Pediatric Trials Network

Antibiotic Safety in Infants with Complicated Intra-Abdominal Infections (SCAMP Trial) NICHD-2013-ABS01

Phase 2/3 Trial

Funding Sponsor:

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

Funding Mechanism: HHSN20100003I

Protocol Date:	24 Sep 2015
Protocol Version:	4.0
IND Number:	108,209
Principal Investigator (IND Sponsor):	Michael Cohen-Wolkowiez, MD, PhD Associate Professor, Department of Pediatrics Duke Clinical Research Institute 2400 Pratt Street., Durham, NC 27715 Telephone: (919) 668-8812 michael.cohenwolkowiez@duke.edu

STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonization (ICH) guideline E6: Good Clinical Practice (GCP): Consolidated Guideline, and the applicable regulatory requirements from the Canadian and United States Federal Regulations. The US regulations include but are not limited to 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), 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 will personally 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 guidelines.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the sponsor or the sponsor's representative. I will discuss this material with study personnel 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 (IRB) or Research Ethics Board (REB) responsible for such matters must approve (or provide favorable opinion) 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 regulations and ICH guidelines. 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. CFR, Title 21, part 312.64 and ICH GCP 4.11

_____
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. Code of Federal Regulations and ICH guidelines.

Michael Cohen-Wolkowiez, MD, PhD
Investigator name (print)

Signature

Date

KEY ROLES

Principal Investigators:	Michael Cohen-Wolkowiez, MD, PhD Associate Professor, Department of Pediatrics Duke Clinical Research Institute 2400 Pratt Street Durham, NC 27715 Telephone: 919-668-8812 Email: michael.cohenwolkowiez@duke.edu Danny Benjamin, MD, PhD, MPH Professor, Department of Pediatrics Duke Clinical Research Institute 2400 Pratt Street Durham, NC 27715 Telephone: 919-668-7081 Email: danny.benjamin@duke.edu
Co-Investigators:	Co-investigators are included in the signature log and consent form(s) at each clinical site.
Medical Monitor:	Robert Lindblad, MD Chief Medical Officer The EMMES Corporation 401 N. Washington Street, Suite 700 Telephone: 301-251-1161 Rockville, MD 20850 Email: bpcasafety@emmes.com
BPCA DCC Principal Investigator:	Ravinder Anand, PhD Senior Investigator The EMMES Corporation 401 N. Washington Street, Suite 700 Telephone: 301-251-1161 Fax: 1-800-784-9044 Rockville, MD 20850 Email: abs@emmes.com
NICHD Contract Officer Technical Representative:	Ekaterina Tsilou, MD Medical Officer 6100 Executive Boulevard, Room 4A01H, MSC 7510 Bethesda, MD 20892-7510 Phone: 301-496-6287 Fax: 301-480-2897 Email: tsiloue@mail.nih.gov

PROTOCOL SYNOPSIS

Protocol Title	Antibiotic Safety in Infants with Complicated Intra-Abdominal Infections
Products	Metronidazole, clindamycin, piperacillin-tazobactam
Phase	2/3
Primary Outcome	Safety of drug regimens (ampicillin, metronidazole, clindamycin, piperacillin-tazobactam, and gentamicin)
Secondary Objectives	• Efficacy of drug regimens including ampicillin, metronidazole, clindamycin, piperacillin-tazobactam, and gentamicin in infants with complicated intra-abdominal infections • Pharmacokinetics (PK) of ampicillin, metronidazole, clindamycin, and piperacillin-tazobactam in infants with complicated intra-abdominal infections • Biomarker association with disease severity and antibiotic exposure • Association between genetic polymorphisms in CYP450 enzymes and metronidazole and clindamycin exposure • Comparison of intestinal microbiota between treatment arms • Cerebrospinal fluid (CSF) PK of metronidazole, clindamycin, and piperacillin-tazobactam in infants
Study Design	Partially randomized, multicenter, open-label, safety study
Study Population	Infants with complicated intra-abdominal infections (Groups 1–4) or those with suspected or confirmed infections (Group 5)
Total Number of Participants	Up to 284 (N~70 in Groups 1–3, N~50 in Group 4, N~24 in Group 5)
Number of Sites	Approximately 60
Duration of Subject Participation	Up to 100 days
Estimated Enrollment Duration	Approximately 36 months
Inclusion Criteria	• Informed consent obtained • ≤33 weeks gestation at birth (Groups 1–3, 5) • ≥34 weeks gestation at birth (Groups 4 and 5) • Postnatal age (PNA) <121 days (Groups 1–5) • Sufficient venous access to permit study drug administration (Groups 1–5) • Presenting physical, radiological, and/or bacteriological findings of a complicated intra-abdominal infection within 48 hours prior to randomization /first study drug dose* (Groups 1- 4). Complicated intra-abdominal infections include secondary peritonitis, necrotizing enterocolitis (NEC) grade II or higher by Bell's criteria, Hirschsprung's disease with perforation, spontaneous intestinal perforation, meconium ileus with perforation, bowel obstruction with perforation, gastroschisis with necrosis and/or perforation, omphalocele with necrosis and/or perforation, neonatal appendicitis, intestinal pneumatosis or

PROTOCOL SYNOPSIS (continued)

Exclusion Criteria

- History of anaphylaxis in response to study drugs (Groups 1–5)
- Serum creatinine >2 mg/dL within 48 hours on measurement prior to and closest to randomization or first study drug dose (Groups 1-5)*
- Known ALT >250 U/L or AST >500 U/L on measurement closest to the time of randomization or first study drug dose (Groups 1-5)
- Any condition that, in the judgment of the investigator, precludes participation because it could affect participant safety (Groups 1–5)*

Dose Schedule

Group	Ampicillin	Gentamicin	Clindamycin	Metronidazole	Piperacillin-tazobactam
1	X	X		X	
2	X	X	X		
3		X			X
4				X	
5			X	X	X

Drug	PNA *days*	GA *wks*	PMA *wks*	Loading dose *mg/kg*	Maintenance dose *mg/kg*	Dosing interval *h*
Ampicillin	≤7	≤34			50	12
	>7 – ≤28	≤34			75	12
	≤28	>34			50	8
	>28	ANY			50	8
Metronidazole			<34	15	7.5	12
			≥34 – ≤40	15	7.5	8
			>40	15	7.5	6
Clindamycin			≤32		5	8
			>32 – ≤40		7	8
			>40 – ≤60		9	8
Piperacillin-tazobactam*			≤30		100	8
			>30		80	6

GA, gestational age; PMA, postmenstrual age. * Dosing based on the piperacillin component.

Estimated Start: December 2013

Estimated Finish: September 2017

*Criteria must be satisfied by whichever comes first, randomization (randomized Group 1-3 participants) or first study drug dose (non-randomized Group 1-3, Group 4 and Group 5)

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Area Under the Curve
BPCA	Best Pharmaceuticals for Children Act
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CL	Clearance
CI	Confidence Interval
CSF	Cerebrospinal Fluid
DBS	Dried Blood Spot
DCC	Data Coordinating Center
DCF	Data Collection Form
DCRI	Duke Clinical Research Institute
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELBW	Extremely Low Birth Weight
FDA	Food and Drug Administration
FiO_2	Fraction of inspired oxygen
g	Grams
GA	Gestational Age
GCP	Good Clinical Practice
h	Hours
HC	Health Canada
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference of Harmonization
ICMJE	International Council of Medical Journal Editors
IDSA	Infectious Diseases Society of America
I-FABP	Intestinal Fatty Acid-binding Protein
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intention-to-treat Population
IV	Intravenous
IVH	Intraventricular Hemorrhage

IVRS	Interactive Voice Response System
Kg	Kilogram
Mg	Milligram
MIC	Minimum Inhibitory Concentration
µL	Microliter
MOP	Manual of Procedures
NEC	Necrotizing Enterocolitis
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
PD	Pharmacodynamic
PHI	Protected Health Information
PK	Pharmacokinetics
PMA	Postmenstrual Age
PNA	Postnatal Age
PTN	Pediatric Trials Network
RCT	Randomized Controlled Trial
REB	Research Ethics Board
SAE	Serious Adverse Event
SNAP	Score for Neonatal Acute Physiology
$T_{1/2}$	Half-life
V	Volume of Distribution
VLBW	Very Low Birth Weight
WBC	White Blood Cell

1. BACKGROUND

1.1. Complicated Intra-Abdominal Infections

Complicated intra-abdominal infections are infectious processes causing either localized or diffuse peritonitis.[1] Complicated intra-abdominal infections can be classified as primary or secondary. Primary complicated intra-abdominal infection develops spontaneously; secondary complicated intra-abdominal infection arises as a consequence of a mechanical breach of the gastrointestinal tract.[2] Primary complicated intra-abdominal infection occurs commonly in adult cirrhotic patients by translocation of pathogens from the gut lumen into the peritoneal cavity. Secondary complicated intra-abdominal infection can be a consequence of appendicitis, diverticulitis, cholecystitis, perforation of gastric or duodenal ulcer, or of prior abdominal surgery, among other causes.[2,3]

1.2. Complicated Intra-Abdominal Infections in Premature Infants

Complicated intra-abdominal infections in premature infants are often a result of necrotizing enterocolitis (NEC). The pathogenesis of NEC involves intestinal mucosal injury, usually associated with intestinal ischemia and bacterial overgrowth.[4] The disease process is most likely due to a combination of several factors including immaturity of the gastrointestinal tract, highly immuno-reactive intestinal mucosa, intestinal ischemia, and infectious agents.[5] It is also believed that NEC occurs in response to a dysbiosis or shift in the microbiota. Studies of the fecal microbiota have demonstrated decreased diversity around the time of NEC,[6,7] which is exaggerated in the context of prolonged antibiotic therapy. Decreased diversity and shifts in constitution of the microbiota can increase susceptibility to colonization by more pathogenic species that are pro-inflammatory, thus instigating the onset of NEC. Recent data suggest that differences in intestinal colonization precede the onset of NEC by 3 days or more.[6,8] NEC severity is graded by the Bell's criteria using a combination of clinical signs and radiologic findings (Table 1).[9,10] Stage I (suspected NEC) presents with nonspecific systemic and gastrointestinal symptoms, and abdominal plain radiographs show distension with mild ileus; stage II (definite NEC) includes persistent occult or gross bleeding and persistent "rigid" bowel loops, pneumatosis intestinalis, or portal venous gas; stage III (advanced NEC) includes deterioration of vital signs, evidence of septic shock or marked gastrointestinal hemorrhage, and evidence of pneumoperitoneum.[10,11]

Table 1. Modified Bell's criteria for the diagnosis of NEC in premature infants

Stage	Name	Systemic signs	Intestinal findings			Radiological signs		
			Abdomen	Blood in stool	Absent bowel sounds	Pneumatosis intestinalis	Portal vein gas	Abdominal cavity gas
I A	Suspected	Mild	Mild distention	Occult				
I B	Suspected	Mild	Mild distention	X				
II A	Mild	Mild	Above + **tenderness**	X	X	X		
II B	Moderate	Metabolic acidosis	Above + **cellulitis**	X	X	X	X	

Stage	Name	Systemic signs	Intestinal findings			Radiological signs		
			Abdomen	Blood in stool	Absent bowel sounds	Pneumatosis intestinalis	Portal vein gas	Abdominal cavity gas
III A	Severe	Shock	Above + **peritonitis**	X	X	X	X	
III B	Severe + perf	Shock	Above + **peritonitis**	X	X	X	X	X

Perf: perforation.

1.3. Organisms in Complicated Intra-Abdominal Infections

Organisms in complicated intra-abdominal infections are similar in adults and infants. Complicated intra-abdominal infections, including NEC, are usually polymicrobial and are caused by gram-positive and gram-negative, aerobic and anaerobic bacteria.[12,13] The organisms identified in 96 adult patients with bloodstream infection of abdominal origin included 59% gram-negative bacteria, 26% gram-positive bacteria, 7% fungi, and 9% anaerobes.[3] NEC has been associated with a number of similar bacteria including *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Salmonella*, *Clostridium perfringens*, *C. difficile*, and *C. butyricum*.[12–15]

1.4. Management of Complicated Intra-Abdominal Infections Is Similar in Adults and Infants

Management of complicated intra-abdominal infections is similar regardless of age or cause of the disease. The guidelines of the Surgical Infection Society and the Infectious Diseases Society of America (IDSA) recommend fluid resuscitation, antimicrobial therapy, and surgical intervention (laparotomy or drainage) if needed.[16] Recommended antibiotics for complicated intra-abdominal infections in infants include combinations of ampicillin, gentamicin, and cefotaxime with or without anaerobic coverage with metronidazole, piperacillin-tazobactam, or meropenem.[16,17] In spite of their frequent use, the safety and efficacy of ampicillin, metronidazole, clindamycin, and piperacillin-tazobactam in infants with complicated intra-abdominal infections have not been established.

