FULL PROTOCOL TITLE
Measures of Intestinal Permeability in Preterm Neonates

Other Identifying Numbers:

UMB IRB: HP-00049647

IND 116718

IND Sponsor-Investigator: Rose Marie Viscardi, MD

Version Number: 2.1

6 August 2019

Statement of Compliance

This study will be carried out in accordance with the US Code of Federal Regulations (CFR), local regulations, and Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46; and 21 CFR including part 50 and 56 concerning informed consent and IRB regulations, and 21 CFR 11 concerning electronic records.
- International Conference on Harmonisation (ICH E6); 62 Federal Register 25691 (1997)
- NCCAM Clinical Terms of Award

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any subjects. CVs for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).

∞ **Signature Page 1**

The signature below constitutes approval of this protocol and the attachments, and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations and (ICH E6) guidelines.

Principal Investigator: __Rose Marie Viscardi, M.D._____

Print/Type

Signed: Date:

Name/Title

The Lead Principal Investigator (Protocol Chair) should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of Regulatory documents and a copy should be maintained at the site.

Signature Page 2

The signature below constitutes approval of this protocol and the attachments, and provides the required assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations and (ICH E6) guidelines.

Investigator(s) of Record: ___Sripriya Sundararjaran, M.D._____

Print/Type

Signed: Date:

_____ _____

Name/Title

Additional Investigators: _____

Print/Type

Signed: Date:

_____ _____

Name/Title

The Principal Investigator or Investigator(s) of Record from each participating clinical site should sign the Signature Page 2 as appropriate. This Signature Page 2 should be maintained at each clinical site.

Tool Revision History

Version Number 1.1
Version Date 10/30/13
Summary of Revisions Made:

1) Clarified determination of adverse event severity with reference to neonatal toxicity tables (Appendix D) and additional guideline for assessing laboratory values (Appendix E) (see section 7.4.1).

Version Number 2.0
Version Date 6/30/2018
Summary of Revisions Made:

1) Changes in IND personnel. Dr. Viscardi will assume responsibilities as the sponsor-investigator (see letters from Drs. Fasano and Viscardi). New study personnel have been added.
2) Changed the number and timing of visits. Since the differentiation between preterm infants with normal intestinal barrier maturation and those with delayed maturation was significant at the second timepoint (Study d8) or approximately 7-10 days of age, we will limit the dual sugar solution administration to one administration per subject between d7 and 10 of postnatal age.
3) Eliminate blood draws for zonulin measurement. There was no association of serum zonulin and the gold standard urinary Lactulose/Rhamnose ratio in the initial cohort of 43 subjects, so no further blood draws will be done.
4) Safety Monitoring: The U. Maryland IRB has approved the change from a DSMB to a single safety monitor.
5) Stool microbiome: Addition of stool microbiome analysis in relation to intestinal permeability measured by La/Rh ratio.

Version Number 2.1
Version Date 8/6/2019
Summary of Revisions Made:

1) Increase the sample size: Sample size will be increased to total 200 based on sample size calculation. A total of 200 sample size was calculated to obtain high precision in correlations between features of the stool microbiota and intestinal permeability (IP). Since 83% subjects in the first cohort had high IP (La/Rh ≥ 0.05) at study day 8, a sample size of 166-236 provides marginal error of 0.05 (maximum sampling error of 5%) and alpha of 0.05 (95% confidence level) to detect correlations between features of the microbiota and changes in IP and to achieve an expected sensitivity of 0.8-0.9 and expected specificity at 0.9-0.95.

2) Change in stool collection frequency: Instead of collecting stools twice per day from enrollment until postnatal d21, stools will be collected twice per day from enrollment until postnatal d14 and once on postnatal d21. The rationale for this change is to concentrate stool collections around day of IP measurement by urinary La/Rh ratio to analyze the correlations of stool microbiota composition and IP.
3) Delete site Mercy Medical Center: Due to limited study personnel and adequate enrollment at main site University of Maryland Medical Center, Mercy Medical Center will be dropped as an active site. Enrollment will continue at University of Maryland Medical Center.

Table of Contents

List of Abbreviations

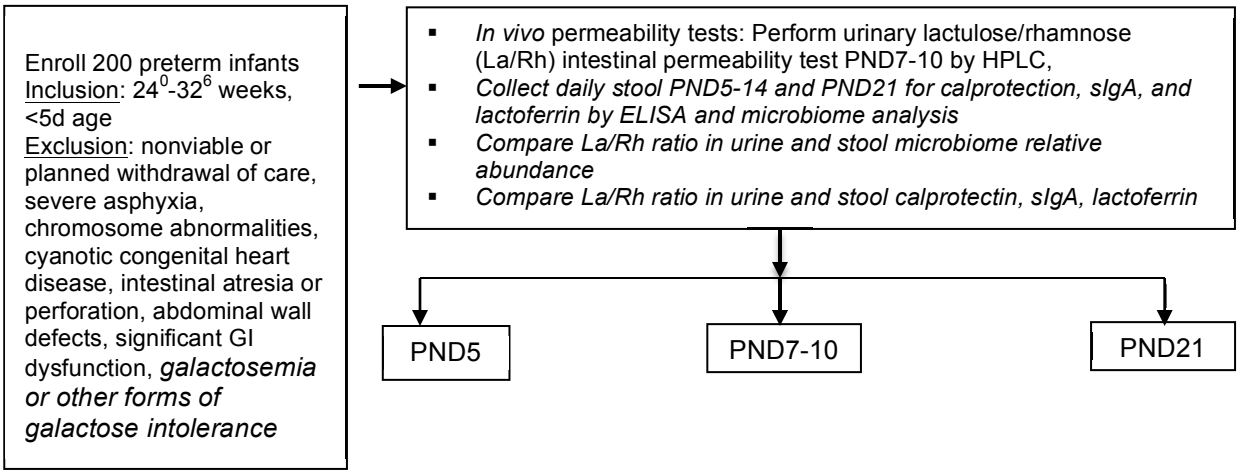
AE	Adverse Event/Adverse Experience
A1AT	Alpha 1 antitrypsin
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendments
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FIH	First in Human
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug
IoR	Investigator of Record
IRB	Institutional Review Board
ISM	Independent Safety Monitor
La/Rh	Lactulose Rhamnose Test
LAR	Legally Authorized Representative
MedDRA♦	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCCAM	National Institute of Complementary and Alternative Medicine
NDA	New Drug Application
NG	Naso-gastric
NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health

