Title:	**Multiple Dose Pharmacokinetic Study of Meropenem in Young Infants (<91 days) with Suspected or Complicated Intra-abdominal Infections**
Protocol Number:	**NICHD-2005-18**
Study Drug:	**Meropenem**
IND Number:	**101,043**
Phase:	**II**
Sponsor:	**NICHD**
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Final Date:	**28 March 2008**

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PROTOCOL SYNOPSIS

Protocol Title	Multiple Dose Pharmacokinetic Study of Meropenem in Young Infants (<91 days) with Suspected or Complicated Intra-abdominal Infections
Sponsor:	NICHD
Product	Meropenem
Objectives:	a. To characterize meropenem single-dose and multiple-dose PK in subjects with suspected or complicated intra-abdominal infections. b. To characterize the safety profile of meropenem in the treatment of suspected or complicated intra-abdominal infections. c. To assess collected efficacy data for meropenem for the treatment of suspected or complicated intra-abdominal infections.
Study Design:	Multi-center, prospective, pharmacokinetic and safety study of meropenem for the treatment of suspected or complicated intra-abdominal infections
Study Population:	Premature and term young infants (<91 days) who have a suspected or early complicated intra-abdominal infection. These subjects must be subdivided into the following four groups: Group 1: GA at birth below 32 weeks - PNA < 2 weeks; Group 2: GA at birth below 32 weeks - PNA ≥ 2 weeks and < 91 days; Group 3: GA at birth 32 weeks or older - PNA < 2 weeks; Group 4: GA at birth 32 weeks or older - PNA ≥ 2 weeks and < 91 days.
Number of Infants:	Approximately 200 infants that complete the study
Number of Sites:	Approximately 25
Treatment:	Meropenem Aminoglycoside should be administered in conjunction with meropenem. Additional antimicrobial coverage may be added per standard of care.
Treatment Duration	At least 3 days; otherwise, per local standard of care (maximum 21 days)
PK/PD:	Minimal sampling and population PK will be employed
Safety:	The protocol will rely on three mechanisms for safety: 1) The DSMB 2) Adverse event and SAE reporting mechanisms 3) The active, daily, real time oversight of the MPODS Clinical Safety Committee All serious adverse events (SAEs) will be closely monitored throughout the course of the study. Seizures will be intensely monitored and clinical laboratories of renal, and hepatic toxicity monitored weekly as available per local standard of care. Safety assessments will include seizure documentation (including correlation of serum meropenem level and seizures), physical examination, clinical laboratory values, LFTs, renal function and nosocomial infections (tracked by pathogen).
Statistical Consideration:	The sample size is designed to assess single and multiple dose PK of meropenem in young infants. All infants who receive meropenem will be analyzed. These infants will comprise the population for the safety analysis, and if any PK samples are obtained, their blood will be evaluated in the PK analysis. Those infants who have an efficacy measurement at Day 28 will also be evaluated for clinical (efficacy) response. Key safety endpoints that will be evaluated in the final analysis: include death, seizures, strictures, perforation, wound dehiscence, short gut, development of extended beta lactamase infection, development of candidiasis, and antimicrobial therapy failure. The proportion of infants affected and 95% CI for each key safety endpoint will be reported. The following PK parameters will be estimated: a) Plasma clearance b) Volume of distribution c) Cmax, Tmax, AUC_{0-T}, (at steady state), Ke and $t_{1/2}$ $AUC_{0-\infty}$ (estimated from the 1st dose)
Inclusion Criteria	a. Written permission from parent or legal guardian b. Age younger than 91 days

	c. Likely to survive beyond the first 48 hours after enrollment d. Sufficient intravascular access (either peripheral or central) to receive study drug AND ONE OF THE FOLLOWING e. 1) Physical, radiological, and/or bacteriological findings of a complicated intra-abdominal infection. These include peritonitis, NEC Grade II or higher by Bell's criteria, Hirschsprung's disease with perforation, spontaneous perforation, meconium ileus with perforation, bowel obstruction with perforation, as evidenced by free peritoneal air on abdominal radiograph, intestinal pneumotosis or portal venous gas on abdominal radiographic examination. OR 2) Possible NEC OR 3) Otherwise receiving meropenem per local standard of care
Exclusion Criteria	a. Renal dysfunction evidenced by urine output <0.5 mL/hr/kg over the prior 24 hours b. Serum creatinine >1.7 mg/dL c. History of clinical seizures or EEG confirmed seizures d. Concomitant treatment with another carbapenem (ertapenem or imipenem) at the time of informed consent e. Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

ACRONYMS AND ABBREVIATIONSAE	Adverse Event
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BUN	Blood Urea Nitrogen
C	Drug Concentration
CBC	Complete Blood Count
CONS	Coagulase Negative Staphylococcus
CRA	Clinical Research Associate
CRF	Case Report Form
CRP	C-reactive Protein
DCRI	Duke Clinical Research Institute
DSMB	Data and Safety Monitoring Board
DOL	Day of Life
ELBW	Extremely Low Birth Weight
ESBL	Extended Spectrum Beta-Lactamases
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GA	Gestational Age
GCP	Good Clinical Practice
GNR	Gram Negative Rod
GPC	Gram Positive Cocci
HIPAA	Health Insurance Portability and Accountability Act
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVH	Intraventricular Hemorrhage
IVRS	Interactive Voice Response System
Kg	Kilogram
LFT	Liver Function Tests
Mcg	Microgram
Mg	Milligrams

MIC	Minimum Inhibitory Concentration
mL	Milliliter
MPODS	Meropenem Off-Patent Drug Studies
MRSA	Methicillin Resistant S. aureus
NEC	Necrotizing Enterocolitis
NICHD	National Institute for Child Health and Human Development
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
PNA	Postnatal Age
PODS	Pediatric Off-Patent Drug Studies
SAE	Serious Adverse Event
SNAP	Score for Neonatal Acute Physiology
Vd	Volume of distribution
WR	Written Request

1.0 Background and Rationale

1.1 Introduction

Meropenem, a carbapenem, belongs to an antibiotic class that possesses one of the broadest spectra of antimicrobial activity available, including most of the bacterial pathogens responsible for serious, life-threatening infections occurring in young (<91 days) infants. Meropenem is stable against hydrolysis by most extended spectrum beta-lactamases and AmpC chromosomal beta-lactamases underscoring the drug's activity against many antibiotic resistant Gram positive (e.g., penicillin-resistant *S. pneumoniae*) and Gram negative (e.g., *P. aeruginosa*) bacteria. Important indications for meropenem involve infections due to multi-drug resistant pathogens and polymicrobial sepsis. Meropenem is FDA-labeled for pediatric subjects from three months of age through adolescence as single agent antimicrobial therapy for bacterial meningitis and complicated intra-abdominal infections. There is substantial off-label use of meropenem in neonates and infants younger than three months of age. This off-label use occurs despite the lack of adequate meropenem PK, dosing, tolerability and safety data for this vulnerable subject group. The present proposal aims to determine PK and safety of meropenem for the treatment of suspected and complicated intra-abdominal in neonates and infants younger than three months of age.

1.2 Metabolism in Adults

Meropenem mean peak plasma concentrations were approximately 23 µg/mL (range 14-26) for 500 mg single dose and 49 µg/mL (range 39-58) for a 1 g single dose in adult volunteers,. Following intravenous doses of 500 mg in adults mean plasma concentrations of meropenem usually decline to approximately 1 µg/mL at 6 hours after administration. In subjects with normal renal function, the elimination half-life of meropenem is approximately 1 hour. Approximately 70% of the intravenously administered dose is recovered as unchanged meropenem in the urine over 12 hours, after which little further urinary excretion is detectable. Urinary concentrations of meropenem in excess of 10 µg/mL are maintained for up to 5 hours after a 500 mg dose. No accumulation of meropenem in plasma or urine was observed with regimens using 500 mg administered every 8 hours or 1 g administered every 6 hours in volunteers with normal renal function. Plasma protein binding of meropenem is approximately 2%. There is one metabolite which is microbiologically inactive.

1.3 Previous Studies in Children

Two large-scale multi-center randomized studies have been published to date. The first of these compared meropenem to cefotaxime, with or without the addition of metronidazole or amikacin in 170 children 3 months to 12 years. (Schuler 1995) Antibiotics were given empirically for presumed serious bacterial infection. Satisfactory clinical response was achieved in 98% of the meropenem-treated subjects and in 93% receiving one of the cefotaxime regimens. Similar results were obtained in a study of 414 children between 1 month and 12 years given either meropenem or cefotaxime with or with out clindamycin or tobramycin. (Arrieta 1997) Children in the meropenem arm received 10 or 20mg/kg; and illnesses included lower respiratory tract infection, urinary tract infection, septicemia, skin infections, and intra-abdominal infections. Meropenem was similar in efficacy to cefotaxime and ceftriaxone in infants and children with bacterial meningitis. (Odio, 1999)

1.4 Safety

The most commonly reported side effects considered related to meropenem in studies in children have been diarrhea (3.3 to 4.7%), nausea and vomiting (0.4 to 1%), rash (0.8%), glossitis (tongue

swelling) (1%), oral thrush (1.9%) or diaper rash from yeast (3.1%), and redness and swelling at the injection site (0.5%). In comparison trials, these reactions occurred in similar frequency in the comparison (cephalosporin) groups. (Schuler 1995, Bradley 1996) Similar results have also been observed in clinical trials of adult subjects.

The potential for adverse CNS effects, particularly seizures, has been carefully studied with meropenem in older subjects. All beta-lactam antibiotics have the potential to cause neurotoxicity. The mechanism for this adverse effect is believed to be competitive inhibition of gamma-aminobutyric acid (GABA). Imipenem has been linked to the development of seizures for several years. In adults, the incidence of this adverse effect has been as high as 3% in some reports. In children, neurotoxicity with imipenem has also been reported. Meropenem has less affinity for GABA receptors and has been found to cause less neurotoxicity than imipenem both in animal models and during clinical trials. In trials comparing meropenem to cephalosporin regimens, the incidence of seizures was not significantly different between groups. The only seizures reported in meropenem-treated pediatric subjects to date have occurred during treatment for meningitis. No cases have been reported in children treated for non-CNS infections. (Bradley 1996)

1.5 Studies in Young Infants

Van Enk et al. reported the results from 7 preterm infants (gestational ages 27-32 weeks, chronological ages 5-44 days) who were treated for infections with meropenem at a dose of 15 mg/kg every 12 hours (Van Enk, 2001). Meropenem PK parameter estimates were highly variable and significantly different from older infants, age 2-5 months (Blumer 1995, Blumer 1996, Parker 1995). Meropenem clearance in these 7 neonates was 40% less and Vd 50% greater than infants, resulting in an average half-life twice that of older infants.

Two studies have been recently completed studies in young infants. The first study evaluated single dose PK of meropenem (10, 20 or 40 mg/kg) in 37 newborn infants (23 preterm and 14 full term) who already were receiving empirical antibacterial treatment for presumed infection (van den Anker). There were no drug-related adverse events reported. Based on the data generated by this single-dose study, it was concluded that a regimen of 20 mg/kg meropenem every 8 hours would result in adequate meropenem systemic exposure; however, few of these infants were <30 weeks gestational in the first week of life. The second recently completed study supports 20-30mg/kg as the initial doses for younger and more mature infants, respectively. This study included a population PK analysis of meropenem in preterm and term infants (Capparelli et al ICAAC 2006). Of the 37 infants enrolled, 22 were preterm and the mean gestational age was 30 weeks. The preponderance of preterm infants in this study complements the van den Anker study. The population analysis demonstrated an effect of post-conception age where post-conception age is defined as post-natal age (PNA) + gestational age (GA) at birth. Additionally, serum creatinine was correlated with meropenem clearance. The model predicted meropenem dosed in preterm infants at 20-30mg/kg q 8-12hr would maintain serum concentrations above 4 mcg/mL for more than 75% of the dose interval in about 3 quarters of simulated infants.

1.6 Rationale for Initial Dosage Selection and Dose Escalation

Meropenem demonstrates time dependent pharmacodynamics. Clinical antimicrobial effects are observed when concentrations exceed the MIC for at least 30-40% of the dose interval in immunocompetent subjects; and for neutropenic subjects, levels should exceed the MIC for >75% of the dose interval. We consider neonates to be relatively immunocompromised. We determined the dose needed to provide exposure above MIC of 2 mcg/ml for >75% of the dosing interval in most of the patients, and above MIC of 4 mcg/ml (upper limit of MIC) for >50% of the

interval for >90% of patients. We suspect that the inflammatory process and capillary leak associated with necrotizing enterocolitis and bowel perforation may increase the volume of distribution of the drug in the sickest infants. We aim to avoid sub-therapeutic exposure in these infants. The following initial meropenem dosing scheme was predicted to achieve the therapeutic exposure metric in a majority of infants in simulation studies using a population PK model for meropenem in neonates. We will dose escalate if we do not achieve concentrations above the MIC 2 mcg/ml for >75% of the dose interval in at least 90% in any age/dose strata.

Initial Dosing:

Infants <32 weeks

<2 weeks PNA	20 mg/kg Q12
≥2 weeks PNA	20 mg/kg Q8

Infants ≥32 weeks

<2 weeks PNA	20 mg/kg Q8
≥2 weeks PNA	30 mg/kg Q8

2.0 Study Objectives

This study will evaluate the safety, tolerability and PK-PD of meropenem in infants <91 days of age with suspected and complicated intra-abdominal infections.

The specific aims of this trial are:

1. To characterize meropenem single-dose and multiple-dose PK in subjects with suspected and complicated intra-abdominal infections.
2. To characterize the safety profile of meropenem in the treatment of suspected and complicated intra-abdominal infections.
3. To assess collected efficacy data for meropenem for the treatment of suspected and complicated intra-abdominal infections.

3.0 Investigational Plan

3.1 Overall Study Design

This is a multi-center, prospective, pharmacokinetic and safety study of meropenem in infants less than 91 days of age for the treatment of suspected or documented complicated intra-abdominal infections.

The dose of meropenem, stratified by gestational age and post natal age, is predicted to provide therapeutic exposure in the majority of infants. Infants will be enrolled in four GA/PNA strata. Safety will be assessed in real time by the Meropenem Off-Patent Drug Studies (MPODS) Clinical Events Safety Committee and by an independent Data Safety Monitoring Board (DSMB). First dose and steady state meropenem PK will be studied in an interim analysis by GA/PNA group once approximately 12 babies have enrolled in a GA/PNA strata. During interim PK analysis, infants in the GA/PNA group under analysis will continue to be enrolled for ongoing safety evaluation and steady state PK. If the interim PK analysis suggests that the initial dosing does not achieve concentrations more than the MIC 2 mcg/ml for more than 75% of the dose interval in at least 90% of infants then we will plan to dose escalate. We will then enroll 12 infants per group using a higher dose regimen. First dose and steady state meropenem PK will be obtained along with ongoing safety evaluation. Subsequent patient enrollment to final goal of 200 infants will occur in safety group with steady state PK.

1) First dose and steady state meropenem PK will be studied in infants <91 days of age using a population PK design.
2) There will be four GA/PNA groups (see 3.2 below)
3) Approximately 200 infants will be enrolled.
    a. First dose and steady state PK
        i. At least 12 infants enrolled in 4 GA/PNA strata at initial dose (48 infants)
        ii. At least 12 infants enrolled in 4 GA/PNA strata at higher dose if interim PK analysis suggests that exposure target is not met in initial 12 subjects (48 infants)
    b. Safety and steady state PK
        i. Up to 152 additional infants will be enrolled for ongoing safety evaluation and further collection of steady state PK data. These safety/steady state infants will receive initial dose of meropenem unless a determination of dose escalation is made. If dose escalation is warranted then ongoing enrollment in this group will be at higher dose.
4) Investigators are encouraged to provide infants with concomitant aminoglycoside therapy. Use of other antimicrobial agents is discouraged, but may be given per local standard of care

The 200 infants in this trial will receive at least 3 days and no more than 21 days of therapy with meropenem. Infants may be given therapy until the attending physician believes that the infant has had presumptive clinical cure.

3.2 Selection of Study Population

Infants who meet the inclusion/exclusion criteria will be enrolled up and including DOL 90, inclusive. Infants will be enrolled in each of the following groups:

Group 1: GA at birth below 32 weeks - PNA < 2 weeks;
Group 2: GA at birth below 32 weeks - PNA ≥ 2 weeks and < 91 days;
Group 3: GA at birth 32 weeks or older - PNA < 2 weeks;
Group 4: GA at birth 32 weeks or older - PNA ≥ 2 weeks and < 91 days.

3.3 Inclusion Criteria Selection of Study Population

a. Written permission from parent or legal guardian
b. Age younger than 91 days
c. Likely to survive beyond the first 48 hours after enrollment
d. Sufficient intravascular access (either peripheral or central) to receive study drug.

AND ONE OF THE FOLLOWING

e. 1) Physical, radiological, and/or bacteriological findings of a complicated intra-abdominal infection. These include peritonitis, NEC Grade II or higher by Bell's criteria, Hirschsprung's disease with perforation, spontaneous perforation, meconium ileus with perforation, bowel obstruction with perforation, as evidenced by free peritoneal air on abdominal radiograph, intestinal pneumotosis or portal venous gas on abdominal radiographic examination.

OR

2) Possible NEC

OR

3) Otherwise receiving meropenem per local standard of care

3.4 Exclusion Criteria

a. Renal dysfunction evidenced by urine output <0.5 mL/hr/kg over the prior 24 hours
b. Serum creatinine >1.7 mg/dL
c. History of clinical seizures or EEG confirmed seizures
d. Concomitant treatment with another carbapenem (ertapenem or imipenem) at the time of informed consent
e. Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

3.5 Withdrawal from Study

Infants may be withdrawn from treatment or from the study at any time. Reasons for infant withdrawal from the study include, but are not limited to:

1. Infant's parent or legal guardian chooses to withdraw the infant for any reason
2. AEs, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the infant's safety
3. Clinical seizure occurring between informed consent and 1st administration of study drug
4. The investigator determines that it is in the infant's best medical interest to be withdrawn.

Detailed reasons for infant withdrawal because of lack of efficacy or because of pre-determined safety concerns are given in the appropriate sections of this protocol. Withdrawn infants will be followed for safety end points to the extent possible.

3.6 Prior and Concomitant Therapy

All antimicrobial agents and all medications received during the 72 hours prior to study administration and for 72 hours following last dose of study drug will be recorded on the appropriate case report form (CRF).