1.5. Understudied Antibiotics in Intra-Abdominal Infections in Premature Infants

In spite of their frequent use, antibiotics are not labeled for use in premature infants with complicated intra-abdominal infections. This is evidenced by the lack of randomized control trials in premature infants with intra-abdominal infections (Table 2).

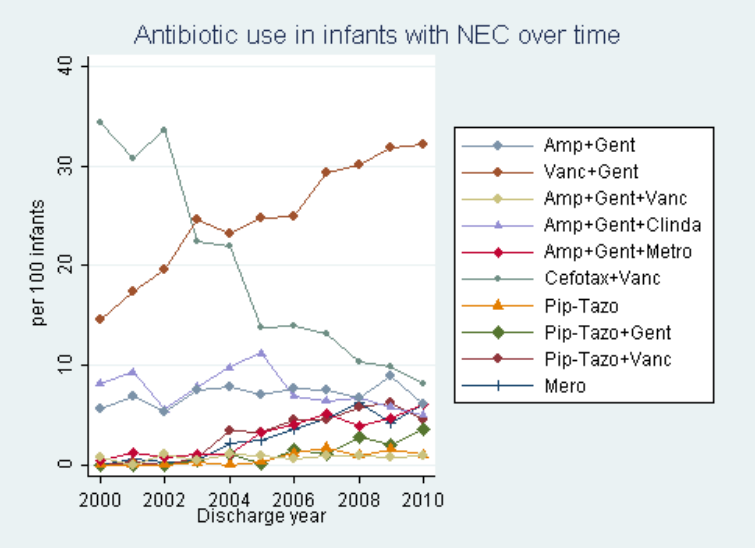
Table 2. Safety of antimicrobials in premature infants with intra-abdominal infections

Drug(s)	N	Author	Year	Gestational age*	Safety findings	Indication	Study design
Ampicillin + gentamicin	20	Hansen	1980	35 (1)	Not assessed	NEC	RCT
Ampicillin + gentamicin	46	Scheifel	1987	Not reported	No AEs	NEC	Observational
Ampicillin + gentamicin + clindamycin	42	Faix	1988	29 (3)	Intestinal strictures	NEC	RCT

AE, adverse event; RCT, randomized controlled trial. *Gestational age is mean (SD).

Among 6,000 premature infants with NEC discharged between 2000 and 2010 from ~300 intensive care nurseries managed by the Pediatrix Medical Group (personal communication, P. Brian Smith), the most commonly used antibiotic combinations included drugs such as ampicillin, gentamicin, metronidazole, clindamycin, and piperacillin-tazobactam (Figure 1). Interestingly, third-generation cephalosporins such as cefotaxime are frequently prescribed in spite of evidence showing increased risk for severe secondary infections in this population.[18] Antibiotics such as vancomycin that provide coverage for resistant gram-positive organisms are also frequently prescribed, in addition to agents active against intestinal pathogens.

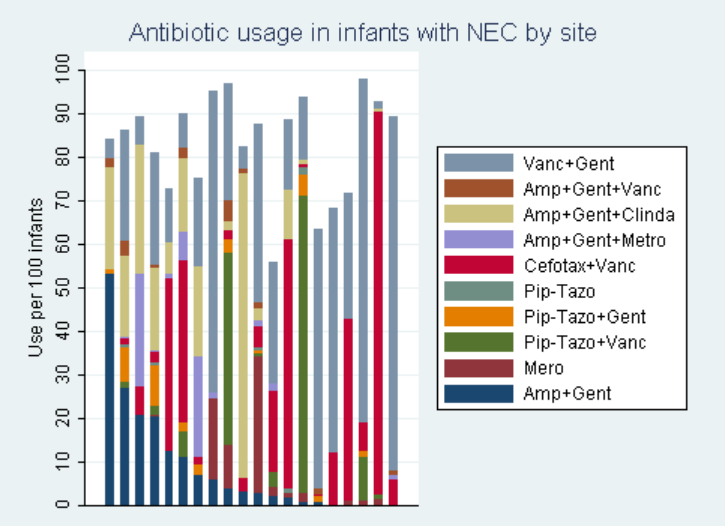
Figure 1. Antibiotic use in premature infants with NEC



Amp, ampicillin; gent, gentamicin; vanc, vancomycin; clinda, clindamycin; metro, metronidazole; cefotax, cefotaxime; pip, piperacillin; tazo, tazobactam; mero, meropenem.

Antibiotic prescribing practices not only varied by year, but also between and within centers. Figure 2 below shows the frequency of use of combination antibiotics in premature infants with NEC among 20 centers in the Pediatrix Medical Group with the highest number of NEC diagnoses. These data suggest that equipoise will be maintained with randomization into the proposed therapeutic groups in this protocol.

Figure 2. Center differences in antibiotic prescribing for premature infants with NEC



Each bar represents 1 site. Amp, ampicillin; gent, gentamicin; vanc, vancomycin; clinda, clindamycin; metro, metronidazole; cefotax, cefotaxime; pip, piperacillin; tazo, tazobactam; mero, meropenem.

Ampicillin

Ampicillin is a beta-lactam widely used in empiric treatment of neonatal sepsis and intra-abdominal infections. This agent is active against the majority of *E. coli* isolates.[19] According to the Food and Drug Administration (FDA) label, ampicillin is indicated in the treatment of infections including intra-abdominal infections caused by susceptible strains of bacteria in children and adults. However, no specific information on indication, dosing, or safety is provided for infants. The label lists hypersensitivity reaction as the major adverse event (AE), though such reactions are very uncommon in neonates. Although only described in adults, another potential serious AE is ampicillin-induced seizure.[20] However, in older children, ampicillin was well tolerated in a pharmacokinetic (PK) trial of 28 children in which no significant AEs were reported.[21] In neonates, an efficacy trial of ampicillin vs. penicillin, in combination with gentamicin, showed ampicillin to be well tolerated in this population at risk for sepsis.[22]

The PK of ampicillin in premature infants is poorly characterized (Table 3). A study conducted by our research team (IND# 113,645) evaluated the PK of ampicillin in 28 premature infants <34 weeks gestational age (GA) at birth. This study demonstrated the importance of postmenstrual age (PMA), composed of postnatal age (PNA) and GA, in ampicillin clearance (CL). Simulations showed that the GA/PNA- based dosing (50 mg/kg every 12 hours for GA ≤34 weeks and PNA ≤7 days; 75 mg/kg every 12 hours for GA ≤34 weeks and PNA ≥8 and ≤28 days; and 50 mg/kg every 8 hours for GA >34 weeks and PNA ≤28 days) was superior to currently recommended ampicillin doses in popular pediatric handbooks (Neofax, Harriet Lane) in regards to the achievement rate of a target trough concentration above a minimum inhibitory concentration (MIC) of ≥8 mcg/ml. This PK study resulted in dosing recommendations stratified by PMA, which will be used in this trial.

Table 3. PK studies of ampicillin in premature infants[23–28]

Author	Year	N	GA	PNA	Findings
Hermans	1975	26	N/A	N/A	$T_{1/2}$ 0.7–4.5 hrs
Colburn	1976	3	N/A	2–4 days	$T_{1/2}$ 2.1–2.9 hrs

Author	Year	N	GA	PNA	Findings
McCracken	1978	29	N/A	2–46 months	$T_{1/2}$ 1.3–1.5 hrs
Sutton	1986	15	Mean (range) 30 (26–41) weeks	N/A	$T_{1/2}$ 9.4 hr, urinary excretion 5–132%
Foulds	1987	53	N/A	1–176 months	CSF:serum concentration 1:3
Giachetto	2004	17	N/A	9–23 months	$T_{1/2}$ 0.50–3.46 hrs

$T_{1/2}$, half-life; CSF, cerebrospinal fluid.

Metronidazole

Metronidazole is a synthetic antibacterial agent with bactericidal activity against most obligate anaerobes.[29] Its use is approved by the FDA for the treatment of adult patients with serious infections caused by susceptible anaerobic bacteria. A common AE reported in the label for adults and potentially relevant to infants with NEC is gastrointestinal reactions including nausea, vomiting, abdominal discomfort, and diarrhea. Several serious neurologic AEs are described, including seizures, encephalopathy, meningitis, and peripheral neuropathy. Metronidazole does not have an FDA label for use in children because of lack of safety and efficacy data in this population. In the medical literature, metronidazole was well tolerated in children (2–16 years of age) suffering from perforated appendicitis.[30] In young infants, its use is restricted to patients with intra-abdominal infection and rare cases of anaerobic bacteremia or central nervous system infection. Two PK studies suggest an acceptable safety profile in premature infants (Table 4).[31,32] However, safety information in this population is still limited.

The PK of metronidazole has been evaluated in small cohorts (N<35) of premature infants (Table 4). Three studies, 2 conducted by our research team (1 under IND# 108,209), suggested that PMA-based dosing achieves drug exposures comparable to adults treated for intra-abdominal infections and achieve surrogate therapeutic targets for efficacy in the majority of infants (Figure 3). In addition, PMA-based dosing was superior to currently recommended metronidazole doses in popular pediatric handbooks (Neofax, Harriet Lane).

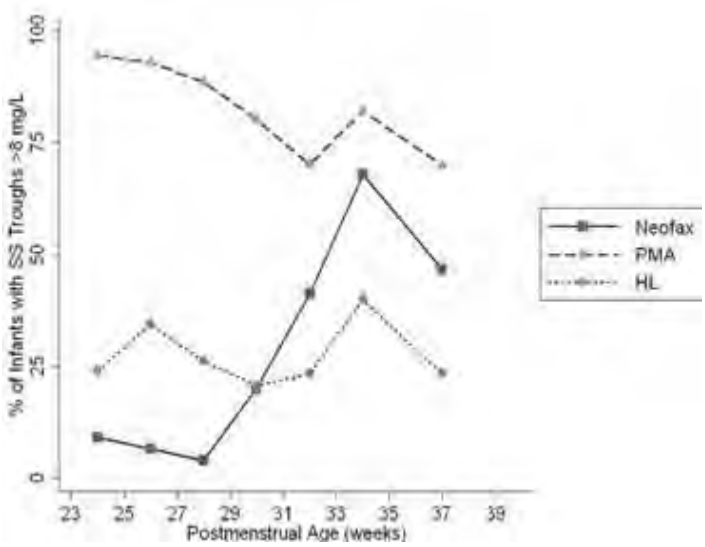
In adults, metronidazole is extensively metabolized by the liver.[33] However, the specific pathways associated with its metabolism have not been fully elucidated. Genetic polymorphisms in liver enzymes can affect drug exposure, and this information is unknown for children and premature infants.

Table 4. PK studies of metronidazole in premature infants[31,32,34,35]

Author	Year	N	GA	PNA	Findings
Hall	1983	24	Mean (range) 30 (25–40) weeks	N/A	$T_{1/2}$ inversely related to GA
Upadhyaya	1988	30	N/A	1–28 days	$T_{1/2}$ 22.5 hrs inversely related to PNA
Suyagh	2011	32	Median (range) 27 (24–37) weeks	1–55 days	CL increased with PMA CL=0.023 L/kg/hr V decreased with PMA V=0.756 L/kg
Cohen-Wolkowiez	2012	32	Median (range) 27 (22–32) weeks	0–97 days	CL increased with PMA CL=0.025 L/kg/hr V=0.71 L/kg

V, volume.

Figure 3. Target attainment rates in premature infants using PMA-based dosing (IND# 108,209)



HL, Harriet Lane Handbook.

Clindamycin

Clindamycin is a lincosamide antibiotic effective against some anaerobic agents, but there is concern about increasing resistance rates.[29,36] Clindamycin has an FDA label for the treatment of serious infections caused by susceptible anaerobic bacteria in adults and children down to 1 month of age. AEs listed include gastrointestinal, hypersensitivity, skin, and liver reactions. It is noted in the label that the product contains benzyl alcohol as a preservative, which has been associated with the fatal "gasping syndrome" in premature infants. However, a toxic effect from this chemical in the clindamycin preparation has not been reported. Clindamycin was well tolerated in a safety/efficacy trial in older children (Table 5).[37] Published data on the safety and PK of clindamycin in premature and term infants are extremely limited (Tables 2 and 5). One randomized controlled trial (N=42) showed that the addition of clindamycin to ampicillin and gentamicin did not reduce bowel perforation in neonatal NEC but was associated with an increased frequency of post-surgical stricture formation.[38] However, the control group was not systematically investigated for the presence of stricture, and this finding has not been confirmed in other trials.