NLM	National Library of Medicine
OCRA	Office of Clinical Research Affairs, NIH, DHHS
OG	Oro-gastric
OHRP	Office for Human Research Protections, DHHS
OHSR	Office for Human Subjects Research, NIH, DHHS
ORA	Office of Regulatory Affairs, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
QM	Quality Management
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

Protocol Summary

Title:	Measures of Intestinal Permeability in Preterm Neonates
Abbreviated Title	Intestinal Permeability in Preterm Neonates
Phase:	1
Population:	A sample of N= 200 inpatient neonates between 24-32 week gestational age. *No gender or ethnic exclusions apply.*
Number of Sites:	1
Study Duration:	24 months
Subject Participation Duration:	28 days
Agent or Intervention:	Lactulose/rhamnose ratio measurement of intestinal permeability in preterm neonates.
Objectives:	To assess intestinal permeability in preterm infants with the lactulose/rhamnose ratio in urine and relationship to stool microbiome characteristics
Endpoints:	Onetime measurement of *in vivo* permeability determined by the urine La/Rh ratio between 7-10 days of age and serial stool microbiome changes between postnatal d5-d14 and at PND21 in a cohort of preterm neonates. The goal is to determine the changes in the intestinal microbiome characteristics in association with impaired intestinal barrier function (La/Rh ≥0.05) at 7-10 days in in preterm neonates.

Descriptive Schematic of Study Design



1 KEY ROLES

List if applicable:

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Safety Monitor	Rose M. Viscardi, M.D., Professor of Pediatrics University of Maryland 29 S. Greene St., RM GS110 Baltimore, MD 21201 Office phone: 410-706-1913 or 410-328-6003 Fax: 410-706-0404 or 410-328-1076 e-mail: rviscard@som.umaryland.edu

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Intestinal barrier: The human intestine is lined by a single layer of cells exquisitely responsive to multiple stimuli, and is populated by a complex climax community of microbial partners. Under normal circumstances, these intestinal cells form a tight, but selective barrier to "friends and foes": microbes and most environmental substances are held at bay, but nutrients are absorbed efficiently (1, 2). Environmental antigen(s) that gain access to the intestinal submucosa via paracellular passage from the intestinal lumen across an aberrant mucosal barrier trigger a host inflammatory response. There may be genetic variation in susceptibility to recognize, and potentially misinterpret, these non-self antigens (1). In all cases, increased permeability precedes disease and causes an abnormality in antigen delivery that triggers the multi-organ process leading to the inflammatory response (1).

Measurement of Intestinal Permeability: The measurement of the urinary excretion of orally administered isotonic solution of non-metabolized sugar probes have been used extensively for 30 years to assess intestinal permeability in health and disease in adults adults (3-9) and in preterm (10-13) and term infants (13-15) as well as older children. Lactulose, a disaccharide is a constituent of infant formulas(10) and is safe treatment for constipation(16) at doses 8-15 times higher than used in the assessment of intestinal permeability. L-Rhamnose is a naturally occurring monosaccharide without known biologic effects. It is found in the polysaccharides of gums, cardiac glycosides, and foods such as oranges, French beans, winter cabbage, and carrots (17) and is used in food flavoring(10). It is not absorbed by the human small intestine and reaches the colon where fermentation may produce propionate. The percent urinary excretion of orally administered lactulose and rhamnose are markers of the intestinal paracellular and transcellular pathways, respectively. The sugar probe tests have been used to safely estimate intestinal permeability in pediatric conditions including celiac disease (18), atopic eczema (19, 20), cystic fibrosis (21, 22), major burns (23) and diarrhea (24-26). The sugar probe has also been safely used to assess intestinal permeability in ill newborns with birth asphyxia (10), necrotizing enterocolitis (NEC) (11) and congenital heart disease (15, 27). A recent review confirmed that the use of the sugar absorbtion tests have been found to be safe in newborns (28). We have summarized the literature of the use of lactulose and L-rhamnose as sugar probes in studies of preterm and term infants (Table 1) and older infants and children (Table 2) (see IND section 6.2).

Intestinal functions and prematurity: Epithelial barrier integrity is itself dynamic and matures over time starting soon after birth, though the mechanisms regulating dynamic permeability are poorly understood. Low birth weight, prematurity, and early postnatal age are associated with a leaky gut (34). Although intestinal permeability (IP) is higher at birth in preterms than term infants, there is usually rapid maturation of the intestinal barrier over the first few days of life in both populations (34). Diet also affects intestinal permeability with breast milk feeding lowering

IP more rapidly in the first month of life compared with cow milk-based formula in term (35) and in preterm neonates (36).