3.7 Assessments

3.7.1 Efficacy Assessments

Limited efficacy data will be obtained. Efficacy data will be based on comparing initial clinical status to clinical status at study Day 28. Initial clinical status will be based on the presenting signs and symptoms of each infant and will be recorded by the local principal investigator (PI) or designee prior to administration of the first dose of study drug . The same physician should record the clinical signs and physical findings on study Day 0 (pre-study drug) and Day 28 in order to derive the efficacy assessments. Success is defined as all of the following:

1) Alive,
2) Negative bacterial cultures from sterile body fluid, and
3) Presumptive clinical cure (details of the presumptive clinical cure are provided below)

Failure will be defined by any of the following:

1. Change in antibiotic therapy while on study drug will be considered a treatment failure except the addition of Gram positive therapy to treat organisms that require it and have been isolated from a non-abdominal source (including CONS, MRSA);
2. Death; or
3. Lack of presumptive clinical cure

The presumptive clinical cure score will be derived by comparing clinical signs and symptoms prior to administration of the first dose of study drug and study Day 28. The clinical, laboratory and radiographic findings are based on the components of the Score for Neonatal Acute Physiology (SNAP) II and other items listed below. It is acknowledged that the SNAP II has not been

validated as a clinical tool beyond the first hours of life, but there is not a clinical tool to predict mortality in serious abdominal infections. The original assessment, efficacy assessment and resulting efficacy interpretation is listed in the following table:

Initial Assessment	Efficacy Assessment	Resulting Efficacy Interpretation and Score
Asymptomatic	Asymptomatic	1
Asymptomatic	Worsening	0
Symptomatic	Worsening	0
Symptomatic	No change	0
Symptomatic	Improved	1
Symptomatic	Asymptomatic	1

If 7 or more of 10 signs receive a score of 1, then the infant will be considered a presumptive clinical cure. The elements of the presumptive clinical score are:

I. Mean blood pressure
II. Temperature
III. PaO_2 (mmHg)/FiO_2
IV. Lowest serum pH
V. Presence or absence of seizures*
VI. Urine output
VII. Cardiovascular inotrope support: Record number and amount of each cardioactive drug
VIII. C-reactive protein (CRP)[#] (prior to study drug, day 3-5, and day 28)
IX. Abdominal girth
X. Findings on abdominal radiograph^

*The presence or absence of seizures will be adjudicated by the MPODS Clinical Events and Safety Committee. The composition, roles and responsibilities of the MPODS Clinical Events and Safety Committee is outlined in the MPODS Clinical Events and Safety Committee charter.

[#]For efficacy assessment, trends in CRP levels will be evaluated on 3 occasions: baseline (72 hours) prior to study drug, between day 3-5, and on day 28 (or at least 7 days after completion of meropenem if day 28 assessment is performed early because infants is nearing discharge). Inability to obtain a CRP level will result in a protocol deviation that will be reported to the study PI but will not disqualify the patient from the study. (see section 5.6)

^The findings on abdominal radiograph will be determined locally.

When there are multiple observations for one aspect of the clinical score recorded at baseline or study Day 28 (e.g., more than one temperature is obtained on Day 28), then the first reading obtained will be used unless the reading is felt by the PI to be spurious and noted as such in the record. Non CRP elements of clinical score that are not otherwise available as part of standard clinical care will be omitted. If the infant is nearing discharge, the score may be recorded prior to study day 28, provided that the infant has been off meropenem for at least 7 days.

3.7.2 Safety Assessments

The collection of safety data is a primary objective of this trial. The safety of meropenem will be a primary focus for monitoring AEs. Safety assessments will include death, seizure documentation, strictures, perforation, wound dehiscence, short gut, development of extended beta lactamase infection, development of candidiasis, and antimicrobial therapy failure. physical examination,

clinical laboratory values and concomitant medications. Vital signs, electrolytes, creatinine, BUN, liver function tests (LFT) and complete blood counts, will be recorded weekly when available per local standard of care. Serious adverse event (SAE) data will continue to be collected for 30 days following completion of study drug administration.

3.7.3 PK Measurements

A population PK approach will be employed. This will include sparse sampling with samples obtained at time intervals rather than at fixed times for assessment of drug concentration. Samples will be no more than 100μL each. Prior to PK sampling (pre study drug assessment), creatinine, BUN, liver function tests (LFT), and urine output over previous 24 hours will be recorded. The complete sampling schedule for assessment of PK parameters is outlined in the PK Appendix. All sample times will be recorded on the appropriate CRF in 24 hour time format. All timing and length of drug infusions before PK sampling is obtained will also be listed on the CRF. Daily weight and all meropenem doses will be recorded on CRF. Infants in each PK cohort will continue to be enrolled to ensure at least 12 infants with at least 3 PK samples around the 1st dose AND 2 samples at steady state.

4.0 Procedures and Study Visits

	Study Day			
PROCEDURE	**0[a]**	**1-27**	**28**	**29 to end of study[a]**
Informed Consent	X			
Abdominal radiographic tests[l]	X	X	X	X
Sterile body fluid cultures[b,c]	X	X	X	X
Abdominal surgical procedures[b]	X	X	X	X
Medical Baseline Conditions	X			
Pertinent Medical History	X			
Physical Exam	X	X	X	
Concomitant Medications[d]	X	X		
Body Weight[e]	X	X	X	
Vital signs, length, head circumference[f]	X	X	X	
Adverse Events[g]	X	X	X	X
Laboratory evaluation[f]	X	X		
CRP[h]	X	X	X	
PK Evaluation[i]		X		
Study drug administration[j]		X		
Clinical Score (see 3.7.1)	X		X	
Efficacy Assessment[k]			X	

a) Day 0 refers to time point prior to start of meropenem but may be the same calendar date as day 1. End of study is 30 days after last administration of study drug
b) Record results from 7 days prior to study drug and 30 days post completion of study drug
c) Record results of sterile body fluid cultures (blood, CSF, Urine, peritoneal fluid) as obtained for clinical care
d) Record from 72 hours prior to first dose and until 72 hours after completion of study drug
e) Assessed prior to first dose and document daily during therapy if available as local clinical care of infant.
f) Assessed prior to first dose and document weekly until 7 days following last dose if available as local clinical care of infant.
g) Record SAEs until 30 days and non serious adverse events until 72 hours following last dose of study drug
h) CRP will be obtained on 3 occasions: 1) within the 72 hours prior to 1st dose of meropenem; 2) between study days 3-5; and 3) on study day 28.
i) Obtain per Appendix 2 and single sample at time of suspicious clinical seizure
j) Treatment for minimum of 3 days and maximum 21 days

k) Obtain at study Day 28; efficacy assessment includes alive, documentation of negative bacterial cultures, and presumptive clinical cure (clinical score elements listed in 3.7.1)
l) Record results from 72 hours prior to first dose and 30 days post completion of study drug

4.1 Procedures Prior to Receipt of First Dose of Study Drug

4.1.1 Parental/Guardian Permission

Prior to the start of any study-related procedure, a signed and dated informed consent and HIPAA authorization must be obtained and documented in the infant's medical record (see Appendix). Once it has been determined that the infant meets all inclusion criteria and no exclusion criteria, the infant will be assigned a subject identification number that will be used on the subject's CRFs and will be considered enrolled. Per inclusion criteria, infants may receive meropenem prior to enrollment.

4.2 Study Drug Administration

4.2.1 Assignment to Therapy Groups

Infants meeting the eligibility will receive meropenem. Concomitant use of an aminoglycoside is suggested. Enrollment and study drug dose will be stratified by GA and PNA as outlined in Sections 1.6 and 3.2. If the infant received meropenem for clinical care within 5 days prior to enrollment date, the first dose kinetics will not be obtained for this study. The infant will be enrolled in the safety-steady-state PK group (see 4.2.5).

After enrollment information for the eligible infant (demographics, stratification criteria, center, etc.) is provided, the dosage assignment and subject number will be allocated. If an infant is assigned a study drug and a number, but does not receive study drug, the subject number will not be used again. The reason for not dosing the subject will be noted on the CRF.

The BPCA-CC will provide instructions for the investigator to obtain each infant's study drug assignment and PK sampling schedule in a timely manner prior to the administration of study drug. The pharmacist will provide the study drug for infusion according to the dosage group.

4.2.2 Dispensing of Study Drug

Study drug will be distributed to the sites by the BPCA-CC or designee. The pharmacist at each site will prepare and dispense the study drug.

Study drug will be dispensed by the pharmacy in appropriate size syringes and administered via a syringe pump at a rate calculated based on the infants' body weight in kilograms (kg) per local standard of care, but with a target of infusing the product over 30 minutes. The pharmacy will supply study syringes wrapped in amber plastic to protect the contents from light. A new bottle of study drug should be used for every dose.

The compatibility of meropenem with other drugs has not been established. Meropenem should not be mixed with or physically added to solutions containing other drugs. Infusion vials of meropenem will be reconstituted and stored per the package insert and local pharmacy requirements. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

4.2.3 Treatments Administered

Meropenem may be administered concomitantly with compatible medications. An in-line filter is not appropriate. The 30 minute infusion must be rate controlled by using appropriate infusion (syringe) pumps.

Dosing and administration of other antimicrobial therapy (e.g., an aminoglycoside) will be administered per local standard of care at the discretion of the infant's neonatologist. If there is a delay in the study drug shipment, sites may use open-label meropenem to protect the safety of the patient.

4.2.4 Treatment Compliance

Treatment compliance will be evaluated by review of information documented on study drug administration and drug accountability forms.

4.2.5 Dosing for infants receiving meropenem per local standard of care

Infants enrolled that were receiving meropenem per local standard of care prior to study entry will be enrolled in the safety-steady state PK group because 1st dose levels will be impossible to obtain. Only steady state PK samples will be drawn (per the MPODS protocol). The infants will continue to receive meropenem at the dosing administered prior to enrollment in the study if the dose is equal to or greater than the dose indicated by MPODS protocol for the infant's GA/PNA.

For example: An infant born at 34 weeks gestation and 4 weeks post natal age has been receiving meropenem at a dose of 40 mg/kg q8 hours and is consented for the study. Based on the open study group, the infant should receive meropenem (30 mg/kg q8 hours): however, the infant will continue to receive 40 mg/kg q8 hours per the local standard of care. Had the same infant (EGA 34 weeks; PNA 4 weeks) been receiving 20 mg/kg q8 hours, the dosing should be increased to 30 mg/kg q8 hours to comply with minimum MPODS protocol dose.

4.3 Protocol Deviations

When a deviation from the protocol is deemed necessary for an individual infant, the investigator or other responsible physician must contact the MPODS PI or BPCA-CC clinical monitor immediately, unless a delay would endanger the subject, so that a timely decision can be made as to whether or not the infant should be enrolled or continue in the study. The deviation from the protocol will be authorized only for that particular infant. A description of the departure from the protocol and the reason(s) for it must be recorded on the appropriate CRF (for PK sampling deviations) or the provided protocol deviation logsheet. Additionally, sites will adhere to local IRB reporting rules for protocol deviations..

5.0 Safety

Safety assessments will include seizure documentation (including correlation of serum meropenem level and seizures), physical examination, and clinical laboratory values as available per standard local care including LFTs, renal function, blood counts and microbiology cultures of sterile body fluids to track nosocomial infections (by pathogen).

5.1 Safety First Plan

The protocol will rely on three mechanisms for safety:

1. The DSMB whose role is outlined below and in the BPCA-CC DSMB charter;
2. AE and SAE reporting mechanisms in accordance with FDA guidance outlined below
3. The active, daily, real time oversight of the MPODS Clinical Events and Safety Committee

5.1.1 Data Safety and Monitoring Plan

The BPCA-CC will establish the Data Safety and Monitoring Plan (DSMP) in compliance with NIH policies for the protection of human subjects in clinical studies. The BPCA-CC DSMP outlines procedures for reporting SAEs and AEs of Special Interest to the BPCA-CC and dissemination of this information from the BPCA-CC to the DSMB and MPODS Clinical Events and Safety Committee.

5.1.2 Data and Safety Monitoring Board

An independent DSMB established in accordance with the "NIH Policy for Data and Safety Monitoring" will monitor the conduct of the trial for performance (e.g., recruitment, flow and quality control of data, adherence to the protocol), patient safety, and efficacy. The DSMB may review the data at any time. At any time and for any reason, the DSMB may recommend to the NICHD Project Officer that the trial be interrupted or discontinued.

5.1.3 AEs

The AEs of Special Interest (1 and 2 below) not otherwise explained by the patient's underlying illness and all SAEs will be submitted in writing (via fax or electronic communication) to the BPCA-CC medical monitor within 24 hours of occurrence. The BPCA-CC will then notify the MPODS PI, MPODS Clinical Events and Safety Committee and the DSMB within one working day after receiving the report from a clinical site. Local IRB guidelines will also be followed.

AEs to be submitted to the BPCA-CC medical monitor within 24 hours of occurrence:

1. Level 2 laboratory AEs (Refer to Table 1) not otherwise explained by the subject's underlying illness
2. Seizures judged not otherwise explained by the subject's underlying illnesses including:
    a. IVH
    b. Meningitis
    c. Electrolyte abnormality
    d. Genetic/metabolic disorder
    e. Drug withdrawal
    f. Hypoxia-ischemia
    g. Cerebral anomalies
3. All SAEs

Table 1: Laboratory AEs of Special Interest

Parameter	Level 1	Level 2
Direct bilirubin		> 5 mg/dL
Indirect bilirubin		>15 if <36 weeks adjusted GA >20 if >36 weeks adjusted GA
AST	Increase 5* (baseline)	Increase 10* (baseline)
ALT	Increase 5* (baseline)	Increase 10* (baseline)
Creatinine	Doubling of baseline serum creatinine and > 1.5 mg/dL	> 2.5 mg/dL

When clinical seizures or seizure-like activity occurs not otherwise explained by the patient's underlying illness, the local PI (or their designee) will record the findings on the CRF. EEG's, if obtained per local standard of care, will be evaluated. The local PI will obtain a copy of the EEG, preferably in digital format, remove all patient identifiers except study ID, and submit as source

documentation for the seizure or seizure like activity. These digital documents and the CRF will be presented to the MPODS Clinical Events and Safety Committee pediatric neurologist. The neurologist will make a final determination of whether or not the infant has had a seizure or seizure like activity.

If seizures occur while on therapy, obtain 100 ul of blood (if possible) to document meropenem level at time of seizure. When possible, obtain up to 2 additional samples, each at least 24 hours apart, during infant seizure activity. Scavenged samples whenever possible are appropriate during infant seizure activity. Time of sample collection will be recorded on CRF. These samples will be sent to the central lab with the other PK samples from the infant.

5.1.4 MPODS Clinical Events and Safety Committee

The roles and responsibilities of the MPODS Clinical Events and Safety Committee are outlined in the MPODS Clinical Events and Safety Committee charter. If interim PK analysis on 12 subject PNA/GA groups reveals that a higher dose is needed to reach the exposure target, then the MPODS Clinical Events and Safety Committee will review AEs and determine if dose escalation can occur by applying the dose escalation safety rules outlined below.

The dose escalation safety rules are to be applied to each GA/PNA group (1-4) separately, thus allowing dosing in each age cohort to progress independently.

1. Escalation of dosage may occur if no more than 3 subjects (or 25% of the group if the size of the group is >12) in an age group develop any of the following:
    a. Level 2 AEs judged by the MPODS Clinical Events and Safety Committee to be related to meropenem
    b. Seizures judged by the MPODS Clinical Events and Safety Committee to be related to meropenem
    c. SAEs that are judged by the MPODS Clinical Events and Safety Committee to be related to meropenem
2. DO NOT escalate dosage if > 3 subjects (or > 25% of the group if the size of the group is >12) in an age group develop any of the following:
    a. Level 2 AEs (defined below) judged by the MPODS Clinical Events and Safety Committee to be related to meropenem
    b. Seizures judged by the MPODS Clinical Events and Safety Committee to be related to meropenem
    c. SAEs that are judged by the MPODS Clinical Events and Safety Committee to be related to meropenem

5.1.5 PK Interim Analysis for Possible Dose Escalation

After obtaining single and steady-state PK samples from approximately 12 infants in each GA/PNA cohort, we will determine if the dose used leads to the pharmacodynamic target exposure. From the PK interim analysis, we will dose escalate if we do not achieve concentrations >2 mcg/ml for >75% of the dose interval in at least 990%in any GA/PNA group (see section 1.6)(Pfaller 1997, Benjamin 2004). We will escalate dosing based on this analysis once safety has been confirmed as described in section 5.1.4. The decision to escalate meropenem dosage will be carried out independently for each GA/PNA group.

5.2 Adverse Events

5.2.1 Definition of Adverse Event

An AE is defined as any untoward medical occurrence such as a sign(s), symptom (s), and/or laboratory finding(s) concurrent with the use of a drug in humans. AEs include worsening of any baseline symptoms. The event may/may not necessarily have a causal relationship with the administration of the drug. AEs may be reported by the subject, or detected by the investigator, or other competent observer. The investigator will also evaluate any change in laboratory values. If the investigator determines a laboratory abnormality to be clinically significant, it is considered a laboratory AE; however, if the laboratory value abnormality is consistent with a current diagnosis, it may be documented accordingly.

5.2.2 Reporting period

AEs will be recorded from the time of informed consent until 72 hours following the last dose of study drug for non SAEs and until 30 days after the last dose of study drug for SAEs. Any AE that occurs between the time informed consent is obtained and the initial dose of study, that is considered related to a protocol specified procedure, must be reported.

5.2.3 Procedures for assessing, recording and reporting AEs

Throughout the duration of the study, the investigator will closely monitor each subject for clinical evidence of drug intolerance and monitor all clinically obtained laboratory values for laboratory evidence of AEs. AEs not explained by the infant's underlying illness which occur during the course of the study will be reported in detail on the appropriate CRFs and followed until resolution or until it becomes stable. All SAEs will be reported to BPCA-CC within 24 hours.

The description of the AE will include description of event, start date, stop date, intensity, if it was serious, and relationship to the study drug. The investigator must verify this information.

The intensity or severity of AEs will be graded as follows:

- **Mild** - awareness of sign or symptom, but easily tolerated. Not expected to have a clinically significant effect on the subject's overall health and well-being. Not likely to require medical attention
- **Moderate** - discomfort enough to cause interference with usual activity or affects clinical status. May require medical intervention
- **Severe** - incapacitating or significantly affecting clinical status. Likely requires medical intervention and/or close follow-up

AEs that increase in intensity will be recorded with a stop date on the AE CRF of the milder AE equal to the date that the condition worsened. A new AE with a start date equal to the date of worsening will then be reported. AEs that decrease in severity need not be reported in this way. The start date will be the date entered above and the date of resolution should be reported as the stop date.

The Investigator is responsible for assessing relationship to study medication using the following definitions:

- **Not related**: An AE that is due to a pre-existing illness or use of another drug, and is not related to the study drug.
- **Possibly related**: An AE that has little or no relationship to the study drug and there exists a more likely alternative cause.

- **Probably related**: An AE that is likely to be related to the administration of the study drug and an alternative cause less likely when compared to the study drug.
- **Definitely Related**: An AE that has a strong temporal relationship to the study drug. AE will recur with continued or repeated use of the study drug, and another cause is unlikely or less likely.

5.2.4 Follow-up of AEs

AEs will be followed until resolution or until stability is reached using good clinical practices.

5.3 Serious Adverse Event

A SAE is defined (21 Code of Federal Regulations part 312.32) as those AEs, which meet any of the following serious outcome criteria:

- Is fatal
- Is life-threatening, meaning, the subject was, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death;
- Is a persistent or significant disability/incapacity, i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions;
- Requires or prolongs inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is an important medical event, based on appropriate medical judgment, that may jeopardize the subject or subject may require medical or surgical intervention to prevent one of the other outcomes above.

5.3.1 Procedures for assessing, recording and reporting SAEs

All SAEs must be reported by facsimile or electronic transmission to BPCA-CC within 24 hours after the onset of the SAE (or the awareness of the investigator of the event). The BPCA-CC then notifies the MPODS PI, MPODS Clinical Events and Safety Committee and the DSMB of SAE within one working day after receiving the report from a clinical site. In addition, a clinical site must report a death or life-threatening event by telephone as soon as possible and within 24 hours to the BPCA-CC.

A SERIOUS ADVERSE EVENT FORM must be completed and signed by the site investigator. All SAEs must also be entered into the AE CRF (select "serious").