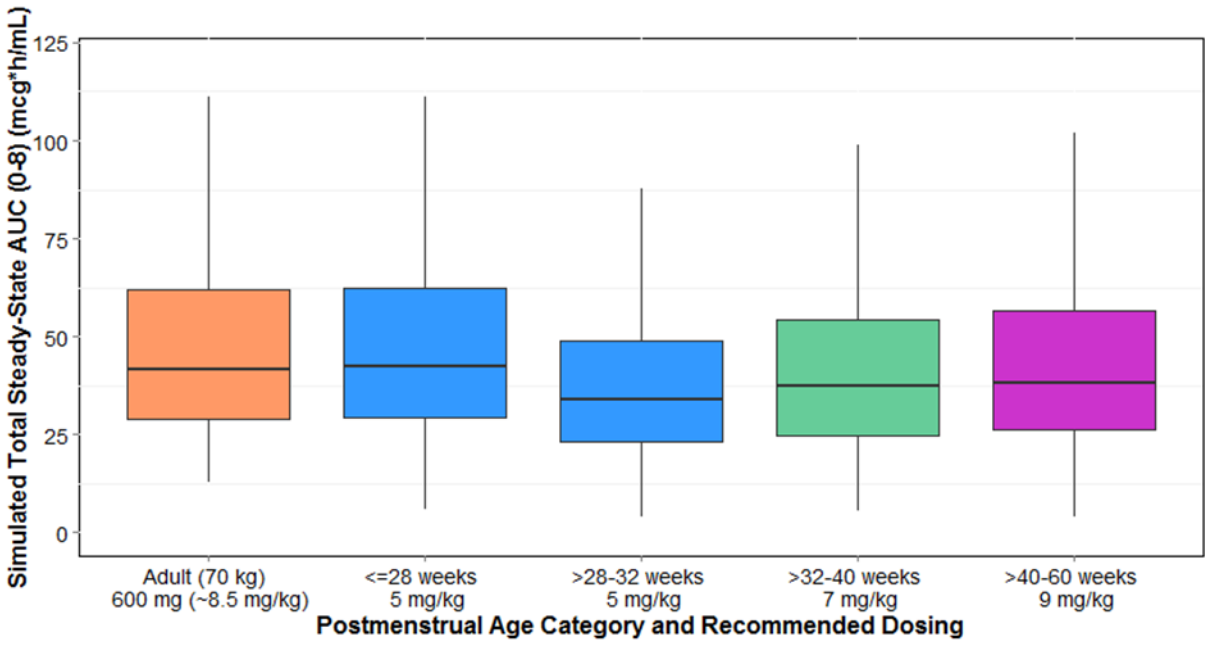
PK information to guide dosing of clindamycin in premature infants is scarce (Table 5). A population PK model was developed using data collected in 2 studies conducted by our research team (IND# 115,396, 113,645). Specifically, the model was developed using data from the Pharmacokinetics of Understudied Drugs Administered to Children per Standard of Care (POPS) trial and evaluated using premature infant data collected in the Pharmacokinetics of Antistaphylococcal Antibiotics in Infants (Staph Trio) trial. The final model was used to perform simulations that would guide infant dosing in the SCAMP trial. Age-based dosing is recommended (all dosing regimens administered every 8 hours): ≤32 weeks PMA, 5 mg/kg; >32–40 weeks, 7 mg/kg; >40–60 weeks, 9 mg/kg (Figure 4).

Clindamycin is metabolized in the liver by CYP450 enzymes in vitro.[39] Specific pathways in infants are still unknown, and genetic polymorphisms could result in exposure variability.

Table 5. PK studies of clindamycin in premature infants[9,40]

Author	Year	N	GA	PNA	Findings
Bell	1984	40	Mean (range) 34 (28–40) weeks	2 days to 51 weeks	CL increased with GA and PNA CL=0.294–1.589 L/kg/hr
Koren	1986	12	26–39 weeks	1–24 days	CL increased with GA Mean CL=0.061 L/kg/hr Mean V=0.567 L/kg

Figure 4. Optimal clindamycin dosing (regimens administered every 8 hours)



Piperacillin-tazobactam

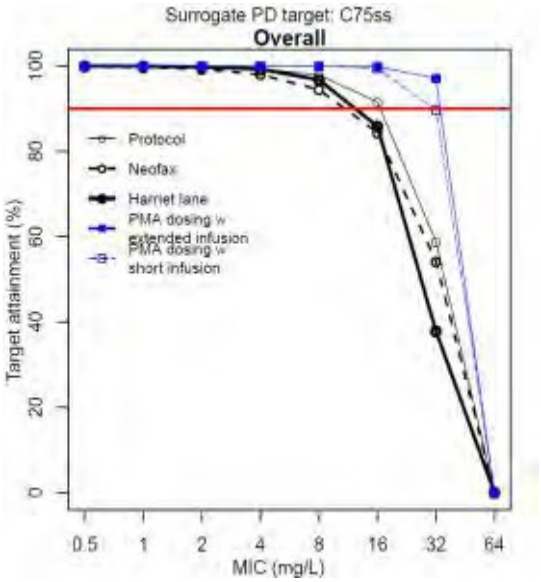
Piperacillin is a beta-lactam from the ureidopenicillin class with enhanced activity against gram-negative aerobic and anaerobic bacteria.[29] Its combination with tazobactam (beta-lactamase inhibitor) further expands the spectrum of activity, which explains its frequent use in poly-microbial intra-abdominal infections. Piperacillin-tazobactam is labeled for use in adults and children down to 2 months of age for infections with susceptible bacteria and for intra-abdominal infections. AEs reported in the label are similar for adults and children and include mild-to-moderate transient events such as skin, gastrointestinal, and allergic reactions. The FDA label does not provide safety or efficacy information for infants less than 2 months of age. Studies in children with intra-abdominal infection demonstrate a good safety profile.[41] In neonates with sepsis, a non-controlled retrospective study of piperacillin-tazobactam in combination with amikacin suggests a favorable safety profile (Table 6).[42] However, the safety profile in infants remains inadequately described due to a lack of high-quality prospective data.

Published PK data of piperacillin-tazobactam in premature infants is virtually non-existent (Table 6). One study conducted by our research team (IND# 104,988) evaluated the PK of piperacillin-tazobactam in 32 premature infants and found that a PMA-based dosing regimen achieved the surrogate therapeutic target in >90% of infants (Figure 5).

Table 6. PK studies of piperacillin-tazobactam in premature infants[43]

Author	Year	N	GA	PNA	Findings
Reed	1994	47 (24 infants)	N/A	2–23 months	CL increased with PNA CL range 0.198–0.282 L/kg/hr Mean V=0.3 L

Figure 5. Target attainment rates in premature infants using PMA-based dosing (IND# 104,988)



PD, pharmacodynamic.

1.6. Biomarkers—Intestinal Fatty Acid-Binding Protein (I-FABP)

Intestinal fatty acid–binding protein (I-FABP) is a protein released from the enterocyte upon cell death. Because of its low molecular weight, I-FABP present in the systemic circulation is filtered by the kidney and can be detected in the urine.[44] To take into account renal function in the interpretation of I-FABP levels, I-FABP is usually reported as a ratio of I-FABP over serum creatinine (I-FABP/creatinine). I-FABP is not expressed in the urinary tract mucosa, thus urinary values provide information about intestinal necrosis. Preliminary data on the use of urinary I-FABP concentration in the diagnosis of NEC among premature infants (N=17) with abdominal signs has shown an increase in I-FABP concentrations in infants with NEC when compared with infants with other diagnoses.[44] However, studies to date are limited by sample size and lack of correlation with antibiotic exposure.

1.7. Assessment of Therapeutic Efficacy in Intra-Abdominal Infections in Premature Infants

Efficacy of a particular antibiotic regimen has not been established in infants with intra-abdominal infections.[45] However, given the similarities in intra-abdominal infections between adults and children, therapeutic efficacy can be extrapolated from adults and older children. In adults, efficacy has been demonstrated with multiple antibiotics using prospective randomized controlled trials, resulting in strong recommendations from the IDSA (recommendation grade AI).[15,16,46–49] In older children, efficacy has been assessed primarily from cohort or case-controlled studies resulting in recommendations from the IDSA graded BII.[16,50–52]

In infants with intra-abdominal infection, a set of criteria to measure therapeutic efficacy based on clinical, laboratory, and radiologic findings has been proposed.[53] In a prospective safety and

efficacy study of meropenem in infants with intra-abdominal infection, success was defined as the absence of death, negative bacterial cultures from sterile body fluid, and a presumptive clinical cure score ≥7 up to 7 days after the end of treatment.[53] The clinical cure score is based on the assessment of 10 elements at baseline and at the end of the treatment. These components are based on the Score for Neonatal Acute Physiology (SNAP) II and include mean blood pressure, temperature, oxygen saturation, serum pH, presence or absence of seizure, urine output, presence of cardiovascular inotrope support, C-reactive protein, abdominal girth, and findings on abdominal radiograph. Although this trial was neither randomized nor powered for efficacy, the success rate was 84%, suggesting meropenem efficacy.

1.8. Public Health Impact and Outcomes of Intra-Abdominal Infections in Premature Infants

Complicated intra-abdominal infections are common and often fatal in premature infants.[54,55] This condition is the most devastating intestinal complication of prematurity. Among all infants, the incidence ranges from 1.7–7%.[54–58] The most consistent risk factor for the development of complicated intra-abdominal infection is prematurity, with an incidence ranging from 3% for those 1251–1500 g birth weight to 14% for neonates born <750 g.[58] NEC has a high overall mortality (15%),[59] and in extremely low birth weight (ELBW, ≤1000 grams birth weight) infants, mortality for surgical NEC is nearly 50%.[60] Survivors often suffer from complications including stricture formation (diagnosed at 1–20 months after the NEC episode)[61] and life-long morbidities such as short bowel syndrome. While stricture formation has been reported in infants with NEC who were treated with clindamycin,[38] short bowel syndrome has not been associated with any particular antibiotic regimen. Finally, infants who had NEC are also at increased risk of poor neurodevelopmental outcomes.[62,63]

1.9. Cerebrospinal Fluid Pharmacokinetics of Metronidazole, Piperacillin-tazobactam, and Clindamycin

Bacterial meningitis is more common in the first month of life than at any point in a patient's lifetime. Mortality is high, and survivors suffer from multiple morbidities including long-term neurodevelopmental impairment. A recent meta-analysis determined that NEC and meningitis in preterm and very low birth weight (VLBW) infants were the 2 most important types of infection that resulted in impairment of mental development, with an associated decrease of the Mental Development Index score of 0.40 SD (6 points) and 0.37 SD (5.6 points), respectively.[64] To prevent mortality and morbidity related to meningitis, it is important to understand the dosing and exposures needed to achieve adequate antibiotic concentrations within the central nervous system compartment. Because of the ethical and logistical challenges of obtaining cerebrospinal fluid (CSF) from the vulnerable infant population for study purposes, there is a lack of studies that characterize CSF PK in infants related to the dosing of antibiotics such as metronidazole, piperacillin-tazobactam, and clindamycin. The FDA has no data on CSF penetration of antibiotics in neonates (verbal communication).

Adult studies on CSF penetration for piperacillin demonstrate poor CSF penetration in patients with uninflamed meninges ($AUC_{CSF}/AUC_{S,}$ 0.034), but an approximately 10-fold increase in CSF penetration in patients with strong meningeal inflammation ($AUC_{CSF}/AUC_{S,}$ 0.32).[65] Tazobactam demonstrated similar CSF penetration in adults without meningeal inflammation ($AUC_{CSF}/AUC_{S,}$ 0.1). Metronidazole, because of its lipophilic nature, demonstrates excellent CSF penetration even in the presence of mild meningeal inflammation, with an average of 46 and 76% of the serum concentration observed in CSF at 1 and 2 hours post-infusion.[66] Clindamycin demonstrates poor CSF penetration in adults, with <2% of drug penetration observed in adults with HIV.[67]

CSF PK data in infants are scarce but suggest similar findings seen in adults for piperacillin and metronidazole (Table 7).[67] No CSF PK data are available for clindamycin or tazobactam in infants and children.[67]

Table 7. CSF PK studies in infants

Drug	N	GA	PNA	Findings
Piperacillin[68]	7	Mean 31 weeks	0–16 d	Concentrations of 2.6–6 mg/L up to 7 hours after 100 mg/kg dose in subjects without meningitis (n=6); concentration of 190 mg/L at 2.5 hours after 200 mg/kg dose in subject with meningitis (n=1)
Metronidazole[69]	1	Premature	30 d	CSF:plasma concentration ratios of 0.89, 0.70, and 1.03 at trough, 1, and 4 hours after dose, respectively

A review of infections seen in >300 NICUs nationwide show that almost all of the most frequently encountered pathogens have susceptibility to piperacillin-tazobactam, clindamycin, or metronidazole (Table 8).