NEC and its pathogenesis: NEC, a life-threatening, GI emergency characterized by increased IP, affects approximately 7 to 10% of preterm neonates, and typically occurs within 7 to 14 days of birth (37, 38) with mortality as high as 30-50% (39). NEC symptoms mainly involve GI dysfunction, such as abdominal distension and feeding intolerance, but the presentation can be non-specific with few warning signs. Current therapies may be invasive, including surgical interventions that are often ineffective due to the rapid progression of the disease. Prematurity is the greatest risk factor for development of NEC (40, 41), due to physiological immaturity of the GI tract and altered levels of the normal GI microbiota. Several studies suggest that the initiation of an intense systemic and local inflammatory cascade leads to intestinal necrosis (42-47). Antenatal exposure to infection/inflammation may predispose the developing intestinal mucosa to subsequent injury or dysregulated inflammatory responses. Previous studies have linked presence of amniotic fluid infection/elevated cytokines, (48) cord blood cytokines, (49, 50) and umbilical cord inflammation (51) with risk for NEC in preterm neonates. In a rat model of NEC, maternal prenatal exposure to microbial LPS led to increased frequency and severity of intestinal injury (52). Taken together, these observations suggest that intestinal injury may be initiated *in utero* and contributes to increased IP at birth in the preterm neonate. Many of the defense mechanisms present in the mature intestine, such as peristalsis and tight junctions between intestinal epithelial cells (37) are decreased in an immature intestine, and thus bacteria normally confined to the intestinal lumen are able to reach systemic organs and tissues. Bacterial translocation triggers the activation of an exaggerated inflammatory response, which leads to further epithelial damage.

2.2 Scientific Rationale

Despite improvements in neonatal intensive care, the birth weight-specific incidence of NEC has not changed over the past 2 decades. However, the total burden of disease is increasing due to increased survival of very immature infants. Mortality remains high and survivors experience significant morbidity including post-surgical short bowel syndrome and its consequences (prolonged dependence on total parenteral nutrition, recurrent infections, poor growth, and liver failure), prolonged hospitalization, and long-term neurodevelopmental impairments. Intestinal barrier immaturity is the proximate cause of susceptibility to NEC in preterm neonates. Although intestinal permeability is higher at birth in preterm compared to term infants (34), *in utero* infection/inflammation and post-natal acquisition of pathogenic bacteria may further exacerbate intestinal injury (48-53). Despite intensive efforts, no clinical factor or routine laboratory test alone or in combination has been described that predicts the 7-10% preterm neonates at-risk for this potentially life-threatening complication (54). Although increased intestinal permeability is a major risk factor for NEC and *in vivo* intestinal permeability has been assessed in infants with the urine LA/MA ratio or urine La/Rh ratio, few preterm infants <30 wk gestation have been included in the prior studies of intestinal permeability.

Since current therapies for NEC may be invasive, including surgical interventions that are often ineffective due to the rapid progression of the disease, there has been considerable focus on developing preventive therapies. Probiotic therapy is a promising, low-cost, and likely safe intervention to reduce intestinal permeability in at-risk infants. There have been at least 11 RCTs and a recent meta-analysis of probiotic supplementation to prevent NEC in preterm neonates (55). Although there was a 30% reduction in NEC incidence in these trials, various formulations, doses, and duration of therapy were used, infants <1000 g BW with the highest NEC incidence were under-represented, and no FDA-approved products are available to assure quality and safety under good manufacturing practices. In preparation for future trials of the efficacy of probiotics to prevent NEC in preterm infants, the proposed observational study will utilize the La/Rh ratio to determine in vivo intestinal permeability in infants 24-32 weeks gestation during the 2 weeks of life.

2.3 Preliminary Data

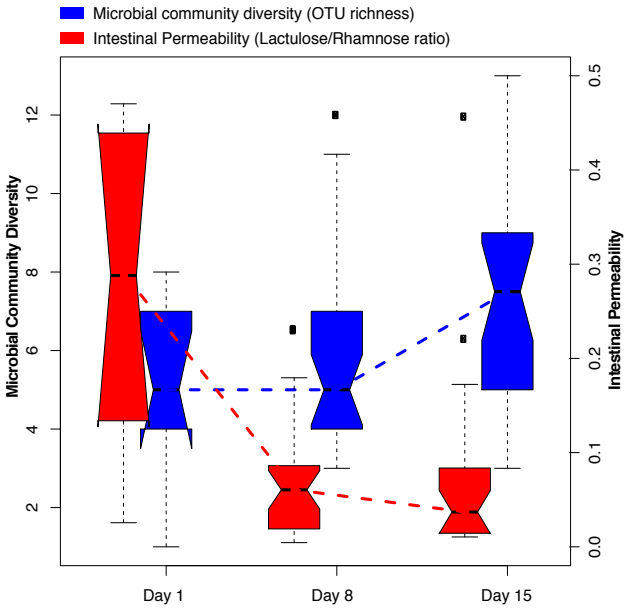

Figure 1. Microbial community diversity and intestinal permeability at study time points day 1, 8, and 15 for a cohort of 43 preterm infants (<33 weeks gestational age). Microbial community diversity is calculated as OTU richness based on 16S rRNA gene amplicon high-throughput sequencing. Intestinal permeability was calculated as the ratio of non-metabolized sugar probes

Our analysis of the initial cohort of 43 preterm infants recruited for the IND protocol, and others' previous studies have shown that IP is high at birth in preterms (<33wk gestation) with a rapid maturation of the intestinal barrier over the first 2 weeks. However, in some infants, high IP persisted and/or recurred in association with altered levels of the normal microbiota (bacteria community composition). Specifically, we observed that (1) rapid maturation of intestinal barrier function, characterized by decreased IP, correlates with increased microbial community diversity (Figure 1), and most outstandingly, the increased abundance of beneficial bacteria *Clostridiales* (Figure 2); (2) *Clostridiales* is highly transcriptionally active and co-active with the probiotic bacterium *Bifidobacterium*; (3) neonatal factors, including early introduction of breast milk, shorter period of antibiotic exposure, and later gestational age, favor the early colonization of the gut microbiota by members of *Clostridiales* and *Bifidobacterium*, which altogether are associated with improved intestinal barrier in preterm infants; (4) low *Clostridiales* spp. abundance (<5%) and early gestational age (<31.7wk) were identified to be the most discriminatory features for elevated IP by supervised learning scheme, reaching an accuracy of 86.1%. (5) *Clostridiales* and *Bifidobacteriales* are the most abundant bacteria groups in later stages (phase II/III at 6-18

months of age) as shown in Figure 2, suggesting a process of gaining prosperity of these two bacterial groups during intestine development after birth. Altogether our preliminary results suggest the early colonization of the natural occurring probiotics strains *Clostridiales* and *Bifidobacterium* strongly associate with rapid maturation of intestinal barrier function, and their measurement are highly promising for early detection and as potential nutritional supplement to prevent NEC in the high-risk preterm population.