The FDA requires that all SAEs that are unexpected and potentially related to the study medication must be reported to the FDA in writing within 15 calendar days of notification of BPCA-CC. SAEs that are unexpected and related to study drug that meet the criteria for death or immediately life-threatening also require BPCA-CC to notify the FDA by telephone, facsimile transmission or in writing as soon as possible but no later than seven calendar days, with a follow-up written report within 15 calendar days. BPCA-CC will prepare an expedited report for the FDA and copies will be distributed to all site investigators. Expedited reports will be placed in the Study Binder by the investigator upon receipt. The investigators will also forward a copy of all expedited reports to their local Investigational Review Boards in accordance with local guidelines .

5.3.2 Follow-up of SAEs

The investigator must complete and submit a follow-up SAE form when important follow-up information (diagnosis, outcome, results of specific investigations, etc.) becomes available after submission of the initial form. Follow-up forms should be submitted according to the same

process used for reporting the initial event as described above (i.e., within 24 hours of knowledge). All SAEs and AEs of Special Interest will be followed until resolution, stabilization or 30 days after the last subject is enrolled, whichever occurs, first. The investigator will be responsible for reporting SAEs to the local IRBs in accordance with local guidelines.

5.4 IRB summary safety reports

As required by the NIH "Guidance of Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multicenter Clinical Trials," the DSMB's summary safety reports will provide feedback at regular and defined intervals to the Institutional Review Boards (IRBs). After each meeting of the DSMB, the executive secretary will send a brief summary safety report to each investigator. The report will document that a review of data and outcomes across all centers took place on a given date and will summarize the Board's review of the cumulative adverse experiences reported from all participating sites without specific disclosure by treatment arm. It will also inform investigators of the study the Board's conclusion with respect to progress or need for modification of the protocol. The clinical site investigators are required to transmit the report to their local IRB as soon as they are received.

5.5 Seizures

Seizures will be closely monitored throughout the trial. The presentation of a seizure can be subtle such as ocular deviation, sucking and lip smacking movements, swimming or 'rowing' or 'bicycling' movements of limbs. They can be tonic/clonic, localized, multifocal, or generalized. They may also be diagnosed by EEG. If seizures (or possible seizure like activities) are present, they are to be recorded on the CRF. Assessment of seizure activity prior to enrollment, at enrollment, while on study drug and up to 30 days post study drug administration are to be recorded on the CRF.

If seizures occur while on therapy, obtain 100 ul of blood (if possible) to document meropenem level at time of seizure. When possible, obtain up to 2 additional samples, each at least 24 hours apart, during additional infant seizure activity. Scavenged samples whenever possible are appropriate during infant seizure activity. Time of sample collection will be recorded on CRF. These samples will be sent to the central lab with the other PK samples from the infant.

The CRF will have a seizure documentation page for every infant thought to have a seizure or seizure-like activity by the local site PI not explained by the infant's underlying illness. When clinical seizures or seizure-like activity occurs, the local PI (or their designee) will record the findings on the CRF. EEG's, if obtained per local standard of care, will be evaluated. The local PI will obtain a copy of the EEG, preferably in digital format, remove all patient identifiers except study ID, and submit as source documentation for the seizure or seizure like activity. These digital documents and the CRF will be presented to the MPODS Clinical Events and Safety Committee pediatric neurologist. The neurologist will make a final determination of whether or not the infant has had a seizure or seizure like activity. The decision by the MPODS Clinical Events and Safety Committee pediatric neurologist will be used to determine whether dosing level is to be advanced in an age group (Centrally Diagnosed Seizure).

Infants with seizures or seizure-like activity will be characterized in four components:
1) Locally Diagnosed Clinical Seizure (yes/no),
2) Locally Diagnosed EEG-confirmed Seizure (yes/no),
3) Centrally Diagnosed Clinical Seizure (yes/no),
4) Centrally Diagnosed EEG-confirmed Seizure (yes/no)

5.6 Blood Volume for PK and Safety Laboratory Tests

Blood samples will be minimized by:

1. Hematology and chemistry laboratory measures will be recorded from laboratories drawn as standard of care and will not be drawn strictly for purpose of this study.
2. No more than 2cc/kg will be obtained for study purposes. First priority for blood acquisition is PK samples (up to 0.7cc of blood). Second priority is CRP levels.
3. CRP levels will be obtained from the patients on 3 occasions: baseline (72 hours) prior to study drug, between days 3-5 and on day 28 of the study. CRP levels can be obtained from residual blood in clinical laboratory if less than 12 hours from time of sample collection. Inability to obtain CRP levels will result in a protocol deviation that will be reported to the MPODS PI but will not disqualify the patient from the study.
4. A limited PK sampling scheme will be employed such that no more than a total of 0.7 mL of blood (7 samples) is obtained from each subject for PK analysis.

6.0 Procedures by Visit

6.1 Pre-Study Drug Administration Procedures (Day 0)

The following procedures will be completed prior to the administration of study drug:

a. Review of Inclusion/Exclusion Criteria prior to infant enrollment
b. Obtain signed and dated informed consent/HIPAA consent.
c. Collect demographic data and medical/surgical history
d. Perform a complete physical examination
e. Obtain and record vital sign measurements, length and head circumference
f. Obtain and record infant weight in grams and medication dosing weight for calculation of appropriate study drug dose if different from actual weight
g. Record results from hematology and serum chemistry labs. Hematology assays will include: hematocrit, hemoglobin, red blood cell count, white blood cell count with differential, and platelet count. Serum chemistry will include glucose, creatinine, blood urea nitrogen, aspartate transaminases (AST), alanine transaminase (ALT), alkaline phosphatase, total and direct bilirubin, sodium, potassium, chloride, calcium, magnesium, total protein, and albumin laboratory evaluations. If these labs have been obtained within 72 hours prior to enrollment in accordance with local standard of care, the results may be used for the baseline values for the study. Use the laboratory values closest to enrollment if there have been multiple tests. **Do not collect additional samples for the purposes of this study**.
h. Record results of sterile body fluid cultures (blood, urine, CSF, peritoneal fluid) obtained as standard clinical care in the 1 week prior to study drug administration. Record Urine cultures only if obtained by sterile catheterization or suprapubic aspiration.
i. Document antimicrobial agents and concomitant medications in the 72 hours prior to study drug administration.
j. Document prior abdominal surgical procedures in the 1 week prior to study drug administration.
k. Document confirmed serious or suspected intra-abdominal infection
l. Document clinical signs of intra-abdominal infection
m. Document the results of abdominal XRAY in 72 hours prior: including A-P and lateral (either cross-table or left lateral decubitis) plain films. This will serve as the 'baseline' film set for the infant.
n. Record the presumptive clinical cure score initial assessment (10 elements of clinical score): mean blood pressure; temperature; PO_2 (mmHg)/FiO_2; lowest serum pH (if

obtained per standard of care); presence or absence of seizures; urine output; cardiovascular inotrope support (record name and dose of each cardioactive agent); CRP (Obtain 1 CRP in the 72 hours prior to enrollment. If multiple CRPs are collected as part of standard of care during this time, record the value closest to the 1st dose of meropenem); abdominal girth; and findings on abdominal XRAY (if obtained per standard of care). If more than one clinical score element result is obtained on Day 0 prior to study drug administration, record the results of the first study obtained that day. Except for CRP, clinical assessments not obtained as local standard of care will not be recorded.

o. Assess and record AEs between the time informed consent is obtained and the initial dose of study drug that are considered related to a protocol specified procedure.

6.2 Procedures During Study Drug Administration

The following procedures or evaluations will be performed during the treatment phase and the data recorded as indicated. The first dose of drug defines the beginning of study Day 1

6.2.1 Study Days 1-27

a. Record study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion, time (24 hour clock) of infusion, and amount given
b. Assess and record AEs from the time of the first dose of study drug through 72 hours following the last dose study drug.
c. Record weight of the patients daily during meropenem administration
d. Record all meropenem drug administration: date, start time, dose: minimum of 3 days and maximum of 21 days.
e. Dosing adjustments should be made on Study Days 7, 14, 21 based on change in PNA or new dosing weight to be determined by the infant's physician
f. Record all concomitant medications administered
g. Collect blood for PK analysis (Schedule provided in Appendix). PK samples are not to be drawn from the lumen of the catheter through which meropenem has been administered. Samples may be collected through the lumens of other catheters, venous sampling, arterial sampling, or capillary heel-sticks.
h. Record result for clinical laboratory assessments obtained per local standard of care (listed in 6.1 g) one time weekly while on study drug (day 1-7, day 8-14, day 15-21, day 22-28) while on therapy and up until one week after therapy completion. These assessments include CBC, AST, ALT, bilirubin (total and direct), creatinine, electrolytes, and BUN. **Do not collect additional samples for the purposes of this study**.
i. Record the results of cultures from sterile body fluids (blood, urine, CSF, peritoneal fluid, or any other sterile body fluid) as obtained per standard of care up to 30 days after last study drug (consistent with section 6.3).
j. Record any abdominal surgical procedures up to 30 days after last study drug (consistent with section 6.3).
k. Record results of any abdominal radiological examinations (e.g., XRAY, ultrasound, CT scan, MRI) up to 30 days after last study drug (consistent with section 6.3).
l. If seizures occur while on therapy, obtain 100µL of blood (if possible) to document meropenem level at time of seizure. These will be sent to the central lab with the other PK samples from the infant. An additional 2 samples, each at least 24 hours apart, during additional infant seizure activity may be obtained. Scavenged samples whenever possible are appropriate during infant seizure activity.

m. Obtain 1 CRP between study Days 3 and 5. If CRPs are collected as part of standard of care between days 3 and 5, these are to be recorded on the appropriate CRF. If more than one CRP is collected on a calendar day, record the first CRP obtained.

6.2.2 Study Day 28: Documentation of Clinical Response

If the infant is nearing discharge, the score may be recorded prior to study day 28, provided that the infant has been off meropenem for at least 7 days.

Record efficacy assessment variables: (3.7.1)

1) Alive
2) Negative bacterial cultures from sterile body fluid
3) Presumptive clinical cure

Record the following and compare to initial evaluation

I. Mean blood pressure
II. Temperature
III. PaO_2 (mmHg)/FiO_2
IV. Lowest serum pH
V. Presence or absence of seizures
VI. Urine output
VII. Cardiovascular inotrope support: Record number and amount of each cardioactive drug
VIII. CRP
IX. Abdominal girth
X. Findings on abdominal radiograph

6.3 Procedures following Study Drug Administration

1. Record concomitant medications for 72 hours after the last dose of study drug.
2. Record the following information for 30 days following the last dose of study drug:
   a. Assess and record SAEs
   b. Record all positive microbiology cultures from sterile body fluids
   c. Record any abdominal surgical procedures and surgical finding
   d. Record any results from abdominal radiological examinations (e.g., XRAY, ultrasound, CT scan, MRI) performed
   e. Record presence or absence of strictures, peritoneal abscesses

7.0 Administration

7.1 Trial Termination

The NICHD, the BPCA-CC, the PODS PI and the Duke Clinical Research Institute (DCRI) will monitor the progression of the trial. Investigator and site participation in the study may be terminated by the PODS PI if there is evidence of an investigator failing to maintain adequate clinical standards or evidence of an investigator or staff failing to comply with the protocol.

7.2 Data Safety and Monitoring Board

To ensure that the welfare of trial subjects receives appropriate consideration, an independent DSMB has been organized by the BPCA-CC to review relevant safety and/or efficacy data during the course of the trial. The DSMB may recommend discontinuation of the study, or modifications to the study protocol for safety reasons. The DSMB charter is available from the BPCA-CC.

7.2.1 Meropenem Escalation

If interim PK analysis reveals that dose escalation is necessary to achieve target exposure then the MPODS Clinical Events and Safety Committee will be responsible for determining if dose escalation can occur based on review of the safety data as described in section 5.1.4. Escalation can occur in one GA/PNA group irrespective of enrollment or trial progression in the other groups.

7.3 Investigational Product

7.3.1 Rationale for Investigational Product

Meropenem will be used as empirical therapy. It provides excellent coverage for most of the bacterial pathogens in isolated from infants in the nursery. It does not provide coverage for methicillin resistant *S. aureus* (MRSA), most coagulase negative staphylococci, or for ampicillin resistant *Enterococcccus*, but these organisms have limited additional mortality in the nursery (Benjamin 2004).

An aminoglycoside is suggested as additional coverage for additional empirical Gram Negative Rod (GNR) coverage. Its administration is based on the uncertainty of the efficacy of the proposed dosages of meropenem. This will ensure that all infants have therapeutic empirical antimicrobial therapy for GNR organism, and that many infants will likely have double coverage for empirical GNR coverage. An aminoglycoside does not provide coverage for MRSA, most coagulase negative staphylococci, or for ampicillin resistant *Enterococcccus*.

GNR coverage: Will be guaranteed by an aminoglycoside and bolstered by meropenem.

Gram Positive Cocci (GPC) coverage: Will likely be provided by meropenem except for methicillin resistant organisms. Attributable mortality in the nursery from these organisms approaches 0. (Benjamin 2004).

Anaerobic coverage: Some coverage will likely be provided by meropenem. Benefits of empirical therapy with anti-anaerobic coverage are not known (Faix et al) and definitive therapy may be added per local standard of care if the infant has an anaerobe isolated from sterile body fluid or the abdomen perforates.

Antifungal coverage: May be provided per local standard of care

7.3.2 Description of Investigational Product

Meropenem is a pyrogen-free, synthetic, broad-spectrum, carbapenem antibiotic for intravenous administration. It is (4R,5S,6S)-3-[[(3S,5S)-5-(Dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate. Its empirical formula is $C_{17}H_{25}N_3O_5S \cdot 3H_2O$ with a molecular weight of 437.52.

7.4 Storage and Disposition of Supplies

The clinical supplies will be stored at controlled room temperature from 15°- 30°C and protected from light in its carton until used. Investigational products are for investigational use only, and are to be used only within the context of this study. Study drug must be maintained under adequate security.

7.5 Drug Accountability

The investigator or his/her designee will verify that study drug supplies are received intact and in the correct amounts. The investigator or his/her designee will document this verification by signing and dating the appropriate shipment request/receipt document. An accurate inventory of study drug will be kept by the site. An overall accountability of the study drug will be performed and verified by the clinical research associate (CRA) throughout the study and at the site close-out visit. All used and unused supplies must be inventoried, accounted for, and disposed at the study site according to the institution's standard operating procedures (SOPs)

following review by the CRA. The investigator agrees not to supply study medication to any persons not enrolled in the study.

8.0 Statistical Methods

8.1 General Considerations of the Statistical and Analytic Plans

The primary objectives of this analysis are to assess the PK and safety of meropenem in young infants.

8.2 Definitions and Populations for Analysis

All infants who receive meropenem will be analyzed. These infants will comprise the population for the safety analysis, and if any PK samples are obtained, their blood will be evaluated in the PK analysis. These infants who have efficacy measurement at Day 28 will also be evaluated for clinical (efficacy) response.

8.3 Demographics

Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented by dosage group for continuous variables (such as age, weight, etc). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by dosage group to summarize discrete variables (such as race, sex, success rates, mortality rates, etc.).

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

8.4 Safety

The number and percentage of infants having treatment-emergent AEs will be tabulated, with a breakdown by group. Descriptive statistics will be provided for clinical chemistry and hematology data, including change from baseline. All subjects who received at least one dose of study product will be included in the safety analyses. AEs will be summarized and tabulated by severity, and relationship to therapy. Deaths and premature termination will be tabulated and summarized. Changes in laboratory parameters will be tabulated and summarized. Laboratory data, such as hematology and serum chemistry data will be tabulated by dosage and age group. Summary statistics for changes from baseline will be presented. AEs will be summarized in tabular form by dosage and age group.

Continuous laboratory measurements will be described at each visit using univariable descriptive statistics (mean, median, etc.); observed values and changes from baseline will be summarized. Lab tests reflective of liver toxicity (e.g., ALT, AST) will be further summarized in terms of the most extreme values and largest changes from baseline (in the appropriate direction) observed from start of study drug through the end-of-therapy lab. Vital signs and physical exam results will be listed.

Key safety endpoints that will be evaluated in the final analysis include: Death, seizures, strictures, perforation, wound dehiscence, short gut, development of extended beta lactamase infection, development of candidiasis, and antimicrobial therapy failure. The key safety endpoints, the proportion expected to develop the endpoints, and the 95%CI are presented in the table below. The proportion of infants affected and 95% CI for each key safety endpoint will be

reported. Safety assessments will also include the tracking standard laboratory assessments of hematologic liver and renal function. Assessments will also include growth parameters (weight, length, and head circumference). Strictures will be defined as previously described (Faix 1988).

Analysis of Seizure: Infants with seizures or seizure-like activity will be characterized in four components:
1) Locally Diagnosed Clinical Seizure (yes/no),
2) Locally Diagnosed EEG-confirmed Seizure (yes/no),
3) Centrally Diagnosed Clinical Seizure (yes/no),
4) Centrally Diagnosed EEG-confirmed Seizure (yes/no)

The proportion of infants in each dosage will be compared. We will derive point estimates and 95% CI for each category. Although estimates for each category will be provided and compared, the definitive categorization of seizure will reside with the MPODS Clinical Safety Committee pediatric neurologist. The neurologist will assess the four components listed above and will make a final 'yes/no' call as to whether or not the infant had seizure.

Safety Table: The key endpoints, applicable sample size, expected proportion of population expected to reach safety endpoint, and subsequent expected 95% confidence intervals are presented. This table presents the 95% CI based on a sample size of 48 infants. The number of infants expected in each dosage of meropenem

Safety Endpoint	Expected Proportion	95% CI
Death	20%	0.10, 0.35
Seizures	2%	0.00, 0.11
Strictures	2%	0.00, 0.11
Antimicrobial Failure	45%	0.31, 0.61
Perforation once enrolled	5%	0.01, 0.14
Short bowel syndrome	5%	0.01, 0.14
Development of ESBL infection	2%	0.00, 0.11
Development of candidiasis	10%	0.03, 0.23

8.5 Pharmacokinetics

The complete PK analysis plan will be presented with the final statistical analysis plan (SAP). The following PK parameters will be estimated:

1. Plasma clearance
2. Volume of distribution
3. Cmax, Tmax, AUC_{0-T}, (at steady state), Ke and $t_{1/2}$
4. $AUC_{0-\infty}$ (estimated from the 1^{st} dose)

The PK parameters and MIC will be used to estimate pharmacodynamic parameters of exposure (time above MIC). For PK analysis, using sparse sampling PK parameters will be estimated for the following cohorts:
Group 1: GA at birth below 32 weeks - PNA < 2 weeks;
Group 2: GA at birth below 32 weeks - PNA ≥ 2 weeks and < 91 days;
Group 3: GA at birth 32 weeks or older - PNA < 2 weeks;
Group 4: GA at birth 32 weeks or older - PNA ≥ 2 weeks and < 91 days.

The plasma concentrations-time profiles of meropenem will be presented in tabular and graphical form by subject, age cohort, and dosage level. The relationship between plasma concentrations and/or PK parameters with demographic factors (weight, sex, age and race), disease severity, toxicity and co-administered medications will be investigated. Analysis of potential relationships between drug and exposure in subjects and the resulting efficacy and/or safety response will be conducted. The exact time and date of sample collection and the dosing history information will be recorded on the appropriate CRF.

8.6 Efficacy

An efficacy assessment will be assessed 28 days after first dose of study drug. Success will be defined by all of the following: Alive and negative bacterial cultures (if obtained) from normally sterile body fluids, and presumptive clinical cure score ≥7. Failure will be defined by any of: Change in antibiotic therapy while on study drug will be considered a treatment failure except the addition of Gram positive therapy to treat organisms that require it, or death, or lack of presumptive clinical cure. This study is not powered to determine efficacy. These data will be used in assessing the feasibility of a potential efficacy study.