Table 8. Bacterial pathogens and sensitivity to selected antibiotics with anaerobic activity*

Organism	N	Clindamycin	Metronidazole	Piperacillin-tazobactam
GBS	205	X		X
E coli	152			X
S aureus	131	X		X
Enterococcus sp.	70			X
Enterobacter sp.	55			X
Klebsiella sp.	44			X
Serratia sp.	36			X
Pseudomonas sp.	26			X
Acinetobacter sp.	12			X
S pneumonia	9	X		X
Citrobacter sp.	7			X
Salmonella sp.	7			X
H influenza	4			X
Listeria sp.	4			
Bacteroides fragilis	3		X	X
Proteus sp.	3			X
Neisseria meningitidis	2			X

Organism	N	Clindamycin	Metronidazole	Piperacillin-tazobactam
Stenotrophomonas sp.	2			
Clostridia sp.	1		X	X

*Data from >300 NICUs (Pediatrix Medical Group, PB Smith, personal communication).

2. OBJECTIVES

Primary Objective

- Evaluate the safety of drug regimens consisting of ampicillin, metronidazole, clindamycin, piperacillin-tazobactam, and gentamicin in infants with complicated intra-abdominal infections.

Secondary Objectives

- Evaluate the efficacy of drug regimens consisting of ampicillin, metronidazole, clindamycin, piperacillin-tazobactam, and gentamicin in infants with complicated intra-abdominal infections.
- Evaluate the population PK of ampicillin, metronidazole, clindamycin, and piperacillin-tazobactam in infants with complicated intra-abdominal infections.
- Explore the association between urinary I-FABP and severity of intra-abdominal infection and antibiotic exposure.
- Explore the association between genetic polymorphisms in CYP450 enzymes and metronidazole and clindamycin exposure.
- Compare intestinal microbiota between treatment arms.
- Evaluate the CSF PK of metronidazole, clindamycin, and piperacillin-tazobactam in infants.

3. STUDY DESIGN AND RATIONALE

The study is designed to evaluate the safety of 3 therapeutic regimens in premature infants (≤33 weeks GA) and 1 therapeutic regimen in late preterm and term infants (≥34 weeks GA) with complicated intra-abdominal infections. This study will also evaluate the CSF PK of metronidazole, clindamycin, and piperacillin-tazobactam in infants with suspected or confirmed infections.

3.1. Study Design

This is a phase 2/3, prospective, open-label, partially randomized, multicenter, safety trial.

3.2. Study Duration

Each participant receiving study drugs for complicated intra-abdominal infections (Groups 1–4) will participate in the study for up to 100 days. Participants receiving study drug for evaluation of CSF PK (Group 5) will participate in the study for up to 17 days. Enrollment for all groups is expected to last approximately 36 months.

3.3. Rationale for Study Design

The most commonly used drugs in infants with complicated intra-abdominal infections are not labeled for use in this population because safety and efficacy data are lacking. The CSF PK for these drugs in this population is virtually non-existent. The proposed study will provide the safety information required for labeling. In addition, the PK of the study drugs has been or will be characterized in premature infants under an IND mechanism.

3.4. Rationale for Dose Selection

Dose selection was determined using PK data from phase I trials in premature infants. In these trials, infant maturation was found to be significantly associated with drug clearance; requiring dosing modifications in this population (see Section **1.** Background.)

4. STUDY POPULATION

4.1. Group Assignment

4.1.1. Premature Infants (Groups 1–3)

Up to 210 premature infants (≤33 weeks gestation at birth) will be assigned to drug regimen groups 1, 2, or 3 according to the plan described in section 5.4.

Group 1 (N-~ 70): ampicillin, gentamicin, and metronidazole
Group 2 (N ~70): ampicillin, gentamicin, and clindamycin
Group 3 (N ~70): piperacillin-tazobactam and gentamicin

At least 40 participants will be assigned to each group by randomization. Participants who meet eligibility criteria for the study and are receiving all drugs in one of the study drug regimens per standard of care prior to study entry can be enrolled and assigned to the corresponding group without randomization. The assignment will be at the discretion of the treating physician based on the antibiotics that are administered per standard of care. Up to 30 non-randomized participants will be enrolled into each Group.

4.1.2. Late Preterm and Term Infants (Group 4)

Approximately 50 late preterm and term infants (≥34 weeks gestation at birth) will be assigned to Group 4. Infants assigned to Group 4 will receive metronidazole in addition to the antibiotic regimens prescribed per standard of care.

Group	Initial regimen	Anaerobic coverage
4	Per standard of care	Metronidazole

4.1.3. Infants Undergoing CSF Collection (Group 5)

Approximately 24 infants with suspected or confirmed infection for which the study drug may provide therapeutic benefit in whom CSF is planned to be collected per standard of care will be assigned to Group 5. Infants in Group 5 will be assigned by the site principal investigator to subgroup(s) (a, b, c) and receive up to 10 days of dosing of study drug. Infants assigned to Group 5 will receive study drug as part of or in addition to the antibiotic regimens prescribed per standard of care. The added antibiotics should have the potential for therapeutic benefit to the participant. If infants are receiving study drugs as standard of care prior to consent, drug administration can continue at the same doses. If study drug is added to the standard-of-care regimen, doses specified in the protocol should be used. Duration of study drug therapy will be determined by the treating physician according to clinical indication.

Group 5a (N~8): metronidazole
Group 5b (N~8): clindamycin
Group 5c (N~8): piperacillin-tazobactam

Assignment to above subgroups will be at the discretion of the treating physician or determined centrally by the study team based on real-time monitoring of enrollment considering number of infants enrolled in each group.

Participants in Groups 1-4 can be co-enrolled into Group 5.

4.2. Inclusion Criteria

1. Informed consent obtained from parent(s) or legal guardian(s) (Groups 1–5)
2. ≤33 weeks gestation at birth (Groups 1–3, 5)
3. ≥34 weeks gestation at birth (Groups 4 and 5)
4. PNA <121 days (Groups 1–5)
5. Sufficient venous access to permit administration of study drug (intravenous [IV]) (Groups 1–5)
6. Presenting physical, radiological, and/or bacteriological findings of a complicated intra-abdominal infection within 48 hours prior to randomization/first study drug dose (Groups 1-4)**. Complicated intra-abdominal infections include secondary peritonitis, NEC grade II or higher by Bell's criteria, Hirschsprung's disease with perforation, spontaneous intestinal perforation, meconium ileus with perforation, bowel obstruction with perforation, gastroschisis with necrosis and/or perforation, omphalocele with necrosis and/or perforation, neonatal appendicitis, intestinal pneumatosis or portal venous gas, free peritoneal air on abdominal radiographic examination, or abdominal abscess.
7. Suspected or confirmed infection for which the study drug may provide therapeutic benefit and planned CSF collection per standard of care (Group 5).

4.3. Exclusion Criteria*

1. History of anaphylaxis in response to study drugs (Groups 1–5)
2. Serum creatinine >2 mg/dL within 48 hours on measurement prior to and closest to randomization /first study drug dose (Groups 1- 5)**
3. Known ALT >250 U/L or AST >500 U/L on measurement closest to the time of randomization or first study drug dose (Groups 1-5)**
4. Any condition that, in the judgment of the investigator, precludes participation because it could affect participant safety (Groups 1–5)

*Do not apply for Group 5 participants receiving drug per standard of care.

**Criteria must be satisfied by randomization (randomized Groups 1-3) or first study drug dose (non-randomized Groups 1-3, Group 4 and Group 5), whichever comes first.

4.4. Permitted Treatment

- Participants may continue standard therapy for existing acute or chronic medical conditions (Groups 1–5).
- Participants requiring additional antibiotic coverage for gram-positive organisms (e.g., vancomycin, nafcillin, oxacillin, linezolid) at the discretion of the treating physician may receive these agents per routine medical care (Groups 1–5).
- Participants ≤33 weeks gestation at birth with complicated intra-abdominal infections receiving antibiotics per routine medical care at the time of enrollment will be randomized or assigned (as described in sections 4.1.1 and 5.4) to any of the first 3 drug regimen groups (Groups 1–3).
- Participants requiring alternative aminoglycosides (instead of gentamicin) at the discretion of the treating physician at study entry or during the treatment phase will not be considered protocol deviations (Groups 1–3).
- Participants ≥34 weeks gestation at birth with complicated intra-abdominal infections receiving antibiotics per routine medical care at the time of enrollment will be assigned to the fourth group (Group 4).

5. STUDY DRUG

5.1. Drug Supplies

All study drugs will be standard IV formulations of commercially available products. The study drug products will be "off the shelf" products as determined by each site's formulary. If any products are sequestered for study use, they will not be re-labeled and will retain their approved commercial use labels. Detailed information will be part of the manual of procedures (MOP).

5.2. Study Drug Administration

During hospitalization, study drug will be administered by hospital staff, and drug dosing information will be recorded by study personnel from the medical chart.

5.3. Doses and Regimens

Randomization/study group assignment and study drug administration will be initiated after criteria for entry into the study are met. For Groups 1–4, the antibiotic regimen will be administered as described in Table 9. Duration of therapy will be determined by the treating physician; however, a maximum of 10 days of therapy will be considered the treatment phase per this study. Gentamicin dosing and therapeutic drug monitoring will be performed per routine medical care. Duration of IV infusion will be 30 (+/- 15) minutes for all drugs, all groups. If, at the time of enrollment, participants are receiving the same antimicrobial regimen as that specified in the protocol, doses will be adjusted to meet protocol-defined doses. If an infant is already on metronidazole, doses will be adjusted to meet the maintenance doses, and no loading dose will be administered. For participants with suspected or confirmed meningitis, ampicillin dosing may be increased per standard of care at the discretion of the treating physician for adequate central nervous system coverage. Ampicillin dosing cannot exceed the maximum dose of 300 mg/kg/day.

Dose amounts for participants enrolled in Groups 1-3 who are not randomized must be adjusted to protocol dosing described in Table 9 unless the total daily dose for each antibiotic (excluding aminoglycosides) administered per standard of care is equal or greater to the protocol specified drug dose amount.

For participants in Group 5, at least 1 dose of the drug of interest will be administered prior and if possible ≥1 hour prior to the CSF collection. If the dose is given <1 hour prior to CSF collection, it will not be considered a protocol deviation. Pre-consent doses given per standard of care may count toward PK sampling counts if the infusion (start and stop time, dose amount) is documented for the pre-consent doses. For participants in this group receiving clindamycin, piperacillin-tazobactam, or metronidazole as part of standard of care, the principal investigator may continue the infant on that dose or change the dose to the recommended protocol study dose. For participants in this group who receive clindamycin, piperacillin-tazobactam, or metronidazole as add-on therapy, protocol-specific doses should be used.

Table 9. Dosing schemes

Drug	PNA *days*	GA *wks*	PMA *wks*	Loading dose *mg/kg*	Maintenance dose *mg/kg*	Dosing interval *h*
Ampicillin	≤7	≤34			50	12
	>7 – ≤28	≤34			75	12
	≤28	>34			50	8
	>28	ANY			50	8
Metronidazole			<34	15	7.5	12
			≥34 – ≤40	15	7.5	8
			>40	15	7.5	6
Clindamycin			≤32		5	8
			>32 – ≤40		7	8
			>40 – ≤60		9	8
Piperacillin- tazobactam*			≤30		100	8
			>30		80	6

*Dosing based on the piperacillin component.

5.4. Method of Assigning Participants to Treatment Arms

5.4.1. Randomized Groups 1-3

For participants ≤33 weeks gestation at birth, the participant number and the randomized treatment will be obtained through the AdvantageEDC enrollment module. In the event that AdvantageEDC is not available at the time of randomization, a back-up randomization procedure will be used. If a participant is randomized but does not receive study drug, that participant will not count towards total sample size and will be replaced by a new participant who, in turn, will be assigned a new identification number. Eligible participants will be randomly assigned to the 3 drug regimen groups (Groups 1–3) using a 1:1:1 allocation ratio. Randomization will be stratified by site. The permuted block design of random block sizes will be used to ensure group balance of randomized drug regimen within each block.

