.1 Adverse Events

An **adverse event** is any untoward medical occurrence in humans, whether or not considered drug-related, that occurs during the conduct of a clinical trial. Any change in clinical status (routine labs, physical examinations, etc.) that is considered clinically significant by the study investigator is considered an AE.

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 to suggest 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 IND 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 IND sponsor, at immediate risk of death from the reaction as it occurred and required immediate intervention)
3. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
4. Inpatient hospitalization or prolongation of existing hospitalization
5. Important medical event that may not result in 1 of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent 1 of the outcomes listed in the above definition of serious event

Guidelines for Assessing Severity of an Adverse Event

The investigator should use the following definitions when assessing intensity of an adverse event:

1. MILD: Participant is aware of symptoms or has minor findings, but tolerates them well and no or minimal intervention required
2. MODERATE: Participant experiences enough symptoms or findings to require intervention
3. SEVERE: Participant experiences symptoms or findings that require significant intervention

8.1.2 Unexpected Adverse Event Definition

Any AE, the specificity or severity of which is not consistent with the package insert or investigational brochure.

8.1.3 Identification of Events and Timeframe for Reporting

As all participants in this study will have pre-existing medical conditions and will be currently hospitalized, those pre-existing conditions will not be considered as adverse events. New events that occur or worsening through frequency or intensity of pre-existing conditions will be reported as adverse events. All reportable events as defined above, determined to be an AE based on physical examination, laboratory findings, or other means, will be recorded in the source documents and entered in the electronic CRF (eCRF). Each event will be recorded on an AE case report form starting after consent has been obtained. The investigator will provide date of onset and resolution, severity, action(s) taken, changes in study drug dosing, causality to study drug, and outcome.

Follow-up of AEs

Reportable events that are identified at the last assessment (or the early termination visit) must be recorded on the AE CRF with the status of the AE noted and followed until resolution. All serious suspected adverse reactions will be followed until resolution. All other events that cannot be resolved by 30 days after last study contact will be considered resolved by convention and entered in the electronic data capture (EDC) system as such. Any event beginning more than 7 days after the last dose of study drug will not be captured.

8.1.4 Interim Safety Analysis

Rifampin: The study team will evaluate safety after 3 participants are enrolled in each cohort and receive the protocol dose. The safety review will be performed within 1 week after all data on the third participant in each cohort are entered and there are no pending queries. Enrollment in the cohort will be halted if there are any safety concerns.

Clindamycin: The study team will evaluate safety after 3 participants ≤26 weeks gestation are enrolled in cohort 1. The safety review will be performed within 1 week after all data on the third participant ≤26 weeks gestation in cohort 1 are entered and there are no pending queries. Enrollment for infants ≤26 weeks gestation in cohort 1 will be halted if there are any safety concerns. During the safety review, enrollment in this cohort will continue except for infants ≤26 weeks gestational age at birth.

8.2 Guidelines for Determining Causality

The investigator will use the following question when assessing causality of an adverse event to study drug where an affirmative answer designates the event as a suspected adverse reaction: Is there a reasonable possibility that the drug caused the event? ‘‘Reasonable possibility’’ means there is evidence to suggest a causal relationship between the drug and the adverse event.

8.3 Reporting Procedures

Relevant clinical and safety laboratory data will be entered into the data coordinating center (DCC) AdvantageEDC^{SM} at the study site within 7 business days of data acquisition. The DCC data management personnel will monitor the occurrence of the events listed below and notify the DMC, safety monitors, investigators, NIH/NICHD, and PTN staff if any of the halting criteria are met. A decision to proceed or to terminate the trial will be made by the sponsor and the NIH/NICHD. The DMC may request review of the data if deemed necessary.

8.3.1 Adverse Event Reporting

All AEs will be entered into the safety data system within 7 days of identification. SAEs will be entered into the data system within 24 hours of identification. If there are any technical difficulties, the SAE will be reported to the DCC by telephone or fax communication. At the earliest, the SAE should also be entered into the EDC. Any SAE entered in the EDC will generate an automatic email notification to the DCC, IND sponsor, and funding sponsor. The BPCA medical monitor will review all SAEs at the time they are reported. Investigators must also submit safety reports locally as required by their IRB.

8.3.2 Regulatory Reporting

Any event that requires expedited reporting based on federal regulations will be forwarded to the IND sponsor. The IND sponsor or its representative will submit expedited safety reports (IND safety reports) to the FDA and other regulatory agencies as necessary, and will inform the investigators of such regulatory reports. Site investigators must submit IND safety reports as required by their IRB. Documentation of the submission and receipt by the IRB must be retained for each IND safety report. All serious events designated as “not related” to study product(s) will be reported to the FDA at least annually in a summary format.