8.7 Sample Size

The sample size is designed to assess single and multiple dose PK of meropenem in young infants.

9.0 Ethics

9.1 Ethical conduct of the trial

This study will be conducted according to the protocol, the applicable FDA and HHS Code of Federal Regulations, Good Clinical Practice, the Declaration of Helsinki, and the ICH Harmonized Tripartite Guideline for Good Clinical Practice. It will also adhere to the ethical principles outlined in The Belmont Report.

9.2 Institutional Review Board

Institutional review boards must be constituted according to the applicable State and Federal requirements of each participating site. The investigators and staff of this study and the IRB of each participating institution will rigorously monitor research data to ensure the safety of research subjects, and will protect the privacy and confidentiality of all study subjects.

This protocol must be submitted to appropriate IRBs and their written unconditional approval obtained and submitted to BPCA-CC before commencement of the study. Investigators must also inform IRBs of all subsequent protocol amendments. Verification of IRB unconditional approval of the protocol and the written parental/guardian permission form will be transmitted to BPCA-CC prior to shipment of investigational drugs (if BPCA-CC distributes the drug). This approval must refer to the study by exact protocol title and number, identify documents reviewed, and state the date of review. All correspondence with the IRB should be filed by the investigator.

Institutional review boards must be informed by investigators of all serious or unexpected AEs occurring during the study that are likely to affect the safety of the subjects or the conduct of the study.

9.3 Informed consent and assent

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be

obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation.

Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form and a copy of the signed assent (if applicable).

The parental/guardian permission form generated by the investigator with the assistance of BPCA-CC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA-CC, Regulatory Authorities, and NICHD or its designees.

9.4 Protection of personal health information

All reports and communications relating to subjects in the study will identify each subject only by the subject's initials and the subject's study number. The investigators will agree to maintain records identifying the subjects enrolled in the study, which will be used for the purpose of long-term follow-up.

Investigators at each study site will be responsible for insuring compliance with the Privacy Rule, a Federal regulation under the Health Insurance Portability and Accountability Act (HIPAA), in accordance with the investigator's institution policy. The Privacy Rule establishes the right of a

research subject or subject's legally authorized representative to authorize an investigator to use and disclose subject's personal health information (PHI) for research purposes. This requirement is in addition to the informed consent and assent to participate in the study. A valid Privacy Rule Authorization is a subject's or subject's legally authorized representative signed permission that allows an investigator to use or disclose the subject's PHI for the purposes, and to the recipient or recipients, as stated in the Authorization. The signed Authorization must be retained by the investigator for 6 years from the date of creation or the date it was last in effect, whichever is later.

Authorization can be combined with an informed permission. Whether combined with an informed permission or separate, an Authorization must contain the following specific core elements and required statements stipulated in the Privacy Rule.

9.4.1 Authorization core elements

a. A description of the PHI to be used or disclosed, identifying the information in a specific and meaningful manner
b. The names or other specific identification of the person or persons (or class of persons) authorized to make the requested use or disclosure
c. The names or other specific identification of the person or persons (or class of persons) to whom the covered entity may make the requested use or disclosure
d. A description of each purpose of the requested use or disclosure
e. Authorization expiration date or expiration event that relates to the individual or to the purpose of the use or disclosure ("end of the research study" or "none" are permissible for research, including for the creation and maintenance of a research database or repository)
f. Signature of the individual and date. If the individual's legally authorized representative signs the Authorization, a description of the representative's authority to act for the individual must also be provided

9.4.2 Authorization required statements

a. A statement of the individual's right to revoke his/her Authorization and how to do so, and, if applicable, the exceptions to the right to revoke his/her Authorization or reference to the corresponding section of the covered entity's notice of privacy practices.
b. Whether treatment, payment, enrollment, or eligibility of benefits can be conditioned on
c. Authorization, including research-related treatment and consequences of refusing to sign the Authorization, if applicable
d. A statement of the potential risk that PHI will be re-disclosed by the recipient. This may be a general statement that the Privacy Rule may no longer protect health information disclosed to the recipient

Authorization must be written in plain language and contain the core elements and required statements, and a signed copy must be provided to the individual signing it if an investigator itself is seeking the Authorization. An Authorization obtained for the study need not have a fixed expiration date or state a specific expiration event, the form can list "none" or the "the end of the research project."
Participant or participant's legally authorized representative has the right to revoke the Authorization, in writing, at any time.

10.0 Source Documents and Case Report Form Completion

10.1 Source documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

10.2 Case report forms

Data for individual subjects will be recorded on CRFs provided by the BPCA-CC. All entries must be complete. A case report form must be completed for each subject enrolled, including those removed from the study. If a subject is removed from the study, the reason for removal must be noted on the CRF by the investigator. The principal investigator must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested as part of an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

Because of the potential for errors and inaccuracies in entering data onto CRFs, laboratory and other test results must be kept on file with the subject's study dossier. Case report forms and copies of test results must be available at all times for inspection by the CRA for the site and the FDA.

11.0 Administration

11.1 Steering Committee

The Members of the Steering Committee will include the PODS PI and several of the site PIs (or Co PIs where applicable) at the subcontract sites, a representative(s) from the BPCA-CC, and the NICHD Project Officer. The NICHD Project Officer and the BPCA-CC staff will be non-voting members of the Steering Committee.

The Steering Committee will hold regular teleconferences. All Steering Committee members (or in special circumstances, their designee) will be required to participate in these meetings/teleconferences.

The Steering Committee will seek and accept advice from the NICHD, BPCA-CC and the DSMB, and will receive implementation recommendations from regular study coordinators' teleconference, to which it may delegate authority for minor implementation decisions. It will adopt a publication policy acceptable to all sites and will supervise the publication of results.

Should a problem at any given site arise, the PI (and Co-PIs) at that site will be contacted by one or more members of the Steering Committee to discuss the problem and to develop a plan for its resolution. A timeline and action-plan will be developed. This plan will be reported back to the Steering Committee. The timeline and outcome will then be monitored by the Steering Committee.

11.2 Responsibilities of the clinical investigator

Each of the site investigators will be responsible for the overall conduct of the study at their site. They must supervise all staff participating in each phase of the project, and be responsible for meeting the established timelines to the best of their ability. Finally, all PIs are ultimately responsible for the ethical conduct of the various studies at their sites and for timely completion of this study and communication of the results to the lead site.

12.0 Data Quality Control and Assurance

Prior to the initiation of the study, an investigator's meeting will be held with the BPCA-CC and MPODS network personnel, the investigators and their study coordinators for the study. This meeting will include a detailed discussion of the protocol, performance of study procedures, CRF completion, simulation of study procedures and specimen collection methods, as applicable. In addition to the investigators' meeting, the study personnel at each site will be trained on the study procedures at a study initiation visit.

The CRAs will monitor each site throughout the study. At each visit, 100% source document review will be made against entries on the CRF and a quality assurance check will be performed to ensure that the investigator is complying with the protocol and all applicable regulations.

After completion of the entry process, computer logic checks will be run to check for such items as inconsistent study dates and outlying laboratory values. Any necessary correction will be made to the database and documented via addenda or audit trail. A manual review of selected line listings will also be performed at the end of the study.

12.1 Site monitoring visits

Monitoring visits to the sites will be made periodically during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data collected on the CRFs. Participating sites and investigators will guarantee access to source documents and CRFs to the CRAs. The principal investigator and relevant site personnel will be available during the monitoring visits and will set aside sufficient time for the process.

12.2 Quality assurance and regulatory agency audits

The study sites may also be subject to quality assurance audits by the NICHD or its designees and appropriate regulatory agencies.

12.3 Data processing and data management

Clinical data processing and data management will be employed based on the procedures developed by the BPCA-CC in conjunction with the NICHD. All of the data entered into the study data set (at the BPCA-CC) will be checked for valid values and ranges, between item logical consistency, and within-subject variation. Prior to any analyses, the distributions of the measures will be examined to aid in the selection of appropriate statistical techniques. Data transformations, utilizing nonparametric and semiparametric techniques, will be used in an attempt to normalize any data that is not normally distributed.

12.4 Ensuring confidentiality

A study number will be assigned for each subject. Data forms will be identified by subject number and initials. The database will not contain any personal identifiers other than subject number and initials.

12.5 Record retention

To enable evaluations and/or audits from Regulatory Authorities and NICHD or its designees, the investigators will keep records, including the identification of all medical charts and associated source documents and copies of all CRFs. The investigators will contact NICHD before disposing of any such materials.

13.0 Use of Information and Publication

13.1 Use of information

After the dataset for the study is finalized and main findings have gone into publication, the data from this project will be made available and shared through CD-ROM and/or a website. All project data will be stored without subject identifiers, so that the data that are shared cannot be linked back to any particular subject. The dataset will cover the outcome data on children collected over the course of the study.

13.2 Publication policy

Prior to a manuscript or abstract being submitted for possible publication or presentation, the Steering Committee, BPCA-CC, and NICHD must review the contents of the submission. More specifically, manuscripts, abstracts, and poster submissions must be submitted to the Steering Committee, BPCA-CC, and NICHD. Financial support from the NICHD will be acknowledged in all publications.

13.3 Data sharing plan

The dataset for this study will cover course and outcome data on children collected over the period of study. The data sharing plan will follow guidelines as dictated by institutional rules and approval of local IRBs of participating research sites, local, state and federal laws and regulations including the Privacy Rule. The final data sharing plan that will be employed by the BEST protocols will be developed in conjunction with the individual site PI's, the BPCA-CC, and the NICHD.

14.0 Completion of Study

The MPODS PI and MPODS investigators will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon by both the MPODS PI and the BPCA-CC.

The NICHD may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. An investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the BPCA-CC and NICHD within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

15.0 Investigator Agreement

I have received and reviewed the package insert for meropenem.

I have read the protocol and agree to conduct the study as outlined and in accordance with all applicable local, state, and federal regulation.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Principal Investigator Date

Name of Principal Investigator (printed or typed)

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Appendix 1: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the subject's interest when providing medical care which might have the effect of weakening the physical and mental condition of the subject."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any SAEs. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
10. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
11. The subjects must be volunteers and informed participants in the research project.
12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the subject's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case, the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the subjects who are research subjects.
2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
3. At the conclusion of the study, every subject entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
4. The physician should fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study must never interfere with the subject-physician relationship.
5. In the treatment of a subject, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the subject, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix 2: PK sampling times

Dose 1 PK-odd Group (infants with birthday on an odd date, e.g. 1st, 3rd, 5th, etc.)
1) Pre: anytime in the 24 hours prior to the first dose
2) Peak: 30 minutes to 1 hour after completion of first dose
3) 3-4 hours after completion of first dose
4) Trough: 7-8 hours after completion of first dose if Q8 hour dosing, or 10-12 hours after completion of first dose if Q12 hour dosing

Dose 1 PK-even Group (infants with birthday on an even date, e.g. 2nd, 4th, 6th etc.)
1) Pre: anytime in the 24 hours prior to the first dose
2) Peak: 1-2 hours after completion of first dose
3) 4-6 hours after completion of first dose
4) Trough: 7-8 hours after completion of first dose if Q8 hour dosing, or 10-12 hours after completion of first dose if Q12 hour dosing

Dose 5 PK-steady state (around 5th dose if possible, but may be done around the 4th, 6th, 7th, 8th, 9th or 10th dose)
1) Pre: anytime in the 3 hours prior to the 5th dose
2) Peak: 15 minutes-2.5 hours after completion of 5th dose
3) 4-12 hours after completion of fifth dose

Safety Steady-state Group
1. No Dose 1 PK samples should be drawn
2. Follow Dose 5 PK-steady state instructions above.

Appendix 3: Dosing Regimen

Low Dose

Infant	GA <32 weeks:	
	< 2 week PNA:	20 mg/kg q 12hr
	≥ 2 weeks PNA:	20 mg/kg q 8hr
Infant	GA ≥32 weeks:	
	< 2 week PNA:	20 mg/kg q 8hr
	≥ 2 weeks PNA:	30 mg/kg q 8hr

High Dose

Infant	GA <32 weeks:	
	< 2 week PNA:	20 mg/kg q 8hr
	≥ 2 weeks PNA:	30 mg/kg q 8hr
Infant	GA ≥32 weeks:	
	< 2 week PNA:	30mg/kg q 8hr
	≥ 2 weeks PNA:	40mg/kg q 8hr

Appendix 4: Study Definitions

Complicated intra-abdominal infections are defined as outlined in the WR. Complicated intra-abdominal infections are characterized by systemic inflammation and an intra-abdominal process extending into the peritoneal space that necessitates a surgical or percutaneous drainage procedure. The post-procedure findings of purulent exudates with inflamed or necrotic exudates with inflamed or necrotic tissue confirm the diagnosis. Examples of intra-abdominal processes in the youngest subjects that can result in peritonitis include:

- Necrotizing enterocolitis,
- Bowel obstruction with perforation,
- Hirschsprung's disease with perforation,
- Meconium ileus with perforation, and
- Spontaneous perforation

Suspected complicated intra-abdominal infections are defined as Necrotizing Enterocolitis (NEC) Stage 2 or greater by modified Bell's Criteria. This includes infants with portal venous gas or pneumatosis intestinalis by abdominal radiograph. These infants are eligible for study.

Empirical antibacterial therapy is defined as the antibacterial therapy that is administered at when cultures of normally sterile body fluid are negative, or results of normally sterile body fluid cultures are pending. Infants will receive empirical meropenem and aminoglycoside. The aminoglycoside is to be administered per local standard of care. The use of other antimicrobial agents is discouraged but may be added per local standard of care.

Empirical antibacterial therapy with meropenem per local standard of care: if an infant is given meropenem as empirical therapy, and such therapy is given in accordance with local standard of care, these infants are eligible for enrollment. In these cases, the infants do not require Stage 2 NEC, but the reasons for empirical therapy with meropenem should be documented in the CRF.

Antibacterial therapy for perforated bowel: If an infant has evidence of perforation or requires surgery, metronidazole should not be added given the anaerobic coverage of meropenem.

Definitive antibacterial therapy: If an infant has a positive culture from normally sterile body fluid (blood, CSF, peritoneal fluid, urine from in/out catheterization, etc.) then the infant may receive definitive therapy per local standard of care based on the sensitivity of the blood culture.

Definition of escalated empirical antibacterial therapy: if at any time the attending physician determines that a study participant's clinical condition has deteriorated while receiving initial empirical antibiotic therapy, the infant may receive additional empirical therapy as outlined in the escalation parameters below.

Escalation of antibacterial therapy will be considered a 'treatment failure'. These data will not be a primary endpoint for this trial, but will be used for planning subsequent trials. The following are guidelines that may be used to escalate therapy

Escalation for presumptive Gram negative rod (GNR) therapy: normally sterile body fluid yields Gram-negative bacilli and clinical deterioration. If the infant's condition has stabilized or

improves during empirical treatment, then empirical therapy should not be changed. The broad outline that constitutes a deterioration of clinical status is:

a. **Cardiopulmonary deterioration**: infants with refractory hypotension and evidence of tissue hypo-perfusion or who require escalation in the level of respiratory support after first dose of study medication. Refractory hypotension includes infants who require >20 mL/kg crystalloid or plasma and/or initiation of a vasopressor/inotrope medication. Evidence of tissue hypoperfusion includes oliguria and/or metabolic acidosis with a base deficit of 7 or greater on two blood gases not less than 4 hours apart. Escalation in respiratory support may include change from supplemental oxygen only to assisted ventilation with nasal CPAP or mechanical ventilation. For infants already receiving mechanical ventilation an increase in the mean airway pressure greater than 2 cm/H_20 constitutes escalation in respiratory support.

b. **Disseminated intravascular coagulation** (DIC): includes infants with thrombocytopenia (platelet count <100K) or elevation in prothrombin time >1.5 times the reference standard with elevation in fibrin degradation products.

NOTE—the clinician is not obligated to add additional therapy in the face of cardiopulmonary deterioration or DIC, but if the infant fits these broad guidelines, **may** add additional therapy and declare initial therapy failure.

Escalation of empirical GNR therapy: cultures are pending but infant has clinical deterioration as outlined above. NOTE—the clinician is not forced to add additional therapy, but the clinician **may** add additional therapy.

Escalation of definitive GNR therapy: if the infant has a GNR isolate that is not susceptible to either meropenem **or** an aminoglycoside, then appropriate therapy per local standard of care will be given. If the infant has any GNR isolated (by culture or Gram stain) from the CSF, then appropriate therapy per local standard of care will be given.

Escalation of presumptive Gram positive therapy: Gram positive therapy may be added if normally sterile body fluid yields Gram-positive cocci. NOTE—the clinician is not obligated to add Gram positive therapy, but **may** add Gram positive therapy. If the initial Gram-stain is subsequently identified as a resistant Gram-positive organism requiring therapy (e.g. MRSA, ampicillin resistant enterococcus, coagulase-negative staphylococcus) then the infant may be given definitive Gram positive therapy (see below). In these circumstances, the infant will **not** be considered a failure if one of the following occurs: the organism isolated requires definitive Gram positive therapy (see below), or once it is proven that the organism does not require Gram positive therapy (e.g. methicillin sensitive S. aureus), Gram positive therapy is discontinued.

Escalation of empirical Gram positive therapy is discouraged, but is permitted.

Escalation to definitive Gram positive therapy: if the infant has a positive culture from normally sterile body fluid (e.g. blood, cerebrospinal fluid, newly placed peritoneal drain, urine from in/out catheterization or suprapubic aspiration) that requires Gram positive therapy as outlined above, then the infant may receive Gram positive therapy to treat the infection in accordance with local standard of care. The infant is **not** considered a failure in this scenario

Empirical antifungal therapy: is defined as the administration of a systemic antifungal agent with cultures that are negative or pending**.** Empirical and definitive antifungal therapies are both allowed in this study.

Locally Diagnosed Clinical Seizures: behavioral phenomena thought to be seizure, and diagnosed as seizure by the local site Principal Investigator. Examples of these behavior include focal clonic (e.g., involving one limb, one side of the body, or the tongue and face), focal tonic, and generalized myoclonic seizures. Clinical attributes that support the diagnosis of seizure include slower and more rhythmic motor activity that cannot be stopped by restraint.