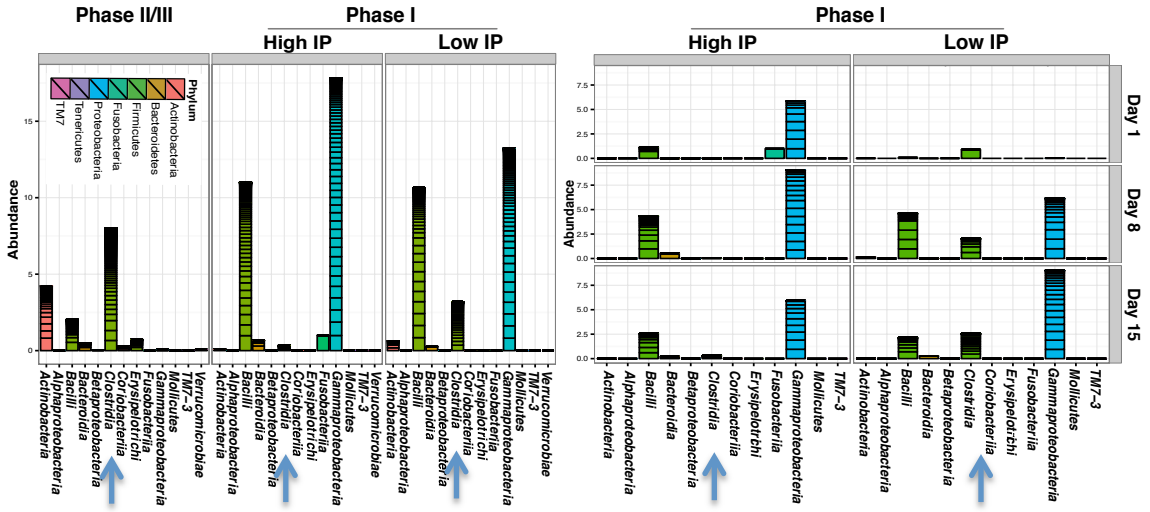

Figure 2: Bar graph of the accumulative relative abundance of bacterial groups. (A) accumulative abundance between phase II/III subjects (6-18 months of age) and phase I infants (within first two weeks of life), (B) stratified abundance profiles by different study day at day 1, 8, and 15 for phase I subjects. The most outstanding difference between high and low IP in preterm infants is in the *Clostridiales* (arrows), the only bacteria group of Clostridia in this study, which is the most abundant bacterial group in phase II/III

We propose in this study to recruit additional 157 preterm subjects and to combine with our existing 43 preterm subjects to form a statistically powered cohort at 200 (justified in study size analysis in research design), to address our hypothesis that the two naturally occurring beneficial bacteria *Clostridiales* and *Bifidobacterium* are rapidly gaining prosperity during normal intestine development in association with improved barrier function measured by La/Rh ratio. Overall, this continuation of the initial study builds on the previous findings that identified commensal bacteria *Clostridiales* and *Bifidobacterium* species as a strong indicator to the lowered IP and rapid maturation of intestinal barrier, to substantiate measurement of these probiotic strains combined with associated neonatal factors to form an accurate, rapid detection of intestinal permeability abnormality.

2.4 Potential Risks and Benefits

2.4.1 Potential Risks

The research involves minimal risks.

Loss of confidentiality presents minimal risk. Specimens will be identified by subject ID only linking data for subject ID will be kept in locked cabinet accessible only to the clinical research staff.

Fecal sample will be collected from the diaper and presents no risk to the participant.

Sugar solution- No risks have been reported in the literature from ingesting an isotonic solution of sugar (3-15). A similar simple sugar, sucrose (56), is given in higher doses for pain relief with fewer adverse effects than water placebo.

2.4.2 Known Potential Benefits

There are no known potential benefits to participants. The ability to measure increased intestinal permeability would indicate an infant has a leaky gut, and may be at increased risk for NEC. The potential risks from specimen collection are equivalent to those of normal clinical care.

2.4.3 Risk/Benefit Ratio

Despite improvements in neonatal intensive care, the birth weight-specific incidence of NEC has not changed over the past 2 decades. However, the total burden of disease is increasing due to increased survival of very immature infants. Mortality remains high and survivors experience significant morbidity including post-surgical short bowel syndrome and its consequences (prolonged dependence on total parenteral nutrition, recurrent infections, poor growth, and liver failure), prolonged hospitalization, and long-term neurodevelopmental impairments. Development of an *in vivo* measurement to identify infants with increased intestinal permeability, who are at high risk for NEC, will facilitate clinical trials of potential preventive therapies. There may be risks from the research that are not known.

2.5 Study Objectives

The proposed study will evaluate the intestinal permeability measured by the urinary La/Rh ratio at one timepoint between d7-10 of life in *200* preterm infants 24-32 weeks gestation in preparation for a future study of probiotics to improve intestinal permeability in this population.