8.4 Halting or Discontinuation

A participant’s parent/guardian may voluntarily discontinue participation in this study at any time. The participant’s parent/guardian is not obligated to state the reason for withdrawal. The reasons for withdrawal, or failure to provide a reason, must be documented by the investigator on the completion/withdrawal section of the CRF.

Participant safety data will be reviewed on an ongoing basis to monitor for halting criteria. The study enrollment and dosing will be halted for 1 or more of the drugs in this study for a safety review if serious suspected adverse reactions or serious adverse reactions occur in 2 or more participants within each drug group. Enrollment to any remaining drug(s) will continue.

This information will be submitted to the BPCA DMC, NICHD, and FDA along with an analysis and future plans for the study.

Furthermore, the NICHD, the IND sponsor, the DMC, and the investigator shall have the right to recommend termination of 1 or more of the drugs in this study at their discretion. Enrollment to any remaining drug(s) will continue. Possible reasons for termination of the study include, but are not limited to:

1. Adverse events
2. Unsatisfactory enrollment with respect to quantity or quality
3. Inaccurate or incomplete data collection
4. Falsification of records
5. Failure to adhere to the protocol

Note: Participants withdrawn from the study due to an AE, whether serious or non-serious, must be followed by the investigator until the clinical outcome from the AE is determined. The AE(s) should be noted on the appropriate eCRFs, and the participant's progress should be followed until the AE is resolved or considered stable. The medical monitor or study PI must be notified if the AE may relate to overdose of study treatment; the package insert should be consulted for details of any specific actions to be taken.

8.5 Safety Oversight

A qualified and experienced physician not otherwise associated with this protocol will serve as the medical monitor. The medical monitor will review all SAEs at the time they are reported. If safety concerns are identified, the medical monitor may request a meeting of the DMC to review safety data. At a minimum, the medical monitor will comment on the outcomes of the SAE and causal relationship of the SAE to the study product. The medical monitor will also indicate whether he/she concurs with the details of the report provided by the study investigator. If no SAEs prompt review at an earlier time point, the DMC will review AEs and SAEs quarterly. The DMC will convene and make recommendations on termination of the study based on review of safety reports and halting rules. The safety data will be compiled by the DCC. Based on the recommendations of the DMC, PTN, and NIH/NICHD, the IND sponsor will make a decision to terminate or continue the study.

9 CLINICAL MONITORING

Site monitoring will be conducted to ensure that human participant protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and DCC or DCRI sponsor standard operating procedures. The IND sponsor, or as detailed in the Transfer of Regulatory Obligations (TORO), the DCC, NIH/NICHD, or its designee will conduct site-monitoring visits as detailed in the monitoring plan or in the manual of procedures.

Site visits will be made at standard intervals as defined by the site monitoring plans and may be made more frequently as directed by the IND sponsor and NIH/NICHD. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, data collection forms (DCFs), informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

10 STATISTICAL CONSIDERATIONS

10.1 Study Outcome Measures

PK of these 3 antimicrobials.

10.2 Sample Size Considerations

The sample size is based on the ability to observe a serious toxicity rate and to describe the PK. A sample size of 32 participants will provide adequate precision in the PK parameter estimates (i.e., CL) that will be dependent on the underlying variability of the parameter (see table below). For example, assuming an inter-individual coefficient of variance (CV%) of 40% in the population CL parameter estimate after weight-based allometric and maturation scaling, a sample size of 32 participants would provide a margin of error of ±14% in the 95% confidence interval (CI) of the CL estimate.

The sample size is sufficient to describe the PK of these 3 antimicrobials.

PK Parameter CV%	95% CI Margin of Error
40%	± 14%
50%	± 17%
60%	± 21%
80%	± 28%

Replacement Participants
If participants have <3 evaluable PK samples (not scavenged or dried blood spots), additional participants may be enrolled to ensure appropriate analysis. Evaluable samples are defined as samples with adequate volume and integrity to generate accurate concentration results as well as their associated dosing and sample times data.

Population for Analysis
All participants who receive at least 1 dose of study drug will be evaluable for safety profile analysis. All participants that provide at least 1 PK sample will be included in the PK analysis.

Pharmacokinetic data from premature infants collected in the Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care (POPS) trial will be combined and co-modeled with data from the Staph Trio trial. Inclusion of POPS data will increase the sample size across all cohorts, allow for model evaluation/validation using data from an independent trial and likely result in more precise estimation of model parameters.

10.3 Analysis Plan

Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented by gestational

age and postnatal age group for continuous variables (such as age, weight, etc.). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by gestational age and postnatal age group to summarize discrete variables (such as race, sex, etc.).

Demographic and Baseline Characteristics
The number of participants completed and discontinued early from study and the reasons for discontinuation will be summarized. Demographic and baseline characteristics will also be summarized. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of study drug. Study drug administration will be summarized in terms of number of days of dosing and reasons for final discontinuation of study drug.