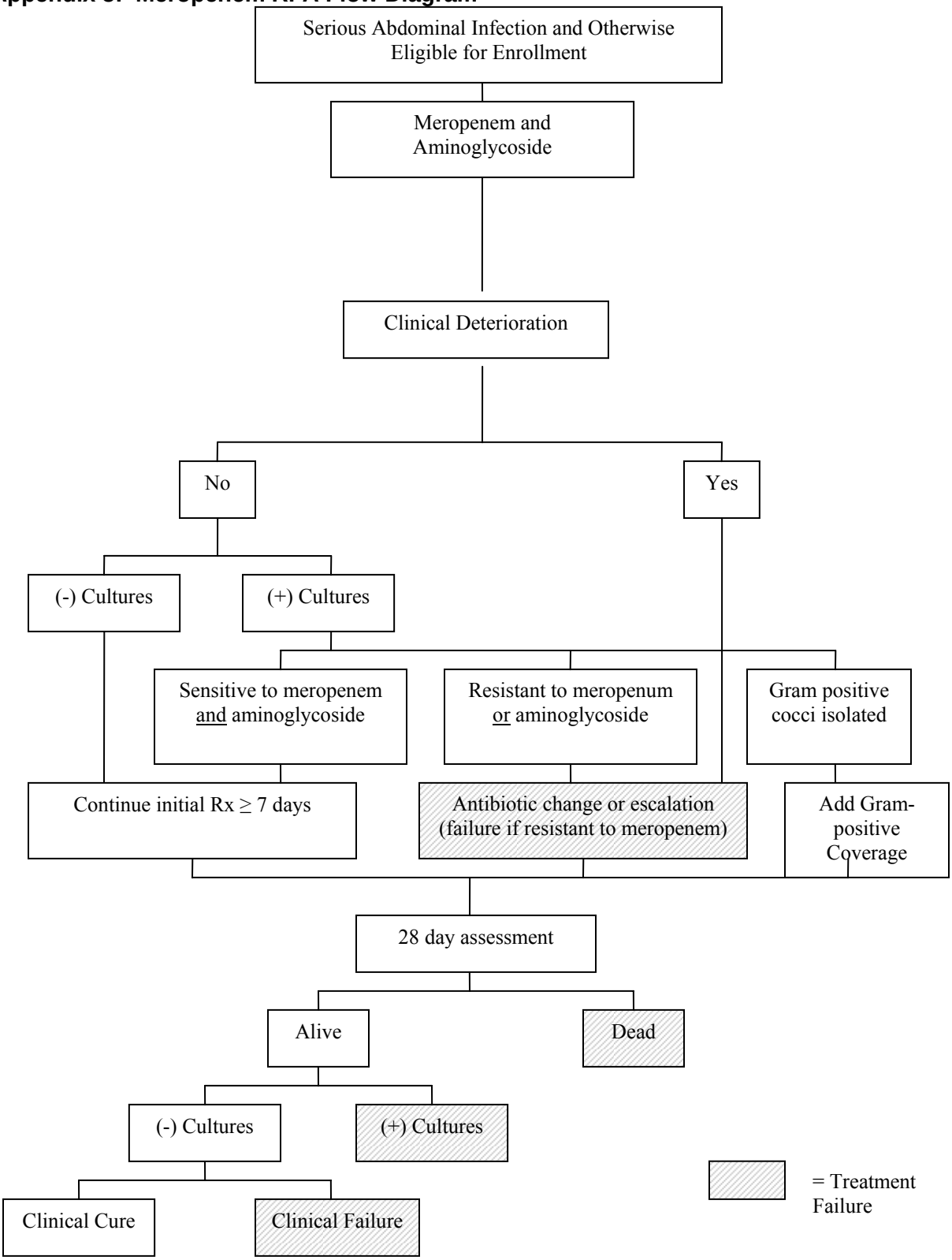
Locally Diagnosed EEG-confirmed Seizures: seizure activity confirmed by electrographic evidence by the local site Principal Investigator in consultation with a neurologist

Centrally Diagnosed Clinical Seizures: similar criteria as locally diagnosed clinical seizures, but also confirmed by the pediatric neurologist of the MPODS Clinical Events and Safety Committee.

Centrally Diagnosed EEG-confirmed Seizures: similar criteria to locally diagnosed EEG-confirmed seizures, but also confirmed by the pediatric neurologist of the MPODS Clinical Events and Safety Committee.

Non epileptic Seizure-Like Events (NELSE): clinical activity that may indicate the existence of neurologic dysfunction, but are not seizures. Clinical attributes that suggest NELSE are tremor, clonus, and motor automatisms that stop with restraint or are induced by touching or loud noises.

Appendix 5: Meropenem RFA Flow Diagram



Appendix 6: Scavenge sampling methods for meropenem pharmacokinetic and genomic analysis in infants

PROTOCOL SYNOPSIS

Product	Meropenem
Objectives:	1. Compare scavenge sampling to traditional sampling for the estimation of PK parameters of meropenem. 2. Collect scavenged blood sample for studies of associations between genetic variation and outcome after intra-abdominal infections 3. Determine the PK of meropenem in the cerebrospinal fluid (CSF) of infants
Study Design:	Add-on study to the multi-center, prospective, dose escalating pharmacokinetic study of meropenem for the treatment of suspected or complicated intra-abdominal infections (MPODS)
Study Population:	Infants enrolled in the primary MPODS protocol
Number of Infants:	All 200 infants in the MPODS will be eligible
Number of Sites:	25
Treatment:	Per MPODS protocol
Treatment Duration:	Per MPODS protocol
Procedures:	Excess plasma and whole blood samples will be saved from infants who have excess laboratory samples obtained in the course of standard care. Additional CSF will be collected from infants who undergo lumbar puncture or ventricular/reservoir tap per local standard of care.
Statistical Consideration:	All infants who have samples obtained will be analyzed. Plasma PK parameters will be calculated separately for the two types of collection methods: population PK timed sampling and scavenge sampling. We will estimate the following PK parameters: Cl, Vd, C_{max}, AUC_{0-T}(AUC at steady state), Ke, $t_{1/2}$, and $AUC_{0-\infty}$ (estimated from the 1^{st} dose). For genetic association studies, exploratory hypothesis generating analyses of the association between common genetic variants (> 10% minor allele frequency) and outcome, could be conducted; the samples would also be available for sharing with investigators interested in the necrotizing enterocolitis phenotype. All infants who have CSF obtained will be analyzed. From the concentrations of meropenem determined in the CSF samples, we will obtain estimates of the following: 1. CSF/plasma meropenem concentration ratio 2. % of dosing interval above MIC_{90} for common pathogens including: *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. 3. Effect of CSF pleocytosis on meropenem concentrations 4. Relationship between meropenem concentration and clinical outcome.
Inclusion Criteria	Enrolled in the MPODS study
Exclusion Criteria	Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
BUN	Blood Urea Nitrogen
C	Drug Concentration
CBC	Complete Blood Count
CFRs	Code of Federal Regulations
CI	Clearance
CNS	Central Nervous System
CRA	Clinical Research Associate
CRF	Case Report Form
CRP	C-reactive Protein
CSF	Cerebrospinal fluid
DCRI	Duke Clinical Research Institute
DNA	Deoxyribonucleic acid
DOL	Day of Life
DSMB	Data and Safety Monitoring Board
EDTA	Ethylenediaminetetraacetic acid
ELBW	Extremely Low Birth Weight
ESBL	Extended Spectrum Beta-Lactamases
FDA	Food and Drug Administration
g/dL	Grams per Deciliter
GA	Gestational Age
GCP	Good Clinical Practice
GNR	Gram Negative Rod
HHS	Health and Human Services
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IVH	Intraventricular Hemorrhage
IVRS	Interactive Voice Response System
Kg	Kilogram
LFT	Liver Function Tests
Mcg	Microgram
Mg	Milligrams
MIC	Minimum Inhibitory Concentration
Min	Minute
mL	Milliliter
MPODS	Meropenem Off-Patent Drug Studies
MRSA	Methicillin Resistant S. aureus
NEC	Necrotizing Enterocolitis
NICHD	National Institute for Child Health and Human Development
NICU	Neonatal Intensive Care Unit

PD	Pharmacodynamic
PHI	Personal Health Information
PI	Principal Investigator
PK	Pharmacokinetic
PNA	Postnatal Age
PODS	Pediatric Off-Patent Drug Studies
SAE	Serious Adverse Event
SNAP	Score for Neonatal Acute Physiology
Vd	Volume of distribution
WR	Written Request

1. Background and Rationale

1.1. Scavenge sampling

The Meropenem Off-Patent Drug Studies (MPODS) will allow for determination of the Pharmacokinetic (PK) of meropenem in preterm infants with gestational ages of less than 30 weeks and collection of specific data on the disposition of meropenem in infants younger than 3 months with complicated intra-abdominal infections. Scavenge samples are samples obtained as part of the normal clinical care of the infants. These samples, once collected, are sent to the clinical laboratories for chemical and hematological assays. Blood sent to the laboratories is often in excess of what is needed for the laboratory assay. Handling of the blood is not standardized and it is often exposed to room temperature for periods of time.

Blood is routinely drawn from infants with complicated intra-abdominal infections (up to 4-6 times per day). As blood sampling for study purposes is limited at many institutions to 7 mL/kg over the period of the study, alternative methods of sampling are needed. The amount of blood allowed for study sampling would be 3.5 mL (<1 teaspoon) for an infant with a weight of 500 grams. These infants are exposed to prolonged courses of numerous antimicrobials while in the Neonatal Intensive Care Unit (NICU) and are at the highest risk of adverse drug events due to an almost complete lack of information regarding PK and safety of therapeutic agents.

1.2. Rationale for collecting scavenged blood for DNA testing.

Epidemiologic data has been gathered for the plausability of a genetic contribution to risk of diseases in extremely preterm birth. Testing a cohort of mono- and di-zygotic twins, investigators recently identified possible familial contribution to risk of Necrotizing Enterocolitis (NEC) and other complications of preterm birth.[1, 2] Only a handful of studies have been done to assess specific genetic variants for associations with risk of NEC in preterm infants. Searches for associations with NEC have also identified a few possible candidate genes, including immune related genes and most recently, a gene involved in arginine metabolism, carbamoyl-phosphate synthetase 1; however,[5, 16, 21, 22] these studies are fraught with limitations including small numbers, in one study only 17 cases and 34 controls were used[16] and varied phenotype definitions (52 of 153 very low birthweight infants in Bokodi's study were classified as NEC cases, with no modified Bell's criteria specified for the cases, while 17 of 46 cases in Treszl's study had stage I NEC.)

The scavenged deoxyribonucleic acid (DNA) samples could be used for comparisons of prevalence of genetic variants among those with different outcomes, and could also be shared with investigators interested in phenotypes of infants whose samples were included in the collection. Samples could also be used to assess the influence of pharmacogenomic variations in the drug metabolic pathways on pharmacokinetic results.[11] A tool such as the multiplex array simultaneously test for 1227 genetic variants in 169 genes involved in drug metabolism, excretion, and transport described by Daly et al, would allow efficient analysis of pharmacogenomic variations and associations with meropenem pharmacokinetics in the study population.[10]

1.3. Rationale for Evaluating Cerebrospinal Fluid PK

The cumulative incidence of meningitis is highest in the first month of life and is higher in preterm infants than term infants.[17] For infants who develop meningitis, the neurodevelopmental consequences are often profound.[20] Empirical antibiotics are often given to sick infants in the NICU. However, blood culture sensitivity may be compromised by the relatively small amount of blood used for the blood culture, 0.5-1 mL in infants vs. >20 mL in adult patients. Unfortunately, many clinicians often delay lumbar puncture in infants for several reasons including: clinical instability of the infant and belief that meningitis is of low probability. However, 30-38% of cases

of meningitis in infants are associated with negative blood cultures. In this setting, use of empirical therapy with poor CNS penetration (aminoglycosides) may not effectively treat undiagnosed CNS infections. Even agents that penetrate the CNS well (3rd generation cephalosporins) may be ineffective in the setting of highly resistant organisms such as extended spectrum beta-lactamase producing organisms (e.g. *Klebsiella spp*).[13, 14]

1.4. Meropenem for treatment of meningitis in infants

The lumbar puncture is often deferred due to the instability of the infant's clinical condition complicating the diagnosis of meningitis. Therefore, higher antimicrobial CSF levels in the setting where cerebrospinal fluid (CSF) results are not known are a public health need. Unlike imipenem, seldom used for meningitis because of the risk of seizures, meropenem's side effect profile allows it to be considered an agent for treatment of CNS infections[12]

In a study of 23 patients (22 pediatric patients) with meningitis given a single dose of meropenem, the CSF/plasma concentration ratio of meropenem ranged from <0.01 to 0.52.[9] CSF concentrations in patients receiving the highest dosage of meropenem (40 mg/kg) ranged from 0.9-6.5 mg/dL. However, many of these patients had been previously treated with other antibiotics and/or steroids prior to obtaining CSF for PK analysis. No data are currently available on the CSF concentrations of meropenem in infants. We have designed these dosages in the MPODS study to achieve meropenem serum concentrations that will likely result in therapeutic levels of meropenem in the CNS. CNS therapeutic levels are desirable because the probability of meningitis is >1% in this population.

2. Study Objectives

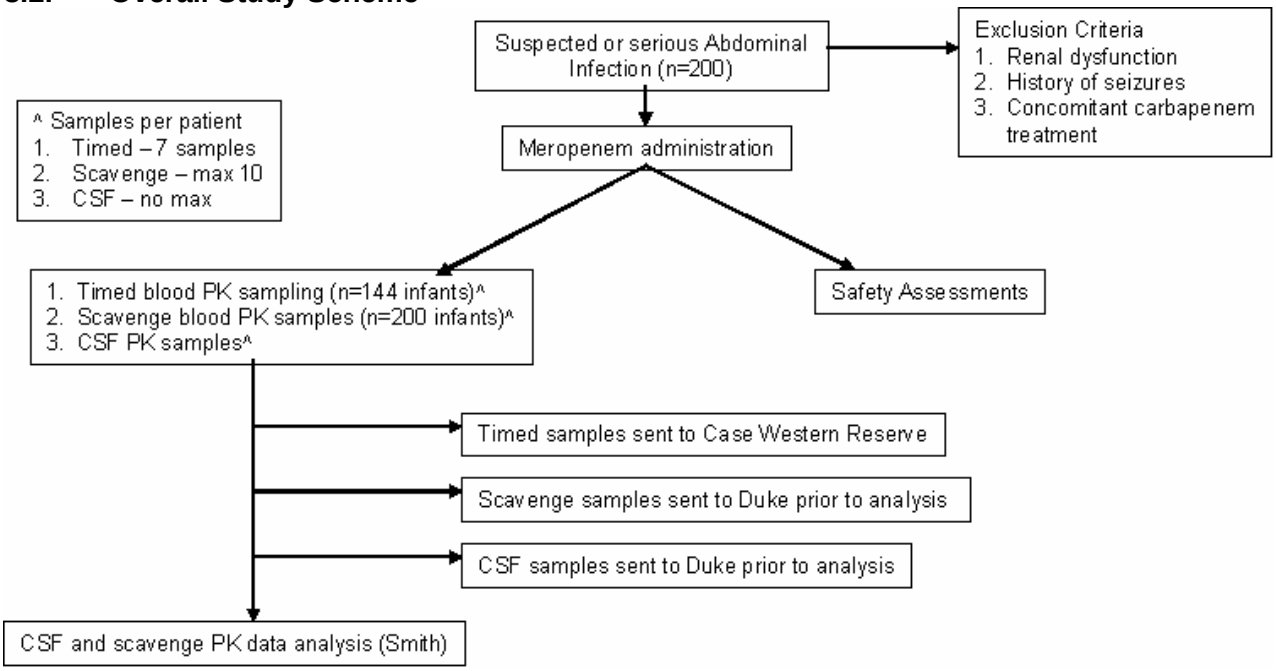
1. Compare scavenge sampling to traditional sampling for the estimation of PK parameters of meropenem.
2. Collect scavenged samples for future DNA extraction and genomics studies
3. Determine the PK of meropenem in the cerebrospinal fluid (CSF) of infants using population PK methodology
4. Determine the CSF/plasma meropenem concentration ratio
5. Determine the % of dosing interval above MIC_{90} for common pathogens including: *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp.
6. Define the effect of CSF pleocytosis on meropenem concentrations
7. Describe the relationship between meropenem CSF concentration and clinical outcome.

3. Investigational Plan

3.1. Overall Study Design

MPODS is a multi-center, prospective, pharmacokinetic study. Infants enrolled in this portion of the study will have scavenge plasma samples for PK analysis and scavenge whole blood samples for genomics analysis obtained if extra plasma or whole blood is available from laboratory samples obtained as part of clinical care. Infants enrolled in this portion of the study will have additional CSF obtained for PK analysis obtained if CSF is obtained as part of clinical care.

3.2. Overall Study Scheme



3.3. Selection of Study Population

Infants enrolled in the MPODS are eligible for inclusion.

3.4. Inclusion Criteria

1. Enrolled in the MPODS study

3.5. Exclusion Criteria

1. Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

3.6. Withdrawal from Study

Infants may be withdrawn from MPODS or from the scavenge study at any time. Reasons for infant withdrawal from the study include, but are not limited to:

1. Infant's parent or legal guardian chooses to withdraw the infant for any reason
2. Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the infant's safety
3. The investigator determines that it is in the infant's best medical interest to be withdrawn.

4. Assessments

4.1. Timed PK Measurements

A population PK approach will be employed for plasma samples obtained as part of the MPODS study.

4.2. Scavenge Samples (PK and Genomics)

1. For the PK analysis, study nurses will collect any remaining plasma from the clinical laboratories from infants receiving meropenem as part of this protocol. For the genomic studies, study nurses will collect remaining whole blood samples, ideally EDTA tube blood.
2. No extra blood/plasma will be needed for this portion of the study.

3. A maximum of 10 plasma samples and 5 whole blood samples will be collected per patient.
4. Whole blood and plasma samples will be stored at -70°C at the study site until shipment to Duke University Medical Center. Shipment of scavenge specimens will occur every 6 months from the sites to the PK specimen repository at Duke University Medical Center.
5. The samples will be stored at Duke prior to meropenem concentration determination, and DNA extraction.

4.3. CSF PK Measurements

1. 50-200 µL of additional CSF will be collected from infants when CSF is obtained as part of clinical care while an infant is receiving study drug (meropenem).
2. CSF may be obtained by the following methods
    a. Lumbar puncture
    b. Ventricular tap
    c. CSF reservoir tap
3. Study nurses will be notified by the care team when clinicians collect CSF from consented patients.
4. 50-200 µL of CSF for meropenem level determination will be collected in a separate sterile polypropylene tube at the time that CSF is collected for diagnostic purposes.
5. The CSF collected for pharmacokinetic analysis will be stored at -70°C at the study site until shipment to Duke University Medical Center.
6. Shipment of CSF specimens will occur every 6 months from the sites to the PK specimen repository at Duke University Medical Center.
7. The samples will be stored at Duke prior to meropenem concentration determination.

5. Procedures and Study Visits

The table below illustrates the schematic representation of assessments:

Table: Schedule of Procedures

	Study Day			
PROCEDURE	0	1-27	28	29 to end of study
Informed Consent	X[a]			
CSF, PK, and genomics evaluation		X		
Meropenem administration		X[b]		
Plasma Pharmacokinetic Evaluation		X[c]		

a) Prior to enrollment and other study activities
b) Treatment for minimum of 3 days per MPODS study
c) Obtained per MPODS protocol

5.1. Prior to the administration of study drug:

Obtain signed and dated informed consent/ Health Insurance Portability and Accountability Act (HIPAA) consent for MPODS study **AND** this protocol

5.2. Study Days 1-27 (while on meropenem therapy)

5.2.1. Plasma scavenge samples

a. Study nurses will obtain scavenge samples from the clinical laboratory (>50 µL of plasma).
b. Record specimen number, study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion(24 hour clock), dose given (mg), and current patient weight (g).
c. Samples must be <96 hours old at the time of collection

d. A maximum of 10 samples will be obtained per patient.

5.2.2. Whole blood genomics samples

a. Study nurses will obtain scavenge whole blood samples remaining in the laboratory samples from the clinical laboratory (>50 µL of whole blood)
    i. EDTA tubes are preferred
    ii. Heparin tubes can be used if no EDTA available
b. Record specimen number and date sample drawn from infant
c. Samples must be <96 hours old at the time of collection
d. A maximum of 5 samples will be obtained per patient.