Primary Objective: To estimate mean, variance, and quantiles in IP measured by urinary Lactulose/Rhamnose ratio at 7-10d of life in neonates born between 24 and 32 weeks of gestational age.

Secondary Objectives*:*

1) To assess stool microbiome characteristics in association with intestinal permeability in preterm infants measured by the urinary lactulose/rhamnose ratio

2) *To analyze the correlation of stool calprotectin, sIgA, and lactoferrin to urinary lactulose/rhamnose ratio and stool microbiome characteristics in neonates born between 24 and 32 weeks of gestational age.*

3 STUDY DESIGN

The study is a specimen collection study. Participants will include neonates admitted to the Neonatal Intensive Care Unit at the University of Maryland Medical Center, Baltimore, MD. The study will not test any type of device or treatment but is designed to accrue information.

3.1 ENDPOINTS

3.2 Study Outcome Measures

The study outcome measures will be intestinal permeability (quantitative) and leaky gut (binary), 1) increased levels of urine Lactulose/Rhamnose [La/Rh] ratio >0.05 will identify infants with increased intestinal permeability (IP).

3.3 **Description of Sub-studies:** Not applicable

4 STUDY POPULATION

All admissions to the University of Maryland Medical Center (UMMC) NICU <5 d age and gestational age 24^0-32^6 weeks will be screened for study eligibility and parental consent of eligible subjects will be obtained. Two hundred total subjects will be recruited. The combined NICU population is approximately 59% male with an ethnic distribution of 65% African-American, 33% Caucasian and 2% other. Subjects will not be excluded based on gender, race or ethnicity.

4.1 Inclusion Criteria

Inclusion criteria will include

1) < 5days

2) Gestational age 24^0-32^6 weeks

4.2 Exclusion Criteria

Exclusion criteria will include:

1) Non-viability or planned withdrawal of life support;
2) Triplet or higher order multiple;
3) Severe asphyxia (Apgar <3 at 5 min and cord pH <7.0);
4) Lethal chromosome abnormalities;
5) Cyanotic congenital heart disease;
6) Intestinal atresia or perforation;
7) Abdominal wall defects;
8) Significant GI dysfunction (e.g. heme-positive stools, abdominal distension (girth >2 cm baseline), or bilious emesis/aspirates.
9) *Infants with galactosemia or other forms of galactose intolerance*

4.3 Randomization

Not randomized

4.4 *Blinding*

Not blinded

4.5 Withdrawal

4.5.1 Reasons for Withdrawal

Subjects may be withdrawn from the study for the following reasons:

1) At the request of the subject's parent(s) or guardian(s), or at the request of other legally authorized representative;

2) If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being;

3) At the request of the IRB or Safety Monitor.

4.5.2 Handling of Withdrawal

Subjects who discontinue due to being withdrawn or lost to death will be replaced. Data and specimens will be de-identified and remain as part of the study.

4.6 Termination of the Study

The study may be terminated:

1) At the request of the IRB or Safety Monitor

2) If the principal investigator is unable to continue with the study and a suitable replacement is not identified.

5 INVESTIGATIONAL PRODUCT(S)

5.1 Study product description

A 1 mL/kg La/Rh solution (8.6 g of lactulose + 140 mg of rhamnose/100 mL) will be used to assess intestinal permeability. Lactulose is a synthetic disaccharide formed from fructose and galactose. Rhamnose is a naturally occurring sugar.

Lactulose will be obtained as Kristalose from Cumberland Pharmaceuticals 2525 West End Avenue, Suite 950, Nashville, TN 37203 (www.cumberlandpharma.com) and is listed in the U.S. Pharmacopoeia.

Rhamnose will be obtained from Saccharides, Inc. 205, 259 Mid Park Way S.E., Calgary Alberta T2X 1M2 Canada.

Detailed instructions for preparation, storage and handling of the sugar solution can be found in the Manual of Procedures.

Administration details can be found in Section 6.

6 STUDY PROCEDURES / EVALUATIONS / SCHEDULE

6.1 Clinical/Laboratory Evaluations and Study Schedule

Informed consent will be obtained before any clinical evaluations are performed. Specific details of assay performance and calibration are listed in the MOP.

6.2 Screening *(include allowable time window)*

After receiving a HIPAA waiver from the IRB, clinical PI and staff will screen all new admittances to the NICU for eligibility criteria. Study interventions must be able to begin within 24 hours of enrollment. Informed consent must be signed before any study procedures are begun.

6.3 Enrollment/Baseline *(include allowable time window)*

After consent and HIPAA forms are signed, chart will be reviewed and history will be recorded. Medical history will be obtained from the chart and eligibility criteria will be confirmed.

Prior to Lactulose/rhamnose sugar probe dosing, the subject will have a complete physical exam including vital signs, weight, height, and head circumference.

6.4 Study Visits *(include allowable time window for each study visit)*

Postnatal day 5 (or within 24 hours of enrollment)

- Begin *collecting available stool samples for microbiome, calprotectin, sIgA, and lactoferrin analysis*
- Medication history will be collected from the chart.
- *Complete physical exam including vital signs, weight, height, and head circumference.*

Postnatal d7-10

- In vivo IP will be determined by means of the urine La/Rh test. Briefly, preterm neonates will be administered a 1 mL/kg La/Rh solution (8.6 g of lactulose + 140 mg of rhamnose/100 mL) via a clinically indicated OG or NG tube. The La/Rh solution will be prepared by the Investigational Drug Pharmacy (IDS) and administered by gavage. Starting at the time of the dose of the La/Rh solution, urine will be collected for a 4-hour period *with cotton balls*. If a participant does not require an OG/NG tube the solution will be administered orally by nippling.
- *Continue collecting available stool samples daily until PND14 and single sample on PND21 for microbiome, calprotectin, sIgA, and lactoferrin analysis. Record daily weight, and weekly height, and head circumference.*

- ∞ Demographic and clinical data will be collected from the chart.
- ∞ Collect adverse events from medical chart.