Safety and Clinical Laboratory Analyses
The number of AEs, suspected adverse reactions, and adverse reactions will be summarized overall, by severity, and by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Prior and concomitant medications will be summarized by World Health Organization (WHO) drug class. Laboratory data, such as hematology and serum chemistry data, will be tabulated by GA and PNA group. Summary statistics for changes from baseline will be presented. Continuous laboratory measurements will be described at each visit using univariable descriptive statistics (mean, median, etc.); observed values and changes from baseline will be summarized. Lab tests reflective of liver toxicity (e.g., ALT, AST) will be further summarized in terms of the most extreme values and largest changes from baseline (in the appropriate direction) observed from start of study drug through the end of therapy.

PK Analysis Plan
PK parameters will be estimated by non-compartmental analysis using WinNonlin software. The plasma concentrations-time profiles of each study drug will be presented in figure form by participant and cohort. The appropriate non-compartmental pharmacokinetic parameters will be computed, including AUC_{0-24}, AUC_{ss}, C_{max}, CL, V_{ss}, and $t_{1/2}$. PK parameters will be summarized by age cohort. The influence of covariates on PK parameters will be explored. If not enough samples are obtained per subject to allow for noncompartmental analysis, a population PK analysis approach will be used.

Interim PK analysis
Rifampin: An interim PK analysis will be performed after enrollment of 3 participants in each cohort for rifampin. If the interim analysis suggests the PK data are sufficient to describe rifampin disposition in this patient population, enrollment will stop after 8 participants are enrolled in each cohort. If additional participants are required, the interim PK data will be used to determine the number of additional participants.

Clindamycin: An interim PK analysis will be performed after enrollment of 3 participants in cohort 1 for participants ≤26 weeks gestational age. If the interim analysis suggests the PK data are sufficient to describe clindamycin disposition in this patient population, enrollment will stop after 8 participants are enrolled in each cohort. If additional participants are required, the interim PK data will be used to determine the number of additional participants.

For both analyses, if the PK analysis suggests that drug exposure is below the maximum tolerated exposure in adults, no dosing modifications will be made.

11 PARTICIPANT CONFIDENTIALITY

Participants will be assigned unique code numbers and will not be identified by name. Participant confidentiality is held strictly in trust by the participating investigators, their staff, the sponsor(s), and their agents. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator. This documentation includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. Clinical study sites will permit access to such records.

The principal investigator will ensure that the use and disclosure of protected health information obtained during this research study complies with the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. The rule provides U.S. federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of participants participating in clinical trials. “Authorization” is required from each research participant (i.e., specific permission granted by an individual to a covered entity for the use or disclosure of an individual’s protected health information). A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization will be combined in the informed consent document (approved by the IRB).

12 INFORMED CONSENT PROCESS

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the participants' families. Consent forms describing in detail the study procedures and risks are given to the participant's legal guardian, and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB-approved, and the participant's legal guardian will be asked to read and review the document. Once the legal guardian has reviewed the document, the investigator will explain the research study to the participant's legal guardian and answer any questions that may arise. The participant's legal guardian will sign and date the informed consent document prior to the participant being enrolled in the study. The participant's legal guardian may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants' legal guardians for their records. The rights and welfare of the participants will be protected by emphasizing to their legal guardians that the quality of their medical care will not be adversely affected if they decline to participate in this study.

The IND sponsor or designee will provide the investigator, in writing, any new information that bears significantly on the participants' risk to receive the investigational product. This new information will be communicated by the investigator to participants' legal guardians who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated, and participants' legal guardians will be re-consented, if necessary.

Site staff may employ IRB-approved recruitment efforts prior to the participant consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility, an informed consent form must be signed.

By signing the informed consent form, the participant's legal guardian agrees that the participant will complete all evaluations required by the trial, unless the participant is withdrawn voluntarily or is terminated from the trial for any reason.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

A case report form (CRF) will be used to record participant data. The CRF will be used for the recording of all historical participant information and study data as specified by this protocol. The CRF must be completed by designated and trained study personnel. The CRF will be signed off (certified) by the principal investigator or designee.

According to ICH E6, source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies). Source documents are defined as original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

It will be the responsibility of the investigator(s) to ensure that the regulatory binder at the site is maintained. The study file will contain, but will not be limited to:

- Current package inserts, and all previous versions
- Final study protocol
- Protocol amendments (if applicable)
- Manual of operations (if applicable)
- Informed consent form (blank)
- Signed informed consent forms
- Revised informed consent forms and/or all addenda (blank)
- DHHS number for IRB or other documentation of IRB compliance with FDA regulations (if applicable)
- Documentation of IRB approval of protocol, consent form, any protocol amendments, and any consent form revisions.
- Annual IRB updates and approvals
- All correspondence between the investigator and IRB

The Manual of Procedures (MOP) describes all the components of the study file in detail.