5.2.3. CSF samples

a. Obtain 50-200 µL CSF at the time of lumbar puncture or ventricular/reservoir tap
b. Record specimen number, meropenem infusion times for the dose before CSF samples are obtained—this includes start/stop times of infusion (24 hour clock), dose given (mg), and current patient weight (g).
c. Collect and record the results of CSF analysis (CSF white and red blood cell counts, CSF glucose, and CSF protein).
d. Record results of CSF cultures obtained at the time of CSF PK sampling
e. Study nurses will collect 1 sample of scavenge plasma available from the day CSF was obtained. No extra blood will be needed for this portion of the study. Samples must have at least 50 µL of plasma to be used for this study. If multiple samples are available, the sample closest in time to the CSF sample will be collected. Samples will be labeled as described above for scavenge samples.

6. Administration

6.1. Trial Termination

The National Institute for Child Health and Human Development (NICHD), the Best Pharmaceuticals for Children Act Coordinating Center (BPCA-CC), the Pediatric Off-Patent Drug Studies (PODS) Principal Investigator (PI) and the Duke Clinical Research Institute (DCRI) will monitor the progression of the trial. Investigator and site participation in the study may be terminated by the PODS PI if there is evidence of an investigator failing to maintain adequate clinical standards or evidence of an investigator or staff failing to comply with the protocol.

6.2. Scavenge Plasma Specimen Handling

4. Collect >50 µl of plasma using individual sterile polypropylene containers.
5. The samples should be stored at -70°C within 4 hours of collection.
6. Label tubes with the specimen number: It will consist of the letter “P”, the site number, the MPODS subject number, and the sample number.

6.3. Scavenge Whole Blood Specimen Handling

1. Collect >50 µl of whole blood using individual sterile polypropylene containers.
2. The samples should be stored at -70°C within 4 hours of collection.
3. Label tubes with the specimen number: It will consist of the letter “B”, the site number, the MPODS subject number, and the sample number.

6.4. CSF Specimen Handling

1. Collect 50-200 µl of CSF using individual sterile polypropylene containers.
2. The samples should be stored at -70°C within 4 hours of collection.

3. Label tubes with the specimen number: It will consist of the letter "C", the site number, the MPODS subject number, and the sample number.

6.5. Specimen Shipping

The specimen shipping form lists all patients, and their identifying information, whose specimens are being sent to Duke University. It is completed by both the staff at the study site center and the staff at Duke University receiving laboratory. It is used to document and identify all scavenge plasma, scavenge whole blood, and CSF specimens that are shipped out by the center. A copy of this form will accompany the shipment from the study site to Duke University. This form is not entered into the computerized database. The following information should be completed on each shipment log.

1. Center Number
2. Number of scavenge samples enclosed
3. Initials of staff at the shipping center completing the form
4. Specimen numbers of specimens contained in the shipment

The samples should be shipped on **a Monday** (or Tuesday after a Monday holiday) in October of 2008, April 2009, and at the end of the study. For the week of Christmas and New Year's, the nurse coordinator will contact Dr. Brian Smith (brian.smith@duke.edu; **919-970-3193**) who will discuss with the nurse coordinator and the technologist the ideal time for sample shipment. Batch the samples in an outer bag, and then pack in dry ice to be shipped to the Duke NICU Clinical Research Office. The Specimen Shipment Log should be filled out and included in the shipment.

SHIP TO:
Sandra Grimes
Box 3179 DUMC
Durham, North Carolina, 27710
Phone: (919) 668-6368
Contact Sandra Grimes at 919-668-3360, or by email at sandra.grimes@duke.edu for questions. Please e-mail the FedEx tracking number to sandra.grimes@duke.edu after you have shipped the package.

7. Statistical Methods

7.1. General Considerations of the Statistical and Analytic Plans

The primary objectives of this analysis are to assess the PK of meropenem in young infants and to validate scavenge sampling in a population where timed sampling is extremely difficult.

7.2. Definitions and Populations for Analysis

All infants who have samples obtained will be analyzed.

7.3. Demographics

Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented by dosage group for continuous variables (such as age, weight, etc). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by dosage group to summarize discrete variables (such as race, sex, success rates, mortality rates, etc.). Demographic and baseline characteristics will be summarized by group and dosage.

7.4. PK parameters

PK parameters will be described by presenting the plasma concentrations-time profiles of meropenem in tabular and graphical form by subject, age cohort, and dosage level. The relationship between plasma concentrations and/or PK parameters with demographic factors (weight, sex, age and race), disease severity, toxicity and coadministered medications will be investigated. Analysis of potential relationships between drug and exposure in subjects and the resulting efficacy and/or safety response will be conducted. PK parameters will be calculated separately for the two types of collection methods: Population PK timed sampling and scavenge sampling. We will estimate the following PK parameters: Cl, Vd, C_{max}, AUC_{0-T}(AUC at steady state), Ke, $t_{1/2}$, and $AUC_{0-\infty}$ (estimated from the 1st dose).

From the concentrations of meropenem determined in the CSF samples, we will obtain estimates of the following:

1. CSF/plasma meropenem concentration ratio
2. % of dosing interval above MIC_{90} for common pathogens including: *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp.
3. Effect of CSF pleocytosis on meropenem concentrations
4. Relationship between meropenem concentration and clinical outcome.

7.5. Sample Size

We anticipate that we will collect 3-4 (maximum of 10) scavenge plasma samples from 100-150 infants enrolled in this portion of the study (300-600 samples total). We anticipate that the PK parameters obtained from scavenge sampling will be similar to the values obtained under timed FDA compliant specifications (relative difference <10%). If scavenge sampling produces PK parameters dissimilar to those collected under time sampling, this proposal will serve as proof of concept that scavenge sampling cannot be used for collection of PK samples.

We expect to collect 2-3 scavenge whole blood samples from 100-150 infants enrolled in this portion of the study (200-450 samples total).

We anticipate that approximately 25 CSF samples will be obtained from 20 infants. We hypothesize that the CSF/plasma meropenem ratio will be >0.25 based on prior work in pediatric patients.[9] We also believe that concentrations of meropenem will exceed the MIC_{90} for >50% of the dosing interval for *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. If the concentration of meropenem are found to be >MIC_{90} for <50% of the dosing interval, we will discourage use of meropenem as therapy for suspected or proven meningitis in this setting.

8. Ethics

8.1. Ethical conduct of the trial

This study will be conducted according to the protocol, the applicable Food and Drug Administration (FDA) and Health and Human Services (HHS) Code of Federal Regulations (CFRs), Good Clinical Practice (GCP), the Declaration of Helsinki, and the International Conference on Harmonisation (ICH) Harmonized Tripartite Guideline for Good Clinical Practice. It will also adhere to the ethical principles outlined in The Belmont Report.

8.2. Adverse event risk and benefits

As this will be a very vulnerable population, we expect that there will be serious adverse events including death. But we anticipate that there will be no adverse events or serious adverse events *related to the study protocol*. As a result of scavenge sampling, no extra blood will be obtained

from the patient. The participants who are enrolled will have no direct benefit, but the study will have substantial benefit to other infants from the description of meropenem pharmacokinetics in this population in addition to validating scavenge sampling of antimicrobials for pharmacokinetic analysis. We expect no adverse events from participation in the scavenged blood for DNA protocol. CSF will only be obtained when done as part of standard of care. No attempt will be made to obtain CSF specifically for the study. AEs and SAEs will be monitored per the MPODS protocol. Samples will be identified by a code number linked to individual patient identifiers in study logs.

8.3. Institutional Review Board

Institutional review boards must be constituted according to the applicable State and Federal requirements of each participating site. The investigators and staff of this study and the IRB of each participating institution will rigorously monitor research data to ensure the safety of research subjects, and will protect the privacy and confidentiality of all study subjects.

This protocol must be submitted to appropriate IRBs and their written unconditional approval obtained and submitted to BPCA-CC before commencement of the study. Investigators must also inform IRBs of all subsequent protocol amendments. This approval must refer to the study by exact protocol title and number, identify documents reviewed, and state the date of review. All correspondence with the IRB should be filed by the investigator. Institutional review boards must be informed by investigators of all serious or unexpected adverse events occurring during the study that are likely to affect the safety of the subjects or the conduct of the study.

8.4. Informed consent

Consent for the scavenged and CSF sample study will be separate from the MPODS study OR will be offered as separate opt-in boxes as part of the MPODS consent form.

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation. Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form.

The parental/guardian permission form generated by the investigator with the assistance of BPCA-CC must be approved (along with the protocol) by the IRB and be acceptable to the Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the ICH Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA-CC, Regulatory Authorities, and NICHD or its designees.

8.5. Protection of personal health information

All reports and communications relating to subjects in the study will identify each subject only by the subject's initials and the subject's study number. The investigators will agree to maintain records identifying the subjects enrolled in the study, which will be used for the purpose of long-term follow-up.

Investigators at each study site will be responsible for insuring compliance with the Privacy Rule, a Federal regulation under the Health Insurance Portability and Accountability Act (HIPAA), in accordance with the investigator's institution policy. The Privacy Rule establishes the right of a research subject or subject's legally authorized representative to authorize an investigator to use and disclose subject's personal health information (PHI) for research purposes. This requirement is in addition to the informed consent to participate in the study. A valid Privacy Rule Authorization is a subject's or subject's legally authorized representative signed permission that allows an investigator to use or disclose the subject's PHI for the purposes, and to the recipient or recipients, as stated in the Authorization. The signed Authorization must be retained by the investigator for 6 years form the date of creation or the date it was last in effect, whichever is later.

Authorization can be combined with an informed permission. Whether combined with an informed permission or separate, an Authorization must contain the following specific core elements and required statements stipulated in the Privacy Rule.

8.6. Authorization core elements

1. A description of the PHI to be used or disclosed, identifying the information in a specific and meaningful manner
2. The names or other specific identification of the person or persons (or class of persons) authorized to make the requested use or disclosure
3. The names or other specific identification of the person or persons (or class of persons) to whom the covered entity may make the requested use or disclosure
4. A description of each purpose of the requested use or disclosure

5. Authorization expiration date or expiration event that relates to the individual or to the purpose of the use or disclosure ("end of the research study" or "none" are permissible for research, including for the creation and maintenance of a research database or repository)
6. Signature of the individual and date. If the individual's legally authorized representative signs the Authorization, a description of the representative's authority to act for the individual must also be provided

8.7. Authorization required statements

1. A statement of the individual's right to revoke his/her Authorization and how to do so, and, if applicable, the exceptions to the right to revoke his/her Authorization or reference to the corresponding section of the covered entity's notice of privacy practices.
2. Whether treatment, payment, enrollment, or eligibility of benefits can be conditioned on
3. Authorization, including research-related treatment and consequences of refusing to sign the Authorization, if applicable.
4. A statement of the potential risk that PHI will be re-disclosed by the recipient. This may be a general statement that the Privacy Rule may no longer protect health information disclosed to the recipient

Authorization must be written in plain language and contain the core elements and required statements, and a signed copy must be provided to the individual signing it if an investigator itself is seeking the Authorization. An Authorization obtained for the study need not have a fixed expiration date or state a specific expiration event, the form can list "none" or the "the end of the research project." Participant or participant's legally authorized representative has the right to revoke the Authorization, in writing, at any time.

9. Source Documents and Case Report Form Completion

9.1. Source documents

Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, pharmacy dispensing records, and recorded data from automated instruments. The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/IEC review, regulatory inspection(s), and will provide direct access to source data documents.

9.2. Case report forms (CRF)

This study will use a single page CRF to record clinical data related to the scavenge sample(s). Other clinical information will be obtained from CRFs in the MPODS trial. Data for individual subjects will be recorded on CRFs provided by the BPCA-CC. All entries must be complete. The principal investigator must review and approve each CRF.

Case report forms must be current to reflect subject status at each phase during the course of the study. Subjects are not to be identified on the CRFs by name; appropriate coded identification and subject initials must be used. The investigator must keep a separate log of subject names and addresses. If requested as part of an FDA inspection, this log may be shown to the FDA investigator, but no copy should be provided so that confidentiality is protected.

10. Administration

10.1. Steering Committee

The Members of the Steering Committee will include the PODS PI and several of the site PIs (or Co PIs where applicable) at the subcontract sites, a representative(s) from the BPCA-CC, and the

NICHD Project Officer. The NICHD Project Officer and the BPCA-CC staff will be non-voting members of the Steering Committee. The Steering Committee will function as described in the MPODS protocol.

10.2. Responsibilities of the clinical investigator

Each of the site investigators will be responsible for the overall conduct of the study at their site. They must supervise all staff participating in each phase of the project, and be responsible for meeting the established timelines to the best of their ability. Finally, all PIs are ultimately responsible for the ethical conduct of the various studies at their sites and for timely completion of this study and communication of the results to the lead site.

11. Data Quality Control and Assurance

11.1. Data processing and data management

Clinical data processing and data management will be employed based on the procedures developed by the BPCA-CC in conjunction with the NICHD. All of the data entered into the study data set (at the BPCA-CC) will be checked for valid values and ranges, between item logical consistency, and within-subject variation.

11.2. Ensuring confidentiality

A study number will be assigned for each subject in the MPODS study which will be retained for identification for this protocol. Data forms will be identified by subject number and initials. The database will not contain any personal identifiers other than subject number and initials.

11.3. Record retention

To enable evaluations and/or audits from Regulatory Authorities and NICHD or its designees, the investigators will keep records, including the identification of all medical charts and associated source documents and copies of all CRFs. The investigators will contact NICHD before disposing of any such materials.

12. Use of Information and Publication

12.1. Use of information

After the dataset for the study is finalized and main findings have gone into publication, the data from this project will be made available and shared through CD-ROM and/or a website. All project data will be stored without subject identifiers, so that the data that are shared cannot be linked back to any particular subject. The dataset will cover the outcome data on children collected over the course of the study.

12.2. Publication policy

Prior to a manuscript or abstract being submitted for possible publication or presentation, the Steering Committee, BPCA-CC, and NICHD must review the contents of the submission. More specifically, manuscripts, abstracts, and poster submissions must be submitted to the Steering Committee, BPCA-CC, and NICHD. Financial support from the NICHD will be acknowledged in all publications.

13. Completion of Study

The MPODS PI and MPODS investigators will complete this study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon by both the MPODS PI and the BPCA-CC.

The NICHD may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. An investigator may also terminate the study at his/her site for reasonable cause, after providing written notice to the BPCA-CC and NICHD within a reasonable amount of time prior to the intended termination date. Advance notice is not required by either party if the study is terminated due to safety concerns.

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Appendix 7: Intestinal Meropenem Metabolism and Disposition in Young Infants (<91 days) with Suspected or Complicated Intra-abdominal Infections

PROTOCOL SYNOPSIS

Protocol Title	Intestinal Meropenem Metabolism and Disposition in Young Infants (<91 days) with Suspected or Complicated Intra-abdominal Infections
Sponsor:	National Institute for Child Health and Human Development
Product	Meropenem
Objectives:	a. To evaluate the metabolism of meropenem (to ICI 213689) by DHP-1 in the intestine b. To identify the role of transporters in the intestinal clearance of meropenem c. To evaluate the distribution of meropenem into body fluids compared to intestinal tissue
Study Population:	Infants enrolled in the primary (MPODS) study who require tissue resection per local standard of care
Number of Infants:	Approximately 200 infants will be enrolled in the main study (MPODS) – approximately 20 infants with intestinal tissue samples are expected for this protocol
Number of Sites:	Approximately 25
Treatment:	Meropenem and additional antimicrobial coverage may be added per standard of care.
Treatment Duration	At least 3 days; otherwise, per local standard of care
Procedure:	Collection of tissue/body fluid samples
PK/PD:	1. Minimal plasma sampling and population pharmacokinetics will be employed (MPODS)
Statistical Consideration:	All infants who receive meropenem and have an intestinal tissue sample obtained will be analyzed. From the intestinal tissue metabolic studies we will determine the following: 1. Metabolism of meropenem (to ICI 213689) by DHP-1 in the intestine 2. Role of transporters in the intestinal clearance of meropenem
Inclusion Criteria	1. Enrolled in the MPODS study 2. Intestinal resection or biopsy
Exclusion Criteria	1. Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

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ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
BLAST	Basic Local Alignment and Search Tool
BPCA-CC	Best Pharmaceuticals for Children Act Coordinating Center
C	Drug Concentration
CD-ROM	Compact Disc read only memory
CFRs	Code of Federal Regulations
CRA	Clinical Research Associate
CRF	Case Report Form
DCRI	Duke Clinical Research Institute
DNA	Deoxyribonucleic acid
DHP-1	Dehydropeptidase enzyme
DSMB	Data and Safety Monitoring Board
EDTA	Ethylenediaminetetraactic acid
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GDP	glycyldehydrophenylalanine
GPDH	Glycerol phosphate dehydrogenase
HHS	Health Human Services
HIPAA	Health Insurance Portability and Accountability Act
hOATs	human organic anion transporters
HPLC	High Pressure Liquid Chromatography
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
LDH	Lactate Dehydrogenase
IEC	Independent Ethics Committee
IRB	Institutional Review Board
Mg	Milligrams
µL	Microliter
mL	Milliliter
M	Molar
µM	Micromolar
MPODS	Meropenem Off-Patent Drug Studies
MRPs	multi-drug resistance protein
NEC	Necrotizing Enterocolitis
NICHD	National Institute for Child Health and Human Development
NICU	Neonatal Intensive Care Unit
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PD	Pharmacodynamic
PHI	Personal Health Information
PI	Principal Investigator
PODS	Pediatric Off-Patent Drug Studies
RNA	ribonucleic acid
RT-PCR	Real-time polymerase chain reaction

SAE	Serious Adverse Event
SD	Standard deviation
Tris-HCl	Tris Hydrochloride
Vd	Volume of distribution

1. Rationale for add-on translational proposal

The studies designed in the proposal (Principal Investigator (PI): Daniel K. Benjamin Jr.) being submitted in response to RFP-NIH-NICH-2005-18 will evaluate safety and pharmacokinetics of meropenem in young infants of the age less than 91 days. In these proposed studies, 200 young infants (<91 days old) with suspected or serious abdominal infections will receive meropenem. Many of these infants will have necrotizing enterocolitis (NEC) stage 2 or higher. The proposed study in this add-on translational research proposal will (i) examine in vitro metabolism of meropenem in the target population, and (ii) assess the role of likely transporters with specificity for anionic compounds (e.g. multi-drug resistance protein (MRPs) and human organic anion transporters (hOATs)) in the clearance of meropenem. The proposed study will ask and answer important question on whether non-renal clearance mechanisms (e.g. intestinal metabolism, secretion) become important in the removal of meropenem and/or its metabolite from the body for young infants; on the role of abdominal infections or NEC (stage 2 or higher) and how it adversely affects non-renal clearance mechanisms; and on distribution of meropenem into body fluids as compared to intestinal tissue.

2. Study Objectives

This study will evaluate the intestinal transport and metabolism of meropenem in infants <91 days of age with complicated intra-abdominal infections.
The specific aims are:

1. To evaluate the metabolism of meropenem (to ICI 213689) by DHP-1 in the intestine of subjects with serious abdominal infections who undergo surgical exploration and intestinal resection.
2. To identify the role of transporters (i.e. hOATs and MRP family) in the intestinal clearance of meropenem in subjects with serious abdominal infections who undergo surgical exploration and intestinal resection.
3. To evaluate the distribution of meropenem and its metabolite into body fluids and compare it to intestinal tissue disposition

3. Investigational plan

3.1 Overall Study Design

This is an add-on translational study to the parent multi-center, prospective, dose escalation safety study of meropenem for the treatment of complicated intra-abdominal infections.
We will obtain informed consent from each family to save samples of intestinal tissue, urine, plasma, pleural and peritoneal fluid for metabolism and distribution analysis if these specimens are obtained in infants undergoing surgical exploration with bowel resection as per local standard of care. Consent for this add-on study will not be required for participation in the main study; consent for this add-on will be provided as an “opt-in” check box. Allowing for opt-in consent and linking specimen collection to standard of care will ensure that this add-on study does not negatively impact enrollment in the main study. Data from these infants will be included within the Case Report Form (CRF) of the parent trial. A separate check box indicating sample collection and documentation of sample information will be recorded on the CRF. No more than 3 samples of intestinal tissue and no more than 1 sample of urine, plasma, pleural and peritoneal fluid will be collected per neonate.