We intend to implement a randomization scheme that will allow continued enrollment during times of drug shortages. In instances of a drug shortage, when a site either restricts the use of, or does not have one of the following study drugs (metronidazole, clindamycin or piperacillin-tazobactam) subjects at the site will be randomized at a 1:1 ratio to one of the unaffected study Groups or assigned to the unaffected study group if 2 drugs are unavailable. Once the drug shortage at the site is over, the original 1:1:1 randomization plan will be re-instituted.

5.4.2 Non- Randomized Groups 1-3, 4 and 5

Premature infants ≤33 weeks gestation at birth may be enrolled into Groups 1-3, without randomization, per criteria described in section 4.1.1.

Participants ≥34 weeks gestation at birth will be assigned to Group 4 without randomization.

For Group 5, the participants will be assigned to a subgroup by the site principal investigator.

6. STUDY PROCEDURES

6.1. Summary of Procedures

Table 10. Schedule of study procedures and assessments (Groups 1–4)

	Baseline	Therapy	Post-therapy		
PROCEDURE	**Day 0***	**Day 1–10^**	**Day 1–3**	**Day 4–30**	**Day 31–90**
Informed consent	X				
Demographics	X				
Physical examination	X				
Medical history	X				
Laboratory evaluation	X	X	X		
Microbiology labs	X	X	X	X	X
Abdominal radiographic findings	X	X	X	X	X
Abdominal surgical procedures	X	X	X	X	X
Study drug administration		X			
PK sampling		X			
Biomarker sampling[†]	X	X	X		
Genetic sampling[‡]	X	X	X	X	X
Stool sampling	X	X		X	
Efficacy assessment	X	X		X	X
Concomitant medications	X	X	X	X	X[§]
Adverse events		X	X	X	
Outcomes of special interest		X	X	X	X

*Day 0 refers to time point prior to start of study drug but may be the same calendar date as day 1.
[†]Urine I-FABP/creatinine.
[‡]Genetic samples will be collected (opt-in informed consent) once at any time during the study.
[§]Antibiotics only (including ampicillin, gentamicin, clindamycin, metronidazole, piperacillin-tazobactam, if applicable). See Appendix I.
^Up to 10 days of study drug.

Table 11. Schedule of study procedures and assessments (Group 5)

	Baseline	Therapy	Post-therapy
PROCEDURE	**Day 0***	**Day 1–10^**	**Day 1–7**
Informed consent	X		
Demographics	X		
Physical examination	X		
Medical history	X		
Laboratory evaluation	X	X	X
Microbiology labs	X	X	X
Study drug administration		X	
PK sampling		X	
Concomitant medications	X	X	X
Adverse events		X	X

*Day 0 refers to time point prior to start of study drug but may be the same calendar date as day 1.
^Up to 10 days of study drug

6.2. Screening

Research staff will screen potential participants for eligibility requirements per local institutional policies.

6.3. Baseline/Pre-Dose Assessment (Day 0/1)

After the parent or legal guardian has signed the IRB/REB-approved consent form and after it has been determined that the participant satisfies all inclusion and no exclusion criteria, participants will be assigned a study ID and to a dosing group, and the following evaluations will be recorded in the electronic case report form (eCRF):

1. Participant demographics, including sex, date of birth, race, ethnicity, gestational age at birth, and birth weight (Groups 1–5).
2. Targeted physical examination: length, actual weight within 24 hours prior to randomization / first study drug dose* (Groups 1- 5).
3. Active medical history (from chart): type of complicated intra-abdominal infection (Groups 1–4) and current medical conditions (Groups 1–5).
4. Laboratory determinations within 48 hours prior to randomization / first study drug dose* (Groups 1- 5) if performed per local standard of care (Section **7.9.** Laboratory Evaluations). If serum creatinine was not collected as standard of care, it will be collected for this study to confirm eligibility (Groups 1–5), except for patients on standard of care dosing in Group 5.
5. Microbiologic determinations within 48 hours prior to randomization / first study drug dose* (Groups 1- 5) if performed per local standard of care (Section **7.9.** Laboratory Evaluations).
6. Abdominal radiographic findings within 48 hours prior to randomization / first study drug dose* (Groups 1- 4) if performed per local standard of care (Section **7.10.** Abdominal Imaging Evaluations).
7. Abdominal surgical procedures within 48 hours prior to randomization / first study drug dose* (Groups 1- 4) if performed per local standard of care (Section **7.11.** Abdominal Surgical Evaluations).
8. Biomarker sampling (urinary I-FABP/creatinine) once within 48 hours prior to randomization / first study drug dose* (Groups 1- 4) (**7.7.** Biomarker Sampling (Urinary I-FABP/Creatinine).
9. Genetic sampling once at any time during the study (Section **7.6.** Whole Blood Sampling for Genetic Analysis) (Groups 1, 2 or4).
10. Concomitant medications (except for antibiotics) within 48 hours prior to randomization / first study drug dose* (Groups 1- 5) and antibiotics from the beginning of the hospital admission (Section **7.12.** Concomitant Medications).
11. Efficacy end point assessments closest to the time of randomization / first study drug dose* (Groups 1- 4) (Section **7.3.** Efficacy)
12. Stool collection within 48h prior to randomization / first study drug dose* (Groups 1- 4) (Section **7.8.** Stool Samples (Intestinal Microbiota).

If several laboratory determinations are obtained within 48 hours prior to the first dose of study drug, record the value closest to study drug dose administration.

*Randomization time should be referenced for participants randomized to Groups 1-3; time of first study drug dose should be referenced for non-randomized participants in Groups 1-5

6.4. Treatment Assessments/Procedures (Day 1–10)

The following assessments will be recorded in the eCRF:

1. Laboratory determinations performed per local standard of care (Section **7.9.** Laboratory Evaluations) (Groups 1–5).
2. Microbiologic determinations performed per local standard of care (Section **7.9.** Laboratory Evaluations) (Groups 1–5).
3. Abdominal radiographic findings performed per standard of care (Section **7.10.** Abdominal Imaging Evaluations) (Groups 1–4).
4. Abdominal surgical procedures performed per standard of care (Section **7.11.** Abdominal Surgical Evaluations) (Groups 1–4).
5. Study medication administration: The start date and time, stop date and time, and amount (total mg) for each dose will be recorded. Day 1 will be day of first dose of study drug. (Groups 1–5).
6. PK sampling will be performed any day of the treatment period when routine laboratory samples are collected (Section **7.5.** PK Sampling—Dried Blood Spot /Plasma, and CSF). If collected following the last dose of study drug, PK samples should be obtained within 24 hours of the last dose of study drug (Groups 1–4).
7. CSF will be collected any day of the treatment period or within 24 hours of the last dose of study drug only if performed per routine medical care (Groups 1–5). Blood PK sampling will be performed within 1 hour of collection of the CSF PK sample (Section **7.5.** PK Sampling—Dried Blood Spot /Plasma, and CSF). This blood sample can be collected for research purposes.
8. Biomarker sampling (urinary I-FABP/creatinine) once daily (Section **7.7.** Biomarker Sampling (Urinary I-FABP/Creatinine) (Groups 1–4).
9. Genetic sampling once at any time during the study (Section **7.6.** Whole Blood Sampling for Genetic Analysis) (Groups 1, 2 or4).
10. Efficacy endpoint assessments on last day of study drug (Section **7.3.** Efficacy) (Groups 1–4).
11. Concomitant medications daily (Section **7.12.** Concomitant Medications) (Groups 1–5).
12. AEs (Section **7.1.** Safety Reporting) (Groups 1–5).
13. Outcomes of special interest (Section **7.4.** Outcomes of Special Interest) (Groups 1–4).
14. Stool collection -(Section **7.8.** Stool Samples (Intestinal Microbiota)) (Groups 1–4).

If available, record laboratory determinations daily; if several laboratory determinations are available for the same day, record the first value of the day. If several abdominal radiographic findings are available for the same day, record the first finding of the day.

6.5. Follow-up Assessments (Day 1–90 post therapy, Groups 1–4; Day 1–7 post therapy, Group 5)

1. Laboratory determinations within 72 hours following last dose of study drug if performed per local standard of care (Section **7.9.1.** Laboratory Determinations) (Groups 1–5).
2. Microbiologic determinations during the 90 days (Groups 1–4) or 7 days (Group 5) following last dose of study drug if performed per local standard of care (Section **7.9.2.** Microbiological Determination) (Groups 1–5).
3. Abdominal radiographic findings during the 90 days following last dose of study drug if performed per local standard of care (Section **7.10.** Abdominal Imaging Evaluations) (Groups 1–4).
4. Abdominal surgical procedures during the 90 days following last dose of study drug if performed per local standard of care (Section **7.11.** Abdominal Surgical Evaluations) (Groups 1–4).
5. Biomarker sampling (urinary I-FABP/creatinine) once within 72 hours following last dose of

study drug (Section **7.7.** Biomarker Sampling [Urinary I-FABP/Creatinine]) (Groups 1–4).
6. Genetic sampling once at any time during the study (Section **7.6.** Whole Blood Sampling for Genetic Analysis) (Groups 1–4).
7. Efficacy assessments: Overall success 30 days and gastrointestinal 90 days following last dose of study drug (Section **7.3.** Efficacy) (Groups 1–4).
8. Concomitant medications (except for antibiotics) within 30 days after last dose of study drug and antibiotics within 90 days after last dose of study drug (Section **7.12.** Concomitant Medications) (Groups 1–4). Group 5 will have concomitant medications recorded for 7 days after the last dose of study drug.
9. AEs and serious adverse events (SAEs) during 72 hours and 30 days, respectively, following last dose of study drug (Section **7.1.** Safety Reporting) (Groups 1–4). Group 5 will have concomitant SAEs for 7 days after the last dose of study drug.
10. Outcomes of special interest 90 days following last dose of study drug (Section **7.4.** Outcomes of Special Interest) (Groups 1–4).
11. Stool collection 2–4 weeks following the last dose of study drug (Section **7.8.** Stool Samples (Intestinal Microbiota). (Groups 1–4).

If several laboratory determinations are obtained within 72 hours following the last dose of study drug, record the value closest to the last study drug administration.

If participant is discharged or transferred prior to the pre-specified time of collection of follow-up assessments, collect information at the time of discharge/transfer.

7. DEFINITIONS AND DETAILED DESCRIPTIONS OF ASSESSMENTS

7.1. Safety Reporting

Safety events include adverse events and outcomes of special interest (Section **7.4.** Outcomes of Special Interest).

Methods and Timing for Collecting Safety Parameters
Safety events and outcomes of special interest will be recorded according to the schedule of study procedures (Table 10, Section **6.1.** Summary of Procedures).

Definitions:

An adverse event or adverse drug reaction 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 (SAE) or **serious suspected 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

The **severity** of an adverse event is determined by the investigator, who should use the following definitions when assessing the 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

An **unexpected** event is when the specificity or severity of the event is not consistent with the package inserts or investigational brochure for the drugs under study.

Causality is determined by the following question, 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 specific adverse event being assessed.

Identification of Safety Events
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 the worsening in frequency or intensity of pre-existing conditions will be reported as adverse events (Table 10, Section **6.1.** Summary of Procedures). 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 eCRF. Each event will be recorded on an AE eCRF starting after first dose of study drug has been delivered. The investigator will provide date of onset and resolution, severity, action(s) taken, changes in study drug dosing, causality to study drug, and outcome.

7.1.1. Follow-up of AEs
Any safety event that is identified at the last assessment (or an early termination) must be recorded on the appropriate eCRF with the status of the safety event noted. All serious suspected adverse reactions and serious adverse reactions will be followed until resolution or until the patient is medically stable. All other events that cannot be resolved by 30 days after the safety monitoring period (30 days for Groups 1-4 and 7 days for Group 5) will be considered resolved by convention and entered in the electronic data capture (EDC) system as such.

7.1.2. Safety Monitoring
The data coordinating center (DCC) will monitor safety during the conduct of the study. The Best Pharmaceuticals for Children Act (BPCA) medical monitor will review all outcomes of special interest and SAEs at the time they are reported. Safety monitoring will be done throughout the study on an ongoing basis. The BPCA data monitoring committee (DMC) will monitor safety on a periodic basis as per the DMC charter. Annual reports will be submitted to federal authorities as required. The DCC data management personnel will monitor the occurrence of the events listed above and notify the DMC, safety monitors, investigators, National Institutes of Health (NIH)/National Institute of Child Health and Human Development (NICHD), and Pediatric Trials Network (PTN) staff if the halting criteria are met. The DMC may request review of the data if deemed necessary. FDA, NIH/NICHD, Health Canada (HC**)** or the principal investigator may terminate the study at any time based on safety concerns. Enrollment will continue during DMC review of safety unless otherwise specified.