6.5 Follow-up Visit(s) *(include allowable time window)*

Not applicable.

6.6 Unscheduled Visit(s)

Not applicable

6.7 Early Termination *(include allowable time window)*

If early termination occurs the assessments from the final visit should be completed if the parent/LAR is willing. The physical exam and data collection regarding adverse events medication history should be collected at a minimum. If possible, the La/Rh should be administered and urine and serum should be collected.

6.8 Final Study Visit *(include allowable time window)*

PND21 (± 2d) or discharge/transfer whichever comes first

- ∞ *Final collection of stool samples for analysis*
- ∞ Medication history including the number of packed red blood cell transfusions and total volume received will be collected from the chart
- ∞ *Record daily weight, and weekly height, and head circumference.*
- ∞ Collection of adverse events from medical chart.

6.9 Product Administration *(include allowable time windows)*

Each enrolled preterm neonates will be administered a 1 mL/kg La/Rh solution (8.6 g of lactulose +140 mg of rhamnose/100 mL) via a clinically indicated OG or NG tube once between 7-10 d of age. The La/Rh solution will be administered by gavage with an enteral feed. Total volume of La/Rh ingested will be recorded. Urine will be collected for a 4-hour period *with cotton balls*. Total volume of urine during the 4 hour collection will be recorded. The urine will be aliquoted and 4 ml of urine saved for analysis. Nursing staff in the NICU will be trained in the procedures prior to study initiation. Only trained NICU staff will be allowed to be responsible for administering the La/Rh solution and collecting the urine. A minimum of 2 ml of urine will be collected. If urine leaks around the cotton balls, then the diaper will be weighed and the diaper weight will be used to estimate the urine volume for the 4 hour collection period. If less than 2 ml urine is collected, then the test will be repeated on another day within the 7-10d window by

administering 1 ml/kg La/Rh solution (8.6g of lactulose + 140 mg of rhamnose/100 mL) via an OG/NG tube followed by a 4 hour urine collection. The urine will be stored at -80°C until processed. The sugars will then be measure in urine by HPLC.

Urine La and Rh will be measured in the lab of our collaborator Dr. Jon Meddings from the University of Calgary, Canada.

Stool calprotectin, sIgA,and lactoferrin will be mesured by commercial ELISA kits.

Rescue Therapy- NA

6.10 Define Specimen Preparation, Handling, and Shipping

6.10.1 Instructions for Specimen Preparation, Handling, and Storage

Specimen management will be detailed in the MOP.

6.10.2 Specimen Shipment

Research staff will transport specimens collected at the University of Maryland Medical Center to the appropriate laboratory on campus. Specimens collected at Mercy Medical Center will be frozen and transported monthly to the laboratory of Dr. Viscardi. Aliquots of urine for La and Rh measurements by HPLC will be shipped to our collaborator Dr. Jon Meddings at the University of Calgary, Canada, for HPLC. All specimens will be accompanied by a shipping invoice indicating the sample ID and the chain of custody. Specific transport procedures are detailed in the MOP.

7 SAFETY REPORTING AND SAFETY MONITORING

Regulatory requirements including the FDA regulations, ICH Guidelines for Good Clinical Practice, and EU Clinical Trials Directive set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

7.1 Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- evaluate subject safety including assessment of adverse events (AEs) for seriousness, severity, and causality,
- notify the sponsor of SAEs immediately,
- provide detailed written reports, including necessary documentation requested by the sponsor or IRB, promptly following immediate initial reports, and
- inform the IRB of AEs as required by applicable regulatory requirements.

7.2 Definitions

Adverse Event (AE)

Any untoward medical occurrence clinical investigation subject who has received a study intervention and that does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Serious Adverse Event (SAE)

An SAE is any adverse event that results in any of the following outcomes:

- Death;
- Life-threatening (immediate risk of death);
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- Congenital anomaly/birth defect;

- ∞ Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unapproved investigational medicinal product).

Expedited Safety Report

Documentation in appropriate form and format summarizing an SAE that meets expedited safety reporting criteria, submitted within the required reporting time frame of applicable regulatory authorities and/or IRBs/IECs of participating countries.

7.3 Safety Reporting Requirements

7.3.1 Reporting Interval

Document all adverse events (AEs) and SAEs from enrollment through PND21.

All SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an adverse event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

The investigator will follow all SAEs until resolution (return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic) even if this extends beyond the study-reporting period.

7.3.2 Notification of the Sponsor of Serious Adverse Events

Any AE that meets a protocol-defined serious criterion that are associated with study product(s) must be submitted within 24 hours of site awareness to the sponsor, and IRB.

Other supporting documentation of the event may be requested by the sponsor and should be provided as soon as possible.

7.3.3 Regulatory Reporting for Studies Conducted Under Sponsored IND

Following notification from the investigator, the sponsor will report events that are both serious and unexpected and that are associated with study product(s) to the Food and Drug Administration (FDA) within the required timelines as specified in 21 CFR 312.32: fatal and life-threatening events within 7 calendar days (by phone or fax) and all other SAEs in writing within 15 calendar days. All serious events designed as "not associated" to study product(s) will be reported to the FDA at least annually in a summary format.

7.4 Investigator's Assessment of Adverse Events

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose adverse event information, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

7.4.1 Assessment of Seriousness

Event seriousness will be determined according to the protocol definition of an SAE (Section 7.2). *Severity will be determined according to neonatal toxicity table (Appendix D) and expanded table of severity for laboratory values (Appendix E)*.

7.4.2 Assessment of Severity

Event severity will be assigned according to the definitions below:

- Mild: events require minimal or no treatment and do not interfere with the study subject's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a study subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life-threatening

7.4.3 Assessment of Association

The association assessment categories that will be used for this study are:

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event.