3.2 Selection of Study Population

The study groups in this proposal are those infants enrolled in the main study—meropenem pediatric off label drug study (MPODS).

3.3 Inclusion criteria

a. Enrolled in the MPODS study
b. Intestinal resection or biopsy per local standard of care

3.4 Exclusion Criteria

a. Any condition which would make the subject or the caregiver, in the opinion of the investigator, unsuitable for the study

3.5 Withdrawal from Study

Infants may be withdrawn from treatment or from the study at any time. Reasons for infant withdrawal from the study include, but are not limited to:

a. Infant's parent or legal guardian chooses to withdraw the infant for any reason
b. Adverse events, conditions, or intercurrent illnesses that preclude compliance with the protocol, particularly if continuation would pose a risk to the infant's safety
c. The investigator determines that it is in the infant's best medical interest to be withdrawn
d. Detailed reasons for infant withdrawal because of lack of efficacy or because of pre-determined safety concerns are given in the appropriate sections

3.6 Assessments (see section 13 for full experiment descriptions)

3.6.1 Intestinal Tissue Samples

Intestinal samples will be collected and stored for metabolic studies. The following parameters will be evaluated:

1. DHP-1 expression in the intestinal tissue will be examined and its contribution in the conversion of meropenem to ICI 213689 will be quantified
2. Expression of DHP-1 gene in intestinal tissue using quantitative PCR will be quantified and expression of the protein using western-blot analysis will be assessed
3. The catalytic activity of DHP-1 using glycyldehydrophenylalanine (GDP) will be determined and the conversion of meropenem to ICI 213689 will be examined in the absence and presence of (a) the DHP-1 inhibitor cilastatin, (b) inhibitory monoclonal DHP-1 antibody
4. The role of transporters (i.e. hOATs and MRP family) will be investigated in the intestinal clearance of meropenem. Expression of hOATs and MRPs by plasmid containing c-DNA constructs for these transporters will be measured and expression of the gene coding for the transporter that is likely to be involved in meropenem clearance will be quantified using quantitative RT-PCR. Expression of the corresponding proteins will be determined using western-blot analysis. Functional activity of the transporter toward meropenem will be determined by assessing uptake of meropenem and its metabolite (ICI 213689) into membrane vesicles prepared from intestinal tissue.

The data on the expression and activity of DHP-1 and target transporters in the intestinal tissue obtained from the patient population will be available for comparison with similar data obtained from intestinal tissues of age-matched subjects and from adults, obtained from tissue banks and tissue procurement institutions.

3.6.2 Urine, Plasma, Pleural, and Peritoneal Fluid PK Measurements

1. 100 µL of urine, plasma, pleural, and peritoneal fluid will be collected from infants undergoing intestinal surgery as part of the normal clinical care while on study drug (meropenem)
2. No more than 1 urine, plasma, pleural, or peritoneal fluid samples will be collected from each infant

3. Time of sample collection will be recorded on the CRF
4. Urine, pleural, and peritoneal fluid hematologic and chemistry parameters (i.e. pH) will be recorded on the CRF
5. Plasma hematologic parameters (white blood cell count, platelets, hemoglobin) will be recorded on the CRF
6. Urine, plasma, pleural, and peritoneal culture results will be recorded on the CRF

Urine, plasma, pleural and peritoneal fluid samples will be collected and stored for drug concentration analysis that will be used to correlate drug disposition at the time of surgery with intestinal tissue experiments.

4. Procedures and Study Visits

The table below illustrates the schematic representation of assessments:

Schedule of Procedures

	Study Day			
PROCEDURE	**0**	**1-27**	**28**	**29 to end of study**
Informed Consent	X[a]			
Antibacterial Therapy	X[b]	X[b]		
Intestinal sample obtained		X		
Urine sample obtained		X		
Plasma sample obtained		X		
Peritoneal fluid sample obtained		X		
Pleural fluid sample obtained		X		
Plasma Pharmacokinetic Evaluation		X[c]		

a) Prior to enrollment and other study activities
b) Treatment for minimum of 3 days
c) Obtained per MPODS protocol

4.1 Prior to the administration of study drug

a. Obtain signed and dated informed consent/HIPAA consent (separate from MPODS parent trial)
b. Document enrollment in parent meropenem trial
c. Document prior abdominal surgical procedures

4.2 Acquisition of intestinal tissue while on meropenem therapy

a. Study nurses will obtain intestinal tissue samples in the operating room at the time of surgery
b. Record specimen number, study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion (24 hour clock), dose given (mg), and current patient weight (g)
c. A maximum of 1 sample will be obtained per patient

4.3 Acquisition of urine samples while on meropenem therapy

a. Study nurses will obtain urine sample within 24 hours of intestinal surgery
b. Record specimen number, study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion (24 hour clock), dose given (mg), and current patient weight (g)
c. A maximum of 1 sample will be obtained per patient

4.4 Acquisition of plasma samples while on meropenem therapy

a. Study nurses will obtain scavenge samples from the clinical laboratory within 24 hours of intestinal surgery
b. Record specimen number, study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion(24 hour clock), dose given (mg), and current patient weight (g)
c. A maximum of 1 sample will be obtained per patient

4.5 Acquisition of pleural and peritoneal fluid samples while on meropenem therapy

a. Study nurses will obtain pleural and peritoneal samples within 24 hours or at the time of intestinal surgery
b. Record specimen number, study drug timing of infusion before the PK samples are obtained—this includes start/stop times of infusion (24 hour clock), dose given (mg), and current patient weight (g)
c. A maximum of 1 sample of each fluid will be obtained per patient

5. Administration

5.1 Trial Termination

The National Institute for Child Health and Human Development (NICHD), the Best Pharmaceuticals for Children Act Coordinating Center (BPCA-CC), the Pediatric Off-Patent Drug Studies (PODS) PI and the Duke Clinical Research Institute (DCRI) will monitor the progression of the trial. Investigator and site participation in the study may be terminated by the PODS PI if there is evidence of an investigator failing to maintain adequate clinical standards or evidence of an investigator or staff failing to comply with the protocol.

5.2 Data Safety and Monitoring Board (DSMB) (MPODS)

To ensure that the welfare of trial subjects receives appropriate consideration, an independent DSMB has been organized by the BPCA-CC to review relevant safety and/or efficacy data during the course of the MPODS trial. The DSMB may recommend discontinuation of the study, or modifications to the study protocol for safety reasons. The DSMB charter is available from the BPCA-CC.

5.3 Intestinal Tissue Specimen Handling

To ensure appropriate sample collection, intestinal tissue samples will be obtained in the operating room at the time of surgery following these steps:

1. Intestinal tissue will be removed by the surgeon. If necrotic intestinal tissue is removed, obtain sample from clear intestinal margins. Immediately soak the tissue in cold Phosphate Buffer Saline (PBS) containing protease inhibitors that do not inhibit DHP-1. PBS containing protease inhibitors will be provided to the site.
2. After 5 minutes, wipe the tissues with tissue papers gently.
3. Place tissue in a 10 mL sterile cryovial, set over dry ice.
4. Tissue sample numbers will consist of the letter “T”, the site number, the MPODS subject number, and the sample number.

Tissues samples will be shipped in dry ice (see section 5.5), and stored at -80 ºC at the coordinating center, Duke Univerisity Medical Center. The tissue will be later homogenized as described in section 13.

5.4.1 Urine and Plasma Specimen Handling

Urine and plasma should be collected within 24 hours of intestinal surgery and stored following these steps:

1. Obtain 100 µl of urine using a bag and collect it in a sterile container.
2. Collect 100 µl sample of scavenge plasma into a sterile polypropylene container. If multiple samples are available, the sample closest in time to the intestinal tissue sample will be collected.
3. Label tubes with the specimen number: It will consist of the letter "U", the site number, the MPODS subject number, and the sample number. The plasma sample number will consist of the letter "P", the site number, the MPODS subject number, and the sample number.
4. The samples should be stored at -70°C within 1 hour of collection.

Samples will be shipped in dry ice (see section 5.5), and stored at -80 ºC at the coordinating center, Duke Univerisity Medical Center.

5.4.2 Pleural and Peritoneal Fluid Specimen Handling

Pleural and peritoneal samples should be collected within 24 hours of intestinal surgery and stored following these steps:

1. Collect 100 µl of peritoneal fluid in a sterile container.
2. Collect 100 µl of pleural fluid in a sterile container.
3. Label tubes with the specimen number: Pleural sample numbers will consist of the letter "L", the site number, the MPODS subject number, and the sample number. Peritoneal sample numbers will consist of the letter "A", the site number, the MPODS subject number, and the sample number.
4. Label tubes with the specimen number (MPODS number followed by the letters "PRF" for peritoneal fluid and "PLF" for pleural fluid), date the specimen was obtained using the mm/dd/yyyy format; the time the specimen was obtained using the 24 hour format; and the date and time of specimen freezing in similar format (use black permanent ink).
5. The sample should be stored at -70°C within 1 hour of collection.

Samples will be shipped in dry ice (see section 5.5), and stored at -80 ºC at the coordinating center, Duke Univerisity Medical Center.

5.5 Specimen Shipping

The sampling log form lists all patients, and their identifying information, whose specimens are being sent to Duke University. It is completed by both the staff at the study site center and the staff at Duke University receiving laboratory. It is used to document and identify all intestinal tissue, urine, plasma, pleural, and peritoneal specimens that are shipped out by the center.. A copy of this form will accompany the shipment from the study site to Duke University. This form is not entered into the computerized database. The following information should be completed on each shipment log.

1. Center Number
2. Number of scavenge samples enclosed
3. Initials of staff at the shipping center completing the form
4. Specimen numbers of specimens contained in the shipment

The samples should be shipped on **a Monday** (or Tuesday after a Monday holiday) in October of 2008, April 2009, and at the end fo the study. For the week of Christmas and New Year's, the nurse coordinator will contact Dr. Brian Smith (brian.smith@duke.edu; **919-970-3193**) who will

discuss with the nurse coordinator and the technologist the ideal time for sample shipment. Batch the samples in an outer bag, and then pack in dry ice to be shipped to the Duke NICU Clinical Research Office. The Specimen Shipment Log should be filled out and included in the shipment.

SHIP TO:
Sandra Grimes
Box 3179 DUMC
Durham, North Carolina, 27710
Phone: (919) 668-6368
Contact Sandra Grimes at 919-668-3360, or by email at sandra.grimes@duke.edu for questions. Please e-mail the FedEx tracking number to sandra.grimes@duke.edu after you have shipped the package.

6. Statistical Methods

The primary objectives of this analysis are to assess the intestinal metabolism and transport of meropenem in infants. All infants with intestinal tissue obtained will be analyzed. The secondary objective of the analysis is to evaluate the disposition of meropenem and its metabolite into body fluids and compare it to intestinal tissue. Descriptive statistics such as number of observations, mean, median, 95% confidence interval, standard deviation, standard error, minimum, and maximum will be presented for continuous variables (such as age, weight, etc).

The following plasma PK parameters will be estimated from the MPODS study.

1. Plasma clearance
2. Volume of distribution
3. Cmax
4. Tmax
5. AUC_{0-T}, (at steady state)
6. Ke
7. $t_{1/2}$
8. $AUC_{0-\infty}$ (estimated from the 1^{st} dose)

From intestinal tissue samples, we will evaluate:

1. Metabolism of meropenem (to ICI 213689) by DHP-1 in the intestine
2. Role of transporters in the intestinal clearance of meropenem

Enzyme activity will be expressed as nmol product/mg protein/min (mean ± SD), and transporter activity will be expressed as nmol flux/mg protein/min (mean ± SD).

From the urine, plasma, pleural, and peritoneal fluid samples we will evaluate:

1. Meropenem and metabolite distribution (drug concentration in body fluids)
2. Intestinal/plasma meropenem and metabolite concentration ratio
3. Peritoneal fluid/plasma meropenem and metabolite concentration ratio
4. Pleural fluid/plasma meropenem and metabolite concentration ratio
5. Urinary excretion of meropenem and metabolite

We anticipate that approximately 20 intestinal tissue samples will be obtained from 20 infants. We hypothesize that the intestinal metabolism of meropenem will account for 20-50% of the total metabolism of meropenem. We also believe that transporters will be involved in the distribution of meropenem within the intestinal tissue.

7. Ethics

7.1 Ethical conduct of the trial

This study will be conducted according to the protocol, the applicable FDA and HHS Code of Federal Regulations, Good Clinical Practice, the Declaration of Helsinki, and the ICH Harmonized Tripartite Guideline for Good Clinical Practice. It will also adhere to the ethical principles outlined in The Belmont Report.

7.2 Adverse event risk and benefits

As this will be a very vulnerable population, we expect that there will be serious adverse events including death. But we anticipate that there will be no adverse events or serious adverse events *related to the study protocol*. The intestinal surgery is being done per standard of care. No intestinal surgery will be made specifically for the study. The second component of the unit of observation (the filling out of the CRF) will not be associated with adverse events, and the third component of the unit of observation will be recording data that is obtained in accordance with good clinical practice. The participants who are enrolled will have no benefit, but the study will have substantial benefit to other infants if the appropriate metabolism, disposition, and transport of meropenem can be determined for infants with complicated intra-abdominal infections.

7.3 Institutional Review Board (IRB)

This protocol must be submitted to appropriate IRBs and written approval obtained before enrollment to this add-on study. Investigators must also inform IRBs of all subsequent protocol amendments and where applicable, obtain approval from the Pathology department for release of tissue samples from the operating room.

7.4 Informed consent

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before protocol-specified procedures are carried out. Informed consent will be obtained and documented in accordance with U.S. 21 CFR Part 50.25, §§ 116, 117 and 408 of 45 CFR Part 46 and all other applicable regulatory requirements.

Prior to any study procedures being performed, the investigator or his/her designee will inform the subject's legally authorized representative (e.g., parent, guardian) of all aspects pertaining to study participation. Information should be given in both oral and written form whenever possible and deemed appropriate by the IRB and should be separate from the parent MPODS trial. The subject's legally authorized representative (parent or guardian) must be given ample opportunity to inquire about details of the study.

The description of the study procedures will include the purpose of the research and procedures, risks and benefits of the research, alternative procedures, confidentiality, legal rights, parental or guardian permission, the contact person and phone number if there are any questions, and the voluntary nature of participation. It will be emphasized that participation is voluntary and participants may withdraw from the study at any time without any effect on standard care. The investigator or his/her designee, and the subject's legally authorized representative must both sign and date the informed permission form. An original signed informed permission form will be retained in the site study records. The subject's legally authorized representative will receive a copy of the signed and dated informed permission form.

The parental/guardian permission form generated by the investigator with the assistance of BPCA-CC must be approved (along with the protocol) by the IRB and be acceptable to the

Steering Committee. Permission forms must be in a language fully comprehensible to the subject's legally authorized representative. Permission shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject's legally authorized representative.

The written parental/legal guardian permission document will embody the elements of informed consent as described in the Declaration of Helsinki, the Code of Federal Regulations, and the International Conference on Harmonisation (ICH) Guidelines and will comply with local regulations. This form may be read to the subject's legally authorized representative, but, in any event, the investigator shall give the representative adequate opportunity to read it before it is signed and dated.

Permission must be documented by the dated signature of the subject's legally authorized representative. The signature confirms the permission is based on information that has been understood. Each signed permission form must be kept on file by the investigators for possible inspection by BPCA-CC, Regulatory Authorities, and NICHD or its designees.

7.5 Protection of personal health information (PHI)

All reports and communications relating to subjects in the study will identify each subject only by the subject's initials and the subject's study number. The investigators will agree to maintain records identifying the subjects enrolled in the study, which will be used for the purpose of long-term follow-up.

Investigators at each study site will be responsible for insuring compliance with the Privacy Rule, a Federal regulation under the Health Insurance Portability and Accountability Act (HIPAA), in accordance with the investigator's institution policy. The Privacy Rule establishes the right of a research subject or subject's legally authorized representative to authorize an investigator to use and disclose subject's PHI for research purposes. This requirement is in addition to the informed consent and assent to participate in the study. A valid Privacy Rule Authorization is a subject's or subject's legally authorized representative signed permission that allows an investigator to use or disclose the subject's PHI for the purposes, and to the recipient or recipients, as stated in the Authorization. The signed Authorization must be retained by the investigator for 6 years form the date of creation or the date it was last in effect, whichever is later.

Authorization can be combined with an informed permission. Whether combined with an informed permission or separate, an Authorization must contain the following specific core elements and required statements stipulated in the Privacy Rule.

7.6 Authorization core elements

1. A description of the PHI to be used or disclosed, identifying the information in a specific and meaningful manner
2. The names or other specific identification of the person or persons (or class of persons) authorized to make the requested use or disclosure
3. The names or other specific identification of the person or persons (or class of persons) to whom the covered entity may make the requested use or disclosure
4. A description of each purpose of the requested use or disclosure
5. Authorization expiration date or expiration event that relates to the individual or to the purpose of the use or disclosure ("end of the research study" or "none" are permissible

for research, including for the creation and maintenance of a research database or repository)

6. Signature of the individual and date. If the individual's legally authorized representative signs the Authorization, a description of the representative's authority to act for the individual must also be provided

7.7 Authorization required statements

1. A statement of the individual's right to revoke his/her Authorization and how to do so, and, if applicable, the exceptions to the right to revoke his/her Authorization or reference to the corresponding section of the covered entity's notice of privacy practices.
2. Whether treatment, payment, enrollment, or eligibility of benefits can be conditioned on
   a. Authorization, including research-related treatment and consequences of refusing to sign the Authorization, if applicable
   b. A statement of the potential risk that PHI will be re-disclosed by the recipient. This may be a general statement that the Privacy Rule may no longer protect health information disclosed to the recipient

Authorization must be written in plain language and contain the core elements and required statements, and a signed copy must be provided to the individual signing it if an investigator itself is seeking the Authorization. An Authorization obtained for the study need not have a fixed expiration date or state a specific expiration event, the form can list "none" or the "the end of the research project." Participant or participant's legally authorized representative has the right to revoke the Authorization, in writing, at any time.

8. Source Documents and Case Report Form Completion

Use of source documents and completion of CRFs for this sub-study will follow the procedures of the primary study. There will be one additional CRF page for this sub study.

9. Administration

The Members of the Steering Committee and their roles are outlined in the main protocol. They will have similar oversight for this substudy. Each of the site investigators will be responsible for the overall conduct of this sub-study at their site consistent with the primary protocol.

10. Data Quality Control and Assurance

Clinical data processing and data management will be employed based on the procedures developed by the BPCA-CC in conjunction with the NICHD as outlined in the main protocol. This sub-study will use the primary study number assigned for each subject in the MPODS main protocol.

11. Use of Information and Publication

After the dataset for the study is finalized and main findings have gone into publication, the data from this project will be made available and shared through CD-ROM and/or a website. All project data will be stored without subject identifiers, so that the data that are shared cannot be linked back to any particular subject. The dataset will cover the outcome data on children collected over the course of the study.