7.2. Reporting Procedures
The clinical site: Clinical and safety laboratory data will be entered into the DCC AdvantageEDC^{SM} at the study site within 7 business days of data acquisition. Outcomes of special interest and SAEs will be entered into the data system within 7 days and 24 hours of identification, respectively. If there are any technical difficulties, SAEs will be reported to the DCC by fax communication. These data will be entered into the EDC as soon as they are available. Investigators must also submit safety reports locally as required by their IRB/REB.

The DCC and study sponsor: Any outcome of special interest or SAE entered in the EDC will generate an automatic email notification to the DCC, IND sponsor, and NIH/NICHD. Any event, excluding outcomes of special interest that requires expedited reporting based on federal regulations will be forwarded to the IND sponsor. or the in-country designee. The IND sponsor or its representative will submit expedited safety reports (e.g. IND safety reports) to the regulatory agencies as necessary, and will inform the investigators of such regulatory reports. Site investigators must submit expedited safety reports as required by their IRB/REB. Documentation of the submission and receipt by the IRB/REB must be retained for each expedited safety report. All serious events designated as “not related” to study product(s) and all pre-specified safety events will be reported to the FDA/regulatory agencies at least annually in a summary format.

7.3. Efficacy

Investigators will complete assessment of efficacy. Two efficacy end points will be assessed:

7.3.1. Gastrointestinal

The following will be recorded

- Time to first full enteral feeds (≥100 mL/kg/ day)

7.3.2. Overall Therapeutic Success

Success will be defined as all of the following:

- Alive
- Negative bacterial blood cultures
- Clinical cure score >4

Failure will be defined by any of the following:

- Death
- Positive bacterial cultures
- Clinical cure score ≤4

The presumptive clinical cure score will be derived using clinical signs and symptoms. The clinical findings are based on the components of the SNAP II and other items listed below. The clinical cure score at 30 days after last dose of study drug will be used to establish efficacy. The blood culture result closest to (prior to) the 30-day assessment will be used to assess overall therapeutic success.

Clinical cure score

The elements of the presumptive clinical score are:

1. Fraction of inspired oxygen (FiO_2)
2. Urine output
3. Cardiovascular inotrope support
4. Need for mechanical ventilation
5. Presence of seizure
6. Lowest serum pH

Record values of each element of the clinical cure score. Scoring will be performed according to Table 12.

Table 12. Elements of the clinical cure score

Element	Score
FiO_2 ≤ baseline FiO_2	1
Urine output ≥1 mL/kg/h for 24-hour period prior to assessment	1
Absence of inotropic support at time of assessment	1
Absence of mechanical ventilation at time of assessment	1
No seizure in 24-hour period prior to assessment	1
pH ≥7.25 or not measured in 24 hours prior to assessment	1

7.4. Outcomes of Special Interest

Outcomes of special interest include:

1. Gastrointestinal surgeries
2. Progression to a higher stage of NEC, if NEC is the cause of the complicated intra-abdominal infection
3. Intestinal strictures
4. Intestinal perforation
5. Positive blood culture (bacterial or fungal)
6. Short bowel syndrome
7. Seizures
8. Death
9. Intraventricular hemorrhage (IVH) grade 3 or 4
10. Feeding intolerance

These will be captured on specific eCRFs to provide uniform data collection and will not be reported separately as AEs or SAEs unless the event(s) are study drug regimen-related. They will be included in all safety reports describing the overall safety of the trial. As these are pre-specified outcomes of special interest, they will not be reported in an expedited manner to the FDA but will be reviewed by the medical monitor and the BPCA DMC convened by NICHD and reported to the FDA in the annual report. Spontaneous reports of other AEs and SAEs will be reported in a standard manner as described above.

Gastrointestinal surgeries: As determined by medical history and confirmed with hospital records. Medical history will be sufficient if medical records are not available. This includes laparotomy, peritoneal drain placement, intestinal resection, ostomy placement, and intestinal anastomosis.

Progression to a higher stage of NEC, if NEC is the cause of complicated intra-abdominal infection: As determined by the scoring in Table 1 (Section **1.** Background).

Intestinal stricture: Radiology reports leading to the diagnosis of intestinal stricture. These include plain abdominal x-rays, upper gastrointestinal series with small bowel follow-through, contrast enema studies, and computed tomography scans of the abdomen and pelvis.

Operative reports documenting surgical procedures leading to the diagnosis and/or treatment of intestinal stricture. These procedures include endoscopy, laparotomy, stricture dilatation, intestinal resection, and ostomy placement.

Intestinal perforation: Radiological reports leading to the diagnosis of intestinal perforation. These include plain chest x-rays, plain abdominal x-rays, ultra-sonograms of the abdomen, contrast studies, and computed tomography scans of the abdomen and pelvis.

Operative reports documenting surgical procedures leading to the diagnosis and/or treatment of intestinal perforation. These include placement of a surgical drain, laparotomy, intestinal resection, and ostomy placement.

Short bowel syndrome: Operative reports documenting resection of bowel, estimated bowel length, and absence/presence of the ileocecal valve.

Operative reports documenting surgical procedures meant to palliate short bowel syndrome. These include serial transverse enteroplasty (STEP) and longitudinal intestinal lengthening and tapering (LILT) procedures.

Documentation of feeding history, including duration on total parenteral nutrition after bowel resection, feeding schedule with volume of enteral feeds given, and evidence of feed intolerance or malabsorption, such as diarrhea or dumping syndrome.

Total parenteral nutrition for >42 consecutive days after bowel resection, or a residual small bowel length of less than 25% expected for gestational age.[70]

Death: The following will be collected in the event of death:

1. Medical records within 3 days of death. Records will include daily progress notes, radiological reports, operative reports, laboratory data (chemistry, hematology, and microbiology), pathological reports, and medication administration records.
2. Hospital discharge summary
3. Autopsy report
4. Brief narrative of events leading to death

Grade 3 IVH: Subependymal hemorrhage with extension into lateral ventricles with ventricular enlargement

Grade 4 IVH: Intraparenchymal hemorrhage

Feeding intolerance:
Documentation of any feedings held for >24 consecutive hours in infants being fed.

7.4.1. Adjudication Committee

A blinded adjudication committee consisting of 3 members will review data provided by the site to make the following determinations for outcomes of special interest: 1) presence of intestinal strictures, intestinal perforation, short bowel syndrome and 2) cause of mortality. This assessment will be done at the end of the study.

7.5. PK Sampling—Dried Blood Spot/Plasma and CSF

PK samples can be collected after consent. PK samples will be obtained at the same time as laboratory tests collected per routine medical care during study treatment (Groups 1–4, see below for Group 5). PK blood samples will not be drawn specifically for research purposes in this study (Groups 1–4). Table 13 below provides optimal PK sampling points that will be used to guide sampling. Every effort should be made to collect PK 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. Elimination samples will only be obtained after the last dose of study drug if the drug is not continued by the treating medical team beyond the study period. Each drug's concentrations in whole blood (dried blood spot) will be measured at a central lab using a

validated bioanalytical assay requiring 60 µL of whole blood for analysis. Whole dried blood spot (DBS) samples will be collected on blotting paper. If whole dried blood spot is not validated for clindamycin by the time the first participant is enrolled, collect plasma and dried blood spot (see study MOP for details) for infants enrolled in Group 2. Plasma samples will be collected in EDTA-containing tubes. A maximum of 7 blood PK samples will be obtained per participant.

Table 13. Optimal PK sampling collection windows for all study drugs by dosing interval (refer to MOP for details regarding prioritization of drugs for Groups 1–3)

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

*After end of flush.

CSF PK samples will be collected per routine medical care, and the sources of CSF can include lumbar puncture, ventriculoperitoneal shunt, or externalized ventricular device. No child will undergo a procedure to collect CSF solely for the purposes of this study. At least 100 µL of CSF will be collected for analysis. One blood PK sample (DBS for Groups 1, 3, 4, 5a, 5c; DBS and plasma for Groups 2 and 5b.) will be collected within 1 hour after collection of each CSF PK sample at the time of standard of care laboratory tests. Group 5 blood PK sample can be collected with standard of care laboratory tests or outside routine medical care (opt-in informed consent). Up to 5 CSF/5 blood samples per infant may be collected up to 24 hours following the last dose of study drug.

7.6. Whole Blood Sampling for Genetic Analysis

One whole blood sample (as an opt-in) will be obtained during the study. Whole blood samples for genetic analysis will not be stored/banked. Leftover sample after genetic analysis will be discarded. The DNA sample will be identified by a code number, and all other identifying information will be removed. A targeted genetic testing approach evaluating genetic polymorphisms of cytochrome P450 enzymes potentially involved with metronidazole or clindamycin metabolism will be conducted. Caregivers of participants will not be informed of genetic results. This test applies only to participants in Groups 1, 2, or 4. Whole blood sample will not be collected from participants in Groups 3 or 5.

7.7. Biomarker Sampling (Urinary I-FABP/Creatinine)

Urine samples for I-FABP/creatinine determination can be collected at any time during the day prior, during, and after the treatment phase according to study procedures (Table 10, Section **6.1.** Summary of Procedures). A maximum of 1 urine sample will be collected per day, with a maximum of 12 total per participant. Every effort should be made to collect biomarker samples; however, lack of collection of these samples will not be considered protocol deviations. Urine samples can be scavenged from the local laboratory within 24 hours of collection. Urine I-FABP/creatinine will be measured using a validated assay at the central laboratory. Sample collection details will be included in the MOP.

7.8. Stool Samples (Intestinal Microbiota)

Stool samples can be collected from diapers before, during, and after the treatment phase according to study procedure (Section **6.1.** Summary of Procedures). A maximum of 3 stool samples per infant will be collected. Intestinal microbiota will be determined by 16s rDNA sequence analysis. Every effort should be made to collect stool samples; however, lack of collection of these samples will not be considered protocol deviations. Sample collection details will be included in the MOP.

7.9. Laboratory Evaluations

7.9.1. Laboratory Determinations

The following hematologic values will be collected: hematocrit, white blood cell (WBC) count, platelet count, and differential. The following serum chemistry values will be collected: blood urea nitrogen (BUN), serum creatinine, potassium, sodium, AST, ALT, total bilirubin, and albumin.

7.9.2. Microbiological Determination

Results for all bacterial cultures obtained from sterile body fluids (blood, CSF, urine obtained by catheterization or supra-pubic tap, peritoneal fluid) will be collected. Anaerobic CSF cultures are encouraged at the site and, if performed per standard of care, results will be recorded.

7.10. Abdominal Imaging Evaluations

The following abdominal findings on imaging evaluations will be recorded if obtained per routine medical care:

1. Pneumatosis intestinalis
2. Portal venous gas
3. Pneumoperitoneum (abdominal cavity gas)
4. Strictures
5. Abscess

7.11. Abdominal Surgical Evaluations

The following abdominal surgeries will be recorded:

1. Laparotomy
2. Peritoneal drain placement
3. Intestinal resection
4. Ostomy placement
5. Intestinal anastomosis

For infants who require laparotomy, extent of disease found at operation will be recorded.

7.12. Concomitant Medications

Concomitant medications and antibiotics will be recorded prior, during and after study drug administration according to the schedule of assessments (see Table 10, Section **6.1.** Summary of Procedures; also Section **19**. Appendix I: Concomitant Antibiotics).

8. 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 BPCA DCC as well as Duke Clinical Research Institute's (DCRI) (sponsor) standard operating procedures. The IND sponsor, or as detailed in the Transfer of Regulatory Obligations, the BPCA DCC, NIH/NICHD, or its designee will conduct site-monitoring visits as detailed in the site monitoring plan or in the MOP.

Site visits will be made at standard intervals as defined by the site monitoring plan 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.

9. STATISTICAL METHODOLOGY

9.1. Study Outcome Measures

9.1.1. Primary Outcomes

Safety: Death within 30 days will be the primary safety end point.

9.1.2. Secondary Outcomes

Safety: The outcomes of special interest as described in Section **7.4.** Outcomes of Special Interest (excluding death) and additional AEs reported during and after study drug administration.

Efficacy:
a. Efficacy end points (Section **7.3.** Efficacy)
b. Success or failure of therapeutic regimen (Section **7.3.** Efficacy)

PK end points:
a. Clearance (CL)
b. Volume of distribution (V)

Biomarker end points:
a. Urine I-FABP/creatinine concentrations

9.2. Sample Size

The study is powered to estimate the incidence of death within 30 days for each drug regimen. The incidence of death in this population is estimated to be 20%. A sample size of 70 will allow us to estimate the 20% death rate with a 95% confidence interval (CI) that has a precision (half-width) of 10% (95% CI; 11.4%, 31.3%).