- ∞ Not Associated – The event is temporally independent of the study product and/or the event appears to be explained by another etiology.

The investigator must provide an assessment of association or relationship of AEs to the study intervention based on:

- ∞ Temporal relationship of the event to the study intervention
- ∞ Whether an alternative etiology has been identified;
- ∞ Biological plausibility;
- ∞ Existing therapy and/or concomitant medications.

7.4.4 Assessment of Reactogenicity *(if applicable)*

Not applicable.

7.5 Safety Monitoring

Safety Monitoring: For the continuation of this observational study, the sponsor-investigator will be the Data Safety Monitor to 1) periodically review and evaluate the accumulated study data for subject safety, study conduct and progress, and, when appropriate, efficacy, validity, and integrity and 2) make decisions concerning the continuation, modification, or termination of the study.

7.5.1 Interruption or Discontinuation of Study Enrollment and Study Product Administration for all Subjects in the Study

Not Applicable

7.6 Halting Criteria/Rules

7.6.1 Stopping Rules for Individual Subjects

A pre-dose checklist for evidence of feeding intolerance will be completed prior to La/Rh administration. The Lactulose/Rhamnose administration will be re-scheduled within the study window if the clinically indicated gastric residual is obtained and measures >50% of the previous nutritive feeding volume, and/or contains bile or gross blood, or is hemoccult-positive. If these GI signs are present throughout the study window, the La/Rh test will not be administered. Other conditions that would result in delay or discontinuation of the La/Rh test include hypotension requiring pharmacologic support, clinical or radiographic signs of intestinal ileus or NEC. The subject will continued to be followed until postnatal day 21±2 d or discharge/transfer which ever occurs first.

Plans for Study Interruption Based on Safety Monitoring:

Since the expected in-hospital mortality rate for infants 24-32 weeks gestation is 10-15% at the UMMC NICU, 5-8 deaths may occur in our study population per year. If more than 10 deaths or 7 confirmed cases of NEC >Stage 2 occur within one year or more than 4 deaths or 3 confirmed NEC cases >Stage 2 in consecutively recruited subjects occur within 30 d of enrollment, the study will be interrupted until reviewed by the safety monitor. The study may also be halted if the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events warrant further investigation. The safety review may require temporary suspension of enrollment or all study interventions. The safety review may determine whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Subsequent review of serious, unexpected, and related AEs by the medical monitor, IRB, the sponsor(s), or relevant local regulatory authorities may also result in suspension of further trial interventions at a site. The study sponsor(s) retain the authority to suspend additional enrollment and study interventions for the entire study, as applicable.

8 CLINICAL MANAGEMENT OF EVENTS

8.1 Adverse Event Management

Study interventions will not influence medical care. Morbidities associated with prematurity are protocol-specified potential serious adverse events. These are events that are anticipated to occur in the preterm population even in the absence of study intervention. Morbidities of prematurity will be recorded on CRFs on study day 21 or discharge/transfer whichever comes first.

8.1.1 Temporary Interruption of Study Product in an Individual Subject

Not applicable.

8..2 Pregnancy *(if applicable)*

Not applicable.

9 CLINICAL MONITORING/SITE MONITORING PLAN

In general, site monitoring is conducted to ensure that:

- ∞ human subjects' rights and well-being are protected;
- ∞ data are accurate, complete, and verifiable from source documents;
- ∞ the study complies with the protocol/amendment(s), ICH Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

The internal monitoring plan will be conducted according to the Quality Management plan in Appendix C.

10 STATISTICAL CONSIDERATIONS

10.5 Sample Size Considerations

Sample size for primary objective: The sample size for the first aim (50 subjects) will provide good precision in the estimate of the distribution of levels of intestinal permeability at each time point. The quantiles will be accurate +/- 10 percentage points (based on a 95% confidence interval). Based on the sites' combined annual admission numbers and survival rates, we expect 120 infants to be eligible in the 6 month planned enrollment period in the first year. The combined NICU population is approximately 59% male with an ethnic distribution of 65% African-American, 33% Caucasian and 2% other.

Sample size for microbiome analysis: The sample size of 200 was calculated to obtain high precision in correlations between features of the stool microbiota and intestinal permeability (IP). Since 83% subjects in the first cohort had high IP (La/Rh $\geq$ 0.05) at study day 8, a sample size of 166-236 provides marginal error of 0.05 (maximum sampling error of 5%) and alpha of 0.05 (95% confidence level) to detect correlations between features of the microbiota and changes in IP and to achieve an expected sensitivity of 0.8-0.9 and expected specificity at 0.9-0.95.

10.2 Randomization *Not applicable*

10.3 Blinding *Not applicable.*

10.4 Planned Interim Analyses *(if applicable)*

Not applicable

10.5 Safety Review *(if applicable)*

10.6 Final Analysis Plan

Primary Objective: To estimate mean, variance, and quantiles in IP measured by urinary Lacutlose/Rhamnose ratio in in neonates born between 24 and 32 weeks of gestational age.

> Sample quantiles of the distribution of IP (as measured by urine LA/Rh ratio) will be calculated at each time point. In addition, we will use mixed effects models to estimate the mean and variance of urine La/Rh. By including other terms in the model we will be able to assess whether high IP (La/Rh ratio >0.05) is affected by gestational age, birthweight, sex or *feeding type (mother's own breast milk only, donor breastmilk, preterm formula only, or both breast milk and preterm formula).*

Secondary Objective: To evaluate the performance of stool calprotectin, sIgA, and lactoferrin as markers for intestinal permeability *in vivo* as established by the gold standard urine La/Rh in preterm neonates.