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the sponsor, its designees, and appropriate regulatory agencies to examine (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, participants' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or

transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Quality Control and Quality Assurance
The principal investigator will provide direct access to all trial-related sites, source data/documents, data collection forms, and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities. The principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

DCC-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the principal investigator, PTN, and NIH/NICHD.

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

14.1 Ethical Standard

The investigator will ensure that the study will be conducted in accordance with the protocol, the ethical principles of Good Clinical Practice (ICH E6) that have their origin in the Declaration of Helsinki, and all applicable local regulations. The investigator will ensure the study is conducted in accordance with the provisions as stated and will comply with the prevailing local laws and customs.

14.2 Institutional Review Board

Prior to enrollment of participants into this trial, the protocol, the informed consent form, and any materials or advertisements presented to participants will be reviewed and approved by the appropriate IRB.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to the DCC. Notification of the IRB's composition and the institution’s Federal-Wide Assurance number will be provided to the DCC.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the investigator for submission to the IRB.

14.3 Informed Consent

The investigator will choose participants in accordance with the eligibility criteria detailed previously. The investigator will not exercise selectivity so that bias is prevented. In this study, a participant’s parent/legal guardian will sign an informed consent for study enrollment. All participants must sign an informed consent form that complies with the requirements of both 21 CFR Part 50 and the Health Insurance Portability and Accountability Act (HIPAA) before entering the trial. A consent form that complies with the requirements of 21 CFR Part 50 and a separate HIPAA-compliant authorization form for the use and disclosure of the participant’s protected health information may be used instead, per institutional standard operating procedures. For details regarding the informed consent process, see section 12.

14.4 Study Discontinuation

If the study is discontinued, enrolled participants will continue to be followed for safety assessments for 7 days. All adverse events must be followed through resolution.

15 DATA HANDLING AND RECORD KEEPING

The investigator is obligated to conduct this study in accordance with U.S. Federal Regulation 21 CFR 312.60-69 as specified on the signed form FDA 1572, applicable state laws, and the International Conference on Harmonization: Good Clinical Practice: Consolidation Guideline. The investigator is responsible for informing the IRB of any safety issues related to the study and the study drug, including reports of serious adverse events, if required, and all IND safety reports.

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs and provided by the DCC to the sites to record and maintain data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF should be consistent with the data collection form/source documents, or the discrepancies should be documented.

The sponsor and/or its designee will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be assessed for severity and causality, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study. The DCC will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

15.3 Types of Data

Data for this study will include safety, laboratory, and outcome measures (e.g., PK data).

15.4 Timing/Reports

The DMC will convene and make recommendations on study continuation based on the safety data collected quarterly.

15.5 Study Records Retention

Records and documents pertaining to the conduct of this study, including data collection forms, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for at least 10 years after the end of the study or per local/state regulations or until subjects reach 21 years, whichever is longer. No study records will be destroyed without prior authorization from the sponsor.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or manual of procedures requirements. The noncompliance may be on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with Good Clinical Practice:
4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1 and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to the sponsor, via the DCC's Internet Data Entry System (IDES).

All deviations from the protocol must be addressed in study data collection forms. A completed copy of the protocol deviation form must be maintained in the regulatory file. Protocol deviations must be submitted to the local IRB/IEC per their guidelines. The site principal investigator/study staff is responsible for knowing and adhering to their IRB requirements.

15.7 Participant Privacy/Authorization

The principal investigator will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule. The rule provides U.S. federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of participants

participating in clinical trials. “Authorization” is required from each research participant (i.e., specific permission granted by an individual to a covered entity for the use or disclosure of an individual’s protected health information). A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization will be combined in the informed consent document (approved by the IRB).

16 PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal under the oversight by the Publication Committee of the Pediatric Trials Network. The PTN Publication Committee comprises representatives of the network cores, thought leaders, DCC, and PTN and is responsible for generation and coordination of the publications that report scientific findings of the network. All public presentations (abstracts, manuscripts, slides and text of oral or other presentations, and text of any transmission through any electronic media) by participating investigators, participating institutions, DCC, and PTN that use PTN data, are intended to represent the PTN, or are supported by the PTN will be reviewed by the Publication Committee per the Publication Committee charter.

The Publication Committee guarantees that the study results are presented by experts in the field that have working knowledge of the study design, implementation, data synthesis/analysis, and interpretation. The committee goals are to ensure that any confidential or proprietary information is protected and that all appropriate statistical analyses have been included.

The PTN Publication Committee will adhere to the trials registration policy adopted by the International Committee of Medical Journal Editors (ICMJE) member journals. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of the IND holder to register this trial in an acceptable registry.

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase I trials), would be exempt from this policy.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication. Refer to: http://publicaccess.nih.gov/ and http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033.html.

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