9.3. Population to be Analyzed

Participants who receive at least 1 dose of study drug will be included in the intention-to-treat (ITT) population used for the safety (Groups 1–5) and efficacy analyses (Groups 1–4). In ITT analyses, participant treatment assignments will be based on the original randomization regardless of changes to the treatment regimen. Safety and efficacy analyses will also be conducted based on final group assignment (per-protocol population). The ITT and per-protocol populations will be separated into full (randomized and non-randomized), randomized, and non-randomized populations.

Infants in any group with at least 1 evaluable PK sample will be included in the PK analysis. Infants who are early terminated or lost to follow-up will not be replaced. All available data from early-terminated infants will be included.

9.4. Statistical Methodology

Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, minimum, and maximum will be presented for continuous variables (such as age, weight, etc.). Other descriptive statistics such as counts, proportions, and/or percentages will be presented to summarize discrete variables (such as race, sex, etc.). All descriptive analyses will be presented by appropriate treatment group (ITT or per-protocol) and overall. A 95% confidence level will be used for confidence intervals. A detailed description of statistical methods and secondary analyses will be prepared and presented in the statistical analysis plan prior to data lock for final analyses.

9.4.1. Demographics and Baseline Characteristics

The number of participants who either completed or discontinued early from the study will be summarized. Demographic and baseline characteristics, will be summarized and compared between treatment arms. Characteristics will be summarized for randomized and non-randomized participants separately and together for Groups 1-3. Study drug administration will be summarized in terms of number of days of dosing.

9.4.2. Safety

The primary safety analyses will describe the incidence of death, and other outcomes of special interest, within each treatment group. Data will be analyzed for both ITT and per-protocol populations. Exact 95% confidence intervals will be presented. No statistical adjustment in confidence levels for multiple outcomes will be performed. The Fisher's exact test will be used to compare outcomes of special interest between treatment groups.

As a secondary analysis of Groups 1-3, logistic regression models may be developed to identify independent predictors of outcomes of special interest and as part of a post-hoc analysis to control for differences between randomized and non-randomized participants. Demographic and baseline characteristics of interest for this analysis may include age, gestational age, birth weight, sex, and race.

Additional safety analyses will include summaries of treatment-emergent AEs, descriptive statistics of laboratory values, frequency distributions and shift tables of laboratory values classified based on toxicity grades, descriptive statistics of vital signs, and frequency distributions of abnormal physical examinations.

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 drug class. Laboratory data, such as hematology and serum chemistry data will be tabulated by treatment group. Summary statistics for changes from baseline will be presented. 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.

9.4.3. Efficacy

Analyses of efficacy of the treatment regimens will include estimation of overall therapeutic success and time to full enteral feeds (see Section **7.3.** Efficacy). Efficacy will be evaluated for both ITT and per-protocol population. Point estimates and 95% confidence intervals will be calculated.

9.4.4. PK Analysis

Population PK methodologies using nonlinear mixed effects modeling in NONMEM will be used to analyze the drug concentration data. The influence of covariates on PK parameters will be explored. Final PK models will be evaluated using bootstrapping and visual predictive checks. Monte Carlo simulations will be used to evaluate optimal drug exposure. The relationship between drug exposure and safety events will be evaluated by calculating the proportion of participants with AEs and SAEs at different exposure levels. The association between genetic polymorphisms in cytochrome P450 enzymes and metronidazole and clindamycin exposure will be explored.

9.4.5. Biomarker Analysis (Urine I-FABP/Creatinine)

The association between urinary I-FABP/creatinine concentrations and severity of complicated intra-abdominal infections and antibiotic exposure will be explored using standard statistical correlation methodologies.

9.4.6. Intestinal Microbiota

Profiles of intestinal microbiota will be described including the number and types of microorganisms. These profiles will be compared between treatment arms.

10. HALTING OR DISCONTINUATION

10.1. Individual Discontinuation

Study drug therapy must be withdrawn if 1 of the following occurs:

1. Participant develops a serious adverse reaction (i.e., an acute anaphylactic reaction) to the study drug
2. Legal guardian declines further study participation
3. The investigator decides it is in the participant's best interest to discontinue treatment with study medication
4. Treating physician adds ampicillin, metronidazole, clindamycin, or piperacillin-tazobactam to the study drug regimen (does not apply to Groups 4 and 5)
5. Treating physician discontinues one of the study products (ampicillin, metronidazole, clindamycin, piperacillin-tazobactam) for >48 hours before completing 10 days (does not apply to Group 5. For Group 4, only metronidazole applies)

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 eCRF.

For Groups 1–4, if the study drug therapy is discontinued, the participant must be followed until at least 90 days after the last dose for safety or until discharge/transfer. For Groups 1–5, 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 (refer to Section **7.1.1.** Followup of AEs). 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 investigator 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.

10.2. Study Halting Criteria

Participant safety data will be reviewed on an ongoing basis to monitor for halting criteria. For Groups 1–4, the study enrollment and dosing will be halted for 1 or more of the drug regimens in this study for a safety review if serious suspected adverse reactions or serious adverse reactions occur in more than 20% of participants after at least 10 participants per cohort have been enrolled. Enrollment to any remaining drug(s) will continue. This information will be submitted to the BPCA DMC, NICHD FDA and HC along with an analysis and future plans for the study.

For Groups 1–5, the NICHD, the IND sponsor, the DMC, and the investigator shall have the right to recommend termination of 1 or more of the drug regimens 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

Termination of the study at any site may also occur at the recommendation of the NICHD, the IND sponsor, the DMC, or the investigator at any time. Possible reasons for termination of the study at a site include, but are not limited to:

1. Inaccurate or incomplete data collection
2. Falsification of records
3. Failure to adhere to the protocol

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 biological sample tests, in addition to the clinical information relating to 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 personal health information (PHI) obtained during this research study complies with the federal privacy regulations. For Canadian sites, these requirements are described in the Canadian Institutes of Health Research (CIHR) Best Practices for Protecting Privacy in Health Research guidelines and in the U.S., the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule applies. The rule provides U.S. federal protection for the privacy of PHI sent to or collected in the U.S. for purposes of this research by implementing standards to protect and guard against the misuse of individually identifiable health information of persons participating in clinical trials. “Authorization” is required from each research participant (i.e., specific permission granted by an individual to a U.S. covered entity for the use or disclosure of an individual’s PHI). A valid authorization must meet the implementation specifications under the CIHR guidelines or the HIPAA Privacy Rule, whichever applies. The relevant privacy authorization will be obtained during the informed consent process.

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/REB-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 and time 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 signed copy of the informed consent document will be given to the participants' legal guardian(s). 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/REB requirements. The informed consent document will be updated, and participants' legal guardians will be re-consented, if necessary.

Site staff may employ IRB/REB-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

An eCRF will be used to record participant data. The eCRF will be used for the recording of all historical participant information and study data as specified by this protocol. The eCRF must be completed by designated and trained study personnel. The eCRF 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, participants' 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, participant 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
- Current study protocol
- Protocol amendments (if applicable)
- MOP (if applicable)
- Informed consent form (blank)
- Signed informed consent forms
- Revised informed consent forms and/or all addenda (blank)
- Department of Health and Human Services number for IRB or other documentation of IRB compliance with FDA regulations (if applicable)
- Documentation of IRB/REB approval of protocol, consent form, any protocol amendments, and any consent form revisions
- Annual IRB/REB updates and approvals
- Correspondence between the investigator and IRB/REB

The 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.

14. 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 documents 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, GCP, 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.

15. ETHICS/PROTECTION OF HUMAN PARTICIPANTS

15.1. Ethical Standard

The investigator will ensure that the study will be conducted in accordance with the protocol, the ethical principles of GCP (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.

15.2. Institutional Review Board/Research Ethics 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 (or provide favorable opinion) by the appropriate IRB/REB.

The responsible official for the IRB/REB will sign the 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/REB's composition and the institution's federal-wide assurance number (if applicable) 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/REB.

15.3. 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 legal guardians must sign an informed consent form that complies with the federal regulatory and privacy requirements before entering the trial. A consent form that complies with the federal requirements and a separate authorization form that complies with federal privacy regulations 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.** Informed Consent Process.

16. 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 federal and provincial laws, and the
ICH: GCP: Consolidation Guideline. The investigator is responsible for informing the IRB/REB of any safety issues related to the study and the study drug, including reports of SAEs, if required, and all expedited 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.

16.1. 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.

16.2. 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.

16.3. Types of Data

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

16.4. Timing/Reports

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

16.5. 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 per federal or provincial regulations. No study records will be destroyed without prior authorization from the sponsor.

16.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP 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 GCP:

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.

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/REB per their guidelines. The site principal investigator/study staff is responsible for knowing and adhering to their IRB/REB requirements.

16.7. 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 federal privacy regulations. For Canadian sites, these requirements are described in the Canadian Institutes of Health Research (CIHR) Best Practices for Protecting Privacy in Health Research guidelines and in the U.S., the HIPAA Privacy Rule applies. The HIPAA Privacy Rule applies to all PHI sent or collected in the U.S. for purposes of this research and provides 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 CIHR guidelines or the HIPAA Privacy Rule, whichever applies. The relevant privacy authorization will be obtained during the informed consent process.

17. PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal under the oversight of the Publications Committee of the Pediatric Trials Network. The PTN Publications 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 Publications Committee per the Publications Committee charter.

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

The PTN Publications 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. Refer to: http://publicaccess.nih.gov/ and http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033.html.

18. REFERENCES

1. Blot S, De Waele JJ. Critical issues in the clinical management of complicated intra-abdominal infections. Drugs. 2005; 65(12): 1611-20.
2. Marshall JC. Intra-abdominal infections. Microbes and infection. Institut Pasteur. 2004; 6(11): 1015-25.
3. De Waele JJ, Hoste EA, Blot SI. Blood stream infections of abdominal origin in the intensive care unit: characteristics and determinants of death. Surg Infect (Larchmt). 2008; 9(2): 171-7.
4. Lee JS, Polin RA. Treatment and prevention of necrotizing enterocolitis. Semin Neonatol. 2003; 8(6): 449-59.
5. Ballance WA, Dahms BB, Shenker N, Kliegman RM. Pathology of neonatal necrotizing enterocolitis: a ten-year experience. J Pediatr. 1990; 117(1 Pt 2): S6-13.
6. Mai V, Young CM, Ukhanova M, et al. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS One. 2011;6:e20647.
7. Wang Y, Hoenig JD, Malin KJ, et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J. 2009;3:944-54.
8. Morrow AL LA, Schibler KR, Taft DH, Yu Z, Wang B, Altaye M, Wagner M, Gevers D, Ward DV, Kennedy MA, Huttenhower C, Newburg DS. Early microbial and metabolomic signatures predict later onset of necrotizing enterocolitis in preterm infants. Microbiome. 2013;1.
9. Bell MJ, Shackelford P, Smith R, Schroeder K. Pharmacokinetics of clindamycin phosphate in the first year of life. J Pediatr. 1984; 105(3): 482-6.
10. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am. 1986; 33(1): 179-201.
11. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978; 187(1): 1-7.
12. Coates EW, Karlowicz MG, Croitoru DP, Buescher ES. Distinctive distribution of pathogens associated with peritonitis in neonates with focal intestinal perforation compared with necrotizing enterocolitis. Pediatrics. 2005; 116(2): e241-6.
13. Brook I, Frazier EH. Aerobic and anaerobic microbiology in intra-abdominal infections associated with diverticulitis. J Med Microbiol. 2000; 49(9): 827-30.
14. Barrett FF, Mason EO, Jr., Fleming D. Brief clinical and laboratory observations. J Pediatr. 1979; 94(5): 796-800.
15. Solomkin JS, Yellin AE, Rotstein OD, Christou NV, Dellinger EP, Tellado JM, et al. Ertapenem versus piperacillin/tazobactam in the treatment of complicated intraabdominal infections: results of a double-blind, randomized comparative phase III trial. Ann Surg. 2003; 237(2): 235-45.
16. Solomkin JS, Mazuski JE, Bradley JS, Rodvold KA, Goldstein EJ, Baron EJ, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis. 2010; 50(2): 133-64.
17. Bell MJ, Ternberg JL, Bower RJ. The microbial flora and antimicrobial therapy of neonatal peritonitis. J Pediatr Surg. 1980; 15(4): 569-73.