Prior to a manuscript or abstract being submitted for possible publication or presentation, the Steering Committee, BPCA-CC, and NICHD must review the contents of the submission. More

specifically, manuscripts, abstracts, and poster submissions must be submitted to the Steering Committee, BPCA-CC, and NICHD. Financial support from the NICHD will be acknowledged in all publications.

12. Completion of Study

The MPODS PI and MPODS investigators will complete this sub-study in compliance with the protocol, and in a manner consistent with the timelines proposed. Continuation beyond published timelines must be mutually agreed upon by both the MPODS PI and the BPCA-CC.

The NICHD may terminate this study prematurely, either in its entirety or at a specific site, for reasonable cause. Written notice must be submitted within a reasonable amount of time prior to the intended termination date. An investigator may also terminate the study at his/her site for reasonable cause. Advance notice is not required by either party if the study is terminated due to safety concerns.

13. Intestinal Metabolism Experiments

Experiment 1: In vitro metabolism of meropenem will be investigated using intestinal tissue homogenates.

Tissue processing: The stored tissue will be homogenized in equal volume of ice cold 75 mM phosphate buffer pH 7.1 . The homogenates will be centrifuged at 1200 x g and aliquots of the supernatant will be snap frozen in liquid nitrogen prior to storage at -80 ºC.

Metabolic stability of meropenem: Metabolic stability of meropenem (i.e. rate of metabolism to ICI 213689) will be conducted by incubating meropenem (10 µM) with tissue homogenate (2.0 mg protein/mL) in 0.1 M Tris-HCl, pH 8.0 (total volume 0.2 mL). The reaction mixture will then be incubated at 37ºC in a shaking water bath for time intervals ranging from 0-120 min. The reaction will be terminated by addition of cold acetonitrile (3X), the reaction mixture will be centrifuged at 14,000 x g, and the supernatant will be analyzed for meropenem and ICI 213689 by HPLC conditions. A similar metabolic stability study will be performed with imipenem that is known to be extensively metabolized by DHP-1 as a positive control to validate the assay conditions. The percentage loss of parent (meropenem) and the appearance of ICI 213689 will be calculated and used in the determination of in vitro T1/2. A more detailed kinetic study will be performed in which the rate of metabolism will be examined as a function of meropenem concentration to estimate its Km and Vmax for intestinal DHP-1.

Data analysis: A Michaelis-Menten model will be fit to the metabolic rate versus concentration data to recover the kinetic parameters (Km, Vmax). Intrinsic clearance (Vmax/Km) will be calculated for intestinal DHP-1 and total intrinsic clearance will be calculated from this parameter as described by Obach et al.[1]

Experiment 2: DHP-1 expression will be examined in intestine and its contribution in the conversion of meropenem to ICI 213689 will be quantified.

Expression of DHP-1 gene: To measure the level of expression, total ribonucleic acid (RNA) will be extracted from intestinal tissue using the RNeasy Mini isolation kit (Qiagen) following the manufacturer's protocol. Tissue segments will be treated with RNA later RNA stabilization reagent (Ambion, Inc., Austin, TX) to prevent changes in gene-expression patterns due to RNA degradation or gene induction post-harvest. Isolated RNA will be treated with DNase I to prevent

residual genomic DNA contamination. First strand cDNA will be reverse transcribed by incubation of 5 ug of the isolated RNA, 50 units of Superscript II Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA), 10 µM random hexamers, 0.5 mM dNTPs, 5 mM MgCl2, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 10 mM dithiothreitol, and 40 units RNase inhibitor at 42ºC for 1 hr. Negative controls will be incubated in the absence of reverse transcriptase. Following cDNA synthesis, RNA will be degraded by addition of RNase H. Real-time PCR analysis of DHP-1 mRNA levels will be performed on ABI prism 7700 sequence detector (Applied Biosystems, Foster City, CA). PCR amplification reactions will be performed in a total volume of 20 µL containing 1 µL of synthesized cDNA, 1 µM forward (5'-GACTCGAGTCGACATCGAT-3' and reverse (5'-TTCCTGACCAGGCGTCAGAT-3') primers specific for the DHP-1 gene, 0.2 µM TaqMan probe (5'-GACTCGAGTCGACATCG-3'), and 10 uL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Specific primers and probes for DHP-1 genes will be synthesized according to the sequence design of Igarashi and Karniski.[2] Sequence specificity for the genes of interest will be confirmed by BLAST search. The fractional PCR threshold cycle number (Ct) will be determined by evaluation of the cycle to cycle fluorescence emission for DHP-1 in samples obtained from various tissue segments. Amount of target RNA in each sample will be estimated from experimental Ct values generated using serial dilutions of standard plasmid DNA for DHP-1 gene. β-actin or GPDH mRNA will be quantified as an internal control and DHP-1 gene expression levels will be normalized to β -actin levels. Ten microliters of the PCR reaction product will be run on 1.6% agarose gel, and visualized with an UV transilluminator using ethidium bromide staining. As a control for the intactness of the mRNA, β -actin mRNA will be detected with specific primers. The resulting PCR product will be isolated from the agarose gel, and the sequence validated by automated sequencing .

Expression of DHP-1 protein: Protein concentration of tissues homogenates prepared as described in specific will be determined using the bicinchoninic acid protein assay reagents and procedure described by the manifacture (Pierce, Rockford, IL) against BSA as a standard. Samples with concentration 10-25 µg will be solubilized in loading buffer (2% sodium dodecyl sulfate, 125 mM Tris-HCl, 20% glycerol), then will separated by polyacrylamide gel electrophoresis. The electrophoretic separated protein will then be transferred on to polyvinylidene difluoride membranes (Immobilon; Millipore, Bedford, MA). The blots will then blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM NaCl, pH 7.5) containing 0.3% Tween 20 (TBS-T) for 3 h at 25°C and then will be incubated with anti-DHP-1 (43 KDa) (will be obtained from Peter Igarashi and Lawrence P. Karniskit), at dilution 1:1000 for 16 h at 4°C. The bound antibody will be detected by BIO RAD VersaDoc Imaging System after incubation with enhanced chemiluminescence with horseradish peroxidase-conjugated anti-rabbit or anti-mouse - secondary antibody and 1:1 (V/V) peroxide buffer:Luminol/Enhancer solution (Pierce, Rockford, IL). The intensity of band corresponding to 43 KDa will be quantitated and the expression will be normalized against the expression of Actin in all samples.

Catalytic activity of DHP-1: Tissue homogenates will be incubated by DHP-1 probe substrate, glycyldehydrophenylalanine (Gdp) as described by Fukasawa, et al.[3] The hydrolysis of Gdp will be measured by spectrophotometry. The activity will be confirmed by inhibition of activity in the presence of DHP-1 potent inhibitor, cilastatin.

Role of DHP-1 vs. other hydrolytic enzymes in the conversion of meropenem to ICI 213689 by intestinal tissue matrix: In order to determine the contribution of DHP-1 to the hydrolysis of meropenem, tissue homogenates will be incubated with 1 mM meropenem (below the Km value estimated to be ~2 mM) in the presence of DHP-1 inhibitor, cilastatin [4] at concentration ranging

from 50 uM to 2 mM. The IC50 value for inhibition of meropenem hydrolysis by cilastatin will be determined by fitting the data (cilastatin concentration with % activity remaining) to Emax model using WinNonLin software, where 100% activity corresponds to zero inhibitor concentration. Inhibition study of imipenem hydrolysis by cilastatin will be conducted in parallel and will be used to validate the assay conditions. Based on the extent of maximum inhibition of the total hydrolytic activity achieved by cilastatin, the role of additional hydrolytic enzymes in the metabolism of meropenem will be assessed. The metabolism of meropenem by DHP-1 will also be evaluated by conducting immuno-inhibition study in which meropenem will be incubated with tissue homogenate in the presence of increasing concentration of the monoclonal antibody for renal DHP-1 enzyme.[2]

Experiment 3. Expression of hOATs and MRPs by plasmid containing c-DNA constructs for these transporters into Xenopus laevis oocytes.

Xenopus laevis oocytes have been extensively used for examining substrates and inhibitors of organic anions and MRP transporters because of the low background of the constitutively expressed transporters in this system. To determine if the model hydrophilic anion is a substrate for any of the OATs and MRPs, individual transporters will be expressed in Xenopus oocytes as described by Sweet and Pritchard[5], and uptake kinetics of the target compounds will be examined in this test system.

Expression of transporters in Xenopus oocytes: Plasmid DNA containing cDNA constructs for hOAT1-4 and MRP1-8 will be obtained from commercial sources and as gifts from several academic labs (e.g. John Pritchard, NIEHS, Research Triangle Park, NC). cRNA will be transcribed in vitro from the respective transporter cDNA templates using the mMESSAGE mMACHINE in vitro transcription kit (Ambion, Austin, TX). Mature stage V and VI Xenopus laevis oocytes will be isolated and defolliculated by treatment with collagenase A and maintained at 18ºC in oocyte ringer's (OR-2) buffer (82.5 mM NaCl, 2.5 mM KCl, 1 mM Na2PO4, 3 mM NaOH, 1 mM CaCl2, 1 mM MgCl2, 1 mM pyruvic acid, and 5 mM HEPES, pH 7.6) supplemented with 0.05 mg/ml gentamicin sulfate, 1.5 mM sodium pyruvate, and 5% heat-inactivated horse serum.[5] Oocytes will be injected with 20 ng of transporter cRNA the day after isolation. Oocytes injected with water will serve as controls. Expression of transporters will be confirmed 3 days after injection by evaluation of the uptake of radiolabel prototypical transporter substrates such as postaglandine (OAT2,4) and acetaminophen glucurionide (MRP2,3), respectively. Also, Western Blot analysis will be conducted for the lysate of the Xenopus oocytes-containing c-RNAs to validate the expression process.

Uptake Studies: The uptake study of radiolabel 14C-meropenem and 14C-ICI 213689 (purchased from Sumitomo Chemical Company Limited, Osaka, Japan) will be conducted with oocytes expressing hOATs and MRPs. Uptake of prototypical substrates or test compound will be initiated by incubation of groups of 6-8 water or transporter injected oocytes with 1 mL of the appropriate compound dissolved in oocyte ringer's buffer (OR-2) (82.5 mM NaCl, 2.5 mM KCl, 1 mM Na2PO4, 3 mM NaOH, 1 mM CaCl2, 1 mM MgCl2, 1 mM pyruvic acid, and 5 mM HEPES, pH 7.6). Uptake will proceed for an appropriate time while slowly rotating on a rotary shaker. Uptake will be stopped by aspiration of the uptake solution followed by rapid washing of the oocytes 3 times with 2 mL of ice-cold OR-2 buffer. Individual oocytes will be dissolved in 0.3 mL 1 N NaOH for 2 hr. After neutralization with 0.3 mL 1N HCl, liquid scintillation cocktail will be added and the radioactivity associated with each oocyte determined by liquid scintillation counting. Substrates of the respective transporters will display significantly increased uptake in oocytes injected with transporter cRNA compared to water injected controls. Uptake kinetics (Jmax, Km) will be

evaluated for meropenem and its metabolite if determined to be hOATs and/or MRP substrates in order to ascertain relative affinities for the respective transporters. Uptake will be determined for several concentrations (1/10th to 10 times the Km) in transporter-expressin and water-injected oocytes. Subtraction of uptake in water-injected ooctyes will allow recovery of the true transporter mediated uptake. A Michaelis-Menten model will be fit to the uptake versus concentration data to recover the kinetic parameters (Jmax, Km).

Experiment 4: Expression of the gene coding for the transporter that is likely to be involved in meropenem clearance will be quantified in intestinal tissue using quantitative RT-PCR

Quantify mRNA for hOATs, MRPs in intestinal epithelium: Tissues will snap-frozen in liquid nitrogen for later extraction of mRNA. The mRNA levels for OATs and MRPs transporters will be detected and quantified in intestinal segments by uantitative real-time PCR using the TaqMan technique (Applied Biosystems, Foster City, CA). Total RNA will be extracted from these tissue segments using the RNeasy Mini isolation kit (Qiagen) following the manufacturer's protocol. Tissue segments will be immediately treated with RNAlater RNA stabilization reagent (Ambion, Inc., Austin, TX) to prevent changes in gene-expression patterns due to RNA degradation or gene induction post-harvest. Isolated RNA will be treated with DNase I to prevent residual genomic DNA contamination. First strand cDNA will be reverse transcribed by incubation of 5 ug of the isolated RNA, 50 units of Superscript II Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA), 10 uM random hexamers, 0.5 mM dNTPs, 5 mM MgCl2, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 10 mM dithiothreitol, and 40 units RNase inhibitor at 42ºC for 1 hr. Negative controls will be incubated in the absence of reverse transcriptase. Following cDNA synthesis, RNA will be degraded by addition of RNase H. Real-time PCR analysis of OATs and MRPs mRNA levels will be performed on a ABI prism 7700 sequence detector (Applied Biosystems, Foster City, CA). PCR amplification reactions will be performed in a total volume of 20 uL containing 1 uL of synthesized cDNA, 1 uM forward and reverse primers specific for the gene of interest, 0.2 uM TaqMan probe, and 10 uL of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Specific primers and probes for hOAT[6] and MRP[7] genes are shown in Tables 1 and 2, respectively. For β-actin mRNA amplification, a commercially available primer pair will be used (Stratagene, La Jolla, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase mRNA will be amplified using the forward primer 5′-CATCTCTGCCCCCTCTGCTGA-3′ and the reverse primer 5′-GGATGACCTTGCCCACAGCCT-3′. Sequence specificity for the genes of interest was confirmed by BLAST search. The fractional PCR threshold cycle number (Ct) will be determined by evaluation of the cycle to cycle fluorescence emission for each gene of interest in samples obtained from various tissue segments. Amount of target RNA in each sample will be estimated from experimental Ct values generated using serial dilutions of standard plasmid DNA for each gene. β-actin or GPDH mRNA will be quantified as an internal control and transporter gene expression levels will be normalized to b-actin levels. Ten microliters of the PCR reaction product will be run on 1.6% agarose gel, and visualized with an UV transilluminator using ethidium bromide staining. As a control for the intactness of the mRNA, β-actin mRNA will be detected with specific primers. The resulting PCR product will be isolated from the agarose gel, and the sequence validated by automated sequencing (Sequencing facility, UNC).

The quantities of β-actin and MRP1–MRP6 mRNAs will be determined using a serial plasmid dilution (human MRP2 cDNA in the expression vector pcDNA3.1, from 1×106 to 1×102 fg) as amplification standard. The mRNA level of each MRP transporter will be normalized by the expression of β-actin mRNA in the respective sample. After quantitative mRNA analysis amplified PCR products will be separated by 0.8% agarose gel electrophoresis, stained with ethidium

bromide, and visualized under UV light. Plasmids containing the respective MRP cDNA sequence served as positive controls for the amplification reactions with each of the MRP primer pairs.

Table 1. Primer sets and probes of organic ion transporters for real-time PCR

	Sequence	Position	Accession Number[a]
hOAT1			
forward primer	GCGCCTTTTTTTGCCTTCT	1030–1048	AB009698
reverse primer	TTCCCGCTTCCCATTGATC	1161–1143	
Taqman probe	CATCTACTCCTGGTTCTTCATTGAGTCGGC	1050–1079	
hOAT2			
forward primer	CCATCCAGGACGTGGAGAGA	1663–1682	AF097518
reverse primer	CCCACTTAGTTCTGGACCTGCTT	1747–1725	
Taqman probe	TGCCCCAACCAGTCTTCAGGAGGAAG	1688–1713	
hOAT3			
forward primer	CACCATCCTCTCCTTAAGCTACCT	1109–1130	AF097491
reverse primer	ACTGTCTCCACGGTCTGCAAGT	1229–1208	
Taqman probe	CATCTTGGCTCTCACCTTTGTGCCCTT	1179–1205	
hOAT4			
forward primer	CAAGCACTTCAGGAGCTCAGAAA	923–945	AB026198
reverse primer	GCTGGACATCAGCACCTCTATG	1009–988	
Taqman probe	TGGCCAGGATAAATGGCCACAAGGA	948–972	

a: from GeneBank database

Table 2. Primer pairs for quantitative RT-PCR analysis of MRP subfamily members in human

MRP	Forward primer	Reverse primer	Accession number
1	5′-CTGACAAGCTAGACCATGAATGT-3′	5′-TCACACCAAGCCGGCGTCTTT-3′	NM_004996
2	5′-CTTCGGAAATCCAAGATCCTGG-3′	5′-TAGAATTTTGTGCTGTTCACATTCT-3′	NM_000392
3	5′-GGACCCTGCGCATGAACCTG-3′	5′-AGGCAAGTCCAGCATCTCTGG-3′	NM_003786
4	5′-GGATCCAAGAACTGATGAGTTAAT-3′	5′-TCACAGTGCTGTCTCGAAAATAG-3′	NM_005845
5	5′-GCTGTTCAGTGGCACTGTCAG-3′	5′-TCAGCCCTTGACAGCGACCTT-3′	NM_005688
6	5′-CACTGCGCTCCAGGATCAGC-3′	5′-CAGACCAGGCCTGACTCCTG-3′	NM_001171

Data analysis: The expression level of transporter genes in intestinal tissue will be determined in triplicate, and mean values of the expression level (copy numbers) in different tissues will be compared.

Experiment 5: Expression of the corresponding (hOAT and MRP) proteins will be determined using western-blot analysis.

Western Blot Analysis will be carried out as described previously elsewhere. Briefly, tissue homogenates at specific protein concentration will be determined using the bicinchoninic acid protein assay reagents and procedure described by the manifacture (Pierce, Rockford, IL) against BSA as a standard. Samples will be solubilized in loading buffer (2% sodium dodecyl sulfate, 125 mM Tris-HCl, 20% glycerol). The samples were separated by polyacrylamide gel electrophoresis on 4-12% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA) and transferred onto

polyvinylidene difluoride membranes (Immobilon; Millipore, Bedford, MA). The blots were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS; 20 mM Tris, 137 mM NaCl, pH 7.5) containing 0.3% Tween 20 (TBS-T) for 3 h at 25°C and will be then incubated with anti-hOATs or, hMRPs, dilution 1:100-1:2000 for 16 h at 4°C. The bound antibody will be detected by BIO RAD VersaDoc Imaging System after incubation with enhanced cheminluminescence with horseradish peroxidase-conjugated anti-rabbit or anti-mouse-secondary antibody and 1:1 (V/V) peroxide buffer:Luminol/Enhancer solution (Pierce, Rockford, IL). The antibodies for MRPs and hOATs will be obtained from Alexis Corp.

Experiment 6: Functional activity of the transporter toward meropenem will be determined by assessing uptake of meropenem and its metabolite (ICI 213689) into membrane vesicles prepared from intestinal tissue.
Preparation of membrane vesicles: The membrane vesicles will be prepared from intestinal tissue according to published procedures.

Vesicle uptake studies: Vesicle uptake studies will be carried out using [14C] meropenem and [14C]ICI 213689 using standard vesicle uptake procedure. Linearity of uptake with respect to time will be established, and then uptake rates will be determined as a function of substrate concentrations. Uptake at 4 °C will be used as a control for non-specific uptake/binding to vesicles. From the uptake rates, kinetic constants Km and Jmax will be determined by fitting a Michaelis-Menten model to the uptake rate versus concentration data to recover the kinetic parameters (Km, Jmax).

14. References

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