18. Cotten CM, McDonald S, Stoll B, Goldberg RN, Poole K, Benjamin DK, Jr. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics. 2006; 118(2): 717-22.
19. Boggan JC, Navar-Boggan AM, Jhaveri R. Pediatric-specific antimicrobial susceptibility data and empiric antibiotic selection. Pediatrics. 2012; 130(3): e615-22.
20. Hodgman T, Dasta JF, Armstrong DK, Visconti JA, Reilley TE. Ampicillin-associated seizures. South Med J. 1984; 77(10): 1323-5.
21. 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.
22. 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.
23. Colburn WA, Gilbaldi M, Yoshioka H, Takimoto M, Riley HD Jr. Pharmacokinetic model for serum concentrations of ampicillin in the newborn infant. J Infect Dis. 1976; 134(1):67-9.
24. Foulds G, McBride TJ, Knirsch AK, Rodriguez WJ, Khan WN. Penetration of sulbactam and ampicillin into cerebrospinal fluid of infants and young children with meningitis. Antimicrob Agents Chemother.1987; 31(11): 1703-5.
25. Giachetto G, Pirez MC, Nanni L, Martinez A, Montano A, Algorta G, et al. Ampicillin and penicillin concentration in serum and pleural fluid of hospitalized children with community-acquired pneumonia. Pediatr Infect Dis J. 2004; 23(7): 625-9.
26. Hermans J, Driessen O, Sorgedrager N, Spoor M. Pharmacokinetic analysis of ampicillin concentration in neonates: comparison of two pharmacokinetic models and of two numerical methods. Arzneimittelforschung. 1975; 25(6): 947-9.
27. McCracken GH, Jr., Ginsburg CM, Clahsen JC, Thomas ML. Pharmacologic evaluation of orally administered antibiotics in infants and children: effect of feeding on bioavailability. Pediatrics. 1978; 62(5): 738-43.
28. Sutton AM, Turner TL, Cockburn F, McAllister TA. Pharmacokinetic study of sulbactam and ampicillin administered concomitantly by intraarterial or intravenous infusion in the newborn. Rev Infect Dis. 1986; 8 Suppl 5: S518-22.
29. Aldridge KE, Ashcraft D, Cambre K, Pierson CL, Jenkins SG, Rosenblatt JE. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of Bacteroides fragilis group, Prevotella, Fusobacterium, Porphyromonas, and Peptostreptococcus species. Antimicrob Agents Chemother. 2001; 45(4): 1238-43.
30. Uhari M, Seppanen J, Heikkinen E. Imipenem-cilastatin vs. tobramycin and metronidazole for appendicitis-related infections. Pediatr Infect Dis J. 1992; 11(6): 445-50.
31. Cohen-Wolkowiez M, Ouellet D, Smith PB, James LP, Ross A, Sullivan JE, et al. Population pharmacokinetics of metronidazole evaluated using scavenged samples from preterm infants. Antimicrob Agents Chemother. 2012; 56(4): 1828-37.
32. Suyagh M, Collier PS, Millership JS, Iheagwaram G, Millar M, Halliday HL, et al. Metronidazole population pharmacokinetics in preterm neonates using dried blood-spot sampling. Pediatrics. 2011; 127(2): e367-74.
33. Jensen JC, Gugler R. Single- and multiple-dose metronidazole kinetics. Clin Pharmacol Ther. 1983; 34(4): 481-7.
34. Hall P, Kaye CM, McIntosh N, Steele J. Intravenous metronidazole in the newborn. Arch Dis Child. 1983; 58(7): 529-31.

35. Upadhyaya P, Bhatnagar V, Basu N. Pharmacokinetics of intravenous metronidazole in neonates. J Pediatr Surg. 1988; 23(3): 263-5.
36. Liu CY, Huang YT, Liao CH, Yen LC, Lin HY, Hsueh PR. Increasing trends in antimicrobial resistance among clinically important anaerobes and Bacteroides fragilis isolates causing nosocomial infections: emerging resistance to carbapenems. Antimicrob Agents Chemother. 2008; 52(9): 3161-8.
37. Dougherty SH, Sirinek KR, Schauer PR, Fink MP, Fabian TC, Martin DH, et al. Ticarcillin/clavulanate compared with clindamycin/gentamicin (with or without ampicillin) for the treatment of intra-abdominal infections in pediatric and adult patients. Am Surg. 1995; 61(4): 297-303.
38. Faix RG, Polley TZ, Grasela TH. A randomized, controlled trial of parenteral clindamycin in neonatal necrotizing enterocolitis. J Pediatr. 1988; 112(2): 271-7.
39. Wynalda MA, Hutzler JM, Koets MD, Podoll T, Wienkers LC. In vitro metabolism of clindamycin in human liver and intestinal microsomes. Drug Metab Dispos. 2003; 31(7):878-87.
40. Koren G, Zarfin Y, Maresky D, Spiro TE, MacLeod SM. Pharmacokinetics of intravenous clindamycin in newborn infants. Pediatr Pharmacol (New York). 1986; 5(4): 287-92.
41. Maltezou HC, Nikolaidis P, Lebesii E, Dimitriou L, Androulakakis E, Kafetzis DA. Piperacillin/Tazobactam versus cefotaxime plus metronidazole for treatment of children with intra-abdominal infections requiring surgery. Eur J Clin Microbiol Infect Dis. 2001; 20(9): 643-6.
42. Flidel-Rimon O, Friedman S, Leibovitz E, Shinwell ES. The use of piperacillin/tazobactam (in association with amikacin) in neonatal sepsis: efficacy and safety data. Scand J Infect Dis. 2006; 38(1): 36-42.
43. Reed MD, Goldfarb J, Yamashita TS, Lemon E, Blumer JL. Single-dose pharmacokinetics of piperacillin and tazobactam in infants and children. Antimicrob Agents Chemother. 1994; 38(12): 2817-26.
44. Derikx JP, Evennett NJ, Degraeuwe PL, Mulder TL, van Bijnen AA, van Heurn LW, et al. Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis. Gut. 2007; 56(10): 1473-5.
45. Shah D, Sinn JK. Antibiotic regimens for the empirical treatment of newborn infants with necrotising enterocolitis. Cochrane Database Syst Rev. 2012; 8: CD007448.
46. Malangoni MA, Song J, Herrington J, Choudhri S, Pertel P. Randomized controlled trial of moxifloxacin compared with piperacillin-tazobactam and amoxicillin-clavulanate for the treatment of complicated intra-abdominal infections. Ann Surg. 2006; 244(2): 204-11.
47. Starakis I, Karravias D, Asimakopoulos C, Kolaras P, Nikolaidis P, Harlaftis N, et al. Results of a prospective, randomized, double blind comparison of the efficacy and the safety of sequential ciprofloxacin (intravenous/oral)+metronidazole (intravenous/oral) with ceftriaxone (intravenous)+metronidazole (intravenous/oral) for the treatment of intra- abdominal infections. Int J Antimicrob Agents. 2003; 21(1): 49-57.
48. Dupont H, Carbon C, Carlet J. Monotherapy with a broad-spectrum beta-lactam is as effective as its combination with an aminoglycoside in treatment of severe generalized peritonitis: a multicenter randomized controlled trial. The Severe Generalized Peritonitis Study Group. Antimicrob Agents Chemother. 2000; 44(8): 2028-33.

49. Jaccard C, Troillet N, Harbarth S, Zanetti G, Aymon D, Schneider R, et al. Prospective randomized comparison of imipenem-cilastatin and piperacillin-tazobactam in nosocomial pneumonia or peritonitis. Antimicrob Agents Chemother. 1998;42(11): 2966-72.
50. Nadler EP, Reblock KK, Ford HR, Gaines BA. Monotherapy versus multi-drug therapy for the treatment of perforated appendicitis in children. Surg Infect (Larchmt). 2003; 4(4): 327-33.
51. Rodriguez JC, Buckner D, Schoenike S, Gomez-Marin O, Oiticica C, Thompson WR. Comparison of two antibiotic regimens in the treatment of perforated appendicitis in pediatric patients. Int J Clin Pharmacol Ther. 2000; 38(10): 492-9.
52. St Peter SD, Tsao K, Spilde TL, Holcomb GW, 3rd, Sharp SW, Murphy JP, et al. Single daily dosing ceftriaxone and metronidazole vs standard triple antibiotic regimen for perforated appendicitis in children: a prospective randomized trial. J Pediatr Surg. 2008; 43(6): 981-5.
53. Cohen-Wolkowiez M, Poindexter B, Bidegain M, Weitkamp JH, Schelonka RL, Randolph DA, et al. Safety and Efficacy of Meropenem in Infants With Suspected or Complicated Intra-abdominal Infections. Clin Infect Dis. 2012; 55(11): 1495-502.
54. Clark RH, Gordon P, Walker WM, Laughon M, Smith PB, Spitzer AR. Characteristics of patients who die of necrotizing enterocolitis. J Perinatol. 2012; 32(3): 199-204.
55. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. Pediatrics. 2012; 129(2): e298-304.
56. Guillet R, Stoll BJ, Cotten CM, Gantz M, McDonald S, Poole WK, et al. Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. Pediatrics. 2006; 117(2): e137-42.
57. Ruegger C, Hegglin M, Adams M, Bucher HU. Population based trends in mortality, morbidity and treatment for very preterm- and very low birth weight infants over 12 years. BMC Pediatr. 2012; 12: 17.
58. Lemons JA, Bauer CR, Oh W, Korones SB, Papile LA, Stoll BJ, et al. Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics. 2001; 107(1): E1.
59. Holman RC, Stoll BJ, Curns AT, Yorita KL, Steiner CA, Schonberger LB. Necrotising enterocolitis hospitalisations among neonates in the United States. Paediatr Perinat Epidemiol. 2006; 20(6): 498-506.
60. Blakely ML, Lally KP, McDonald S, Brown RL, Barnhart DC, Ricketts RR, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: a prospective cohort study by the NICHD Neonatal Research Network. Ann Surg. 2005; 241(6): 984-9; discussion 9-94.
61. Janik JS, Ein SH, Mancer K. Intestinal stricture after necrotizing enterocolitis. J Pediatr Surg. 1981; 16(4): 438-43.
62. Hintz SR, Kendrick DE, Stoll BJ, Vohr BR, Fanaroff AA, Donovan EF, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. Pediatrics. 2005; 115(3): 696-703.
63. Salhab WA, Perlman JM, Silver L, Sue Broyles R. Necrotizing enterocolitis and neurodevelopmental outcome in extremely low birth weight infants <1000 g. J Perinatol. 2004; 24(9): 534-40.

64. van Vliet EO, de Kieviet JF, Oosterlaan J, van Elburg RM. Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. JAMA pediatrics 2013;167:662-8.
65. Nau R, Sorgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clinical microbiology reviews 2010;23:858-83.
66. Nau R, Sorgel F, Prange HW. Pharmacokinetic optimisation of the treatment of bacterial central nervous system infections. Clinical pharmacokinetics 1998;35:223-46.
67. Sullins AK, Abdel-Rahman SM. Pharmacokinetics of antibacterial agents in the CSF of children and adolescents. Paediatric drugs 2013;15:93-117.
68. Placzek M, Whitelaw A, Want S, Sahathevan M, Darrell J. Piperacillin in early neonatal infection. Archives of disease in childhood 1983;58:1006-9.
69. Warner JF, Perkins RL, Cordero L. Metronidazole therapy of anaerobic bacteremia, meningitis, and brain abscess. Archives of internal medicine 1979;139:167-9.
70. Wales PW, de Silva N, Kim J, Lecce L, To T, Moore A. Neonatal short bowel syndrome: population-based estimates of incidence and mortality rates. J Pediatr Surg. 2004; 39(5): 690-5.

19. APPENDIX I: Concomitant Antibiotics

The following antibiotics will be recorded as concomitant medications. All other non-antibiotic concomitant medications will also be recorded.

- Amikacin
- Amoxicillin
- Amoxicillin + Clavulanate
- Ampicillin*
- Ampicillin + Sulbactam
- Azithromycin
- Aztreonam
- Cefaclor
- Cefazolin
- Cefepime
- Cefotaxime
- Cefotetan
- Cefoxitin
- Ceftazidime
- Ceftriaxone
- Cefuroxime
- Cephalexin
- Ciprofloxacin
- Clindamycin*
- Erythromycin
- Gentamicin*
- Imipenem + Cilastatin
- Linezolid
- Meropenem
- Metronidazole*
- Nafcillin
- Oxacillin
- Penicillin G
- Penicillin V
- Piperacillin
- Piperacillin-Tazobactam*
- Rifampin
- Ticarcillin
- Ticarcillin + Clavulanate
- Tobramycin
- Vancomycin

*Report as concomitant medication unless administered as randomized study drug regimen, in which case report on the study drug dosing form.