Assessing the relationships between stool markers and urine La/Rh ratio: To quantify the association between stool calprotectin, sIgA, and lactoferrin, and urine La/Rh ratio, we will calculate Pearson Correlation Coefficients. If the distribution of either variable exhibits substantial skewness we will substitute Spearman Correlation Coefficients. In addition, we will perform sub-group analyses by gestational age or birthweight.

Assessing the sensitivity and specificity of calprotectin, sIgA, and lactoferrin for identifying children with leaky gut syndrome using an ROC analysis. Leaky gut syndrome will be defined as having a urinary La/Rh ratio of greater than 0.05 at 7-10 d (12). Using this definition, we will assess the sensitivity, specificity, and predictive value of calprotectin, sIgA, and lactoferrin on each day for identifying those with leaky gut syndrome. An analogous calculation will be made to estimate specificity. We will perform these calculations for a large range of cut-offs for the stool markers, and summarize the findings using an ROC curve. The area under the ROC curve (AUC) will be calculated using an empirical approach to provide an overall summary measure of the performance of **calprotectin, sIgA, and lactoferrin** as measures for leaky gut syndrome.

Handling missing data or dropouts. Our analysis of the correlation between calprotectin, sIgA, and lactoferrin and IP will be based on an "available data" approach. That is, if a child missing data due to lack of stool sample or sufficient urine collection, information from that time point will not be included in the analysis. We think this is reasonable because in that situation both and IP will be unknown and imputating both would result in very limited additional information. For the analysis of the degree to which calprotectin, sIgA, and lactoferrin can predict leaky gut syndrome, we will initially do an analysis based on the available data, but in a secondary sensitivity analysis we will see how our estimates are affected if we assume that all those children who dropped out due to illness or deteriorating condition actually developed leaky gut syndrome.

Stool bacterial community characterization: DNA will be extracted using the protocols developed and validated at IGS that routinely obtain between 5 and 10 µg of high-quality whole genomic DNA. 16S rRNA gene amplicon sequencing will be performed on Illumina MiSeq platform, using standard methods developed for the NIH Human Microbiome Project and by our group at IGS. Bioinformatics analyses will be performed to calculate bacterial types and their relative abundance. A TaqMan® PanBacterial quantitative PCR assay targeting the 16S rRNA gene, designed and validated by our group at IGS, will be used to estimate the true bacterial abundance per gram of stool. Together the relative proportion and absolute copy number of each individual bacterium will be calculated. Metabolome profiling will be performed using LC/MS (Liquid chromatography/mass spectrometry) by Metabolon, Inc. from 1:2 fecal to PBS solution according the company's sample preparation protocol. Fecal calprotectin biochemical test (CALPR) will be used as an indicator for pathological inflammation of the bowel wall.

Discriminatory classifier computation: Three major procedures are involved to build a discriminatory classifier using supervised machine classifiers to identify high or low intestinal permeability. (1) Data collection. Upon the recruitment of preterm infants at <5d of age and

gestational age (GA) at 24-32 weeks, information including extensive data on demographics, obstetric and neonatal factors, medication exposures, and feeding practices will be obtained. Fecal specimen will be collected to generate bacterial community composition and structure using high-throughput sequencing of 16S rRNA gene amplicon and to calculate bacterial total copy number using quantitative PCR. Urine specimen will be used to measure the non-metabolized sugar lactulose (La) and rhmnose (Rh) concentration using high-pressure liquid chromatography (HPLC), and their ratio will be used to calculate high IP (La/Rh > 0.05) and low IP (La/Rh <= 0.05). (2) Discriminatory classifier generation. The bacterial abundance and neonatal information will be used to form the data matrix and the high or low IP category will be used to "label" a sample as its class, which is used in supervised machine learning scheme with cross-validation (construct model on a sample set and evaluate on a different sample set to avoid over-prediction) to direct generating the classifier after data preprocessing and evaluation. The most discriminatory features (i.e. bacterial species and/or neonatal factors) will be characterized by its contribution to identify a sample in a high or low IP class. (3) We will evaluate the contribution of each discriminatory feature to IP classification using permutation importance measurement (5).

10.7 DATA HANDLING/RECORD KEEPING/Source Documents

All record keeping procedures will maintain participant confidentiality in accordance with ICH-GCP guidelines. Paper records will be kept locked and only study staff will have access to study records. Accuracy will be ascertained through the study quality assurance plan. Details are listed in the Manual of Procedures.

10.7.1 Data Capture Methods

Clinical data (including AEs, and concomitant medications) and clinical laboratory data will be entered on paper CRFs. Clinical data will be entered directly from the source documents. For data analysis the data will be manually entered from CRFs into electronic spreadsheets and processed using SAS 9.2 (Copyright (c) 2002-2008 by SAS Institute Inc., Cary, NC, USA.).

10.7.2 Types of Data

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

10.7.3 Study Records Retention

Each participating site will maintain appropriate medical and research records for this study, in regulatory and institutional requirements for the protection of confidentiality of subjects. Each

site participating in this study will permit authorized representatives of the sponsor, the institutional IRB, and regulatory agencies to examine (and when required by applicable law, copy) clinical records for the purposes of clinical site monitoring, quality assurance reviews, audits, and evaluation of the study safety and progress.

10.7.4 Source Documents

The source document is defined as the first place the data are recorded; i.e., the location where all study-related data are initially recorded. The source could be hard copy paper and/or electronic forms, lab printouts, pharmacy records, medical records, etc, onto which the data are first recorded. In most cases, the source documentation for this study will be the electronic medical record.

The study staff will transcribe research data into the RedCap electronic database from the subject's chart or other medical records. The electronic medical record will be the source document except for data collection forms listed below.

Case Report Forms as Source Documents

Study personnel trained in the proper completion of case report forms (CRFs) will record study data in RedCap database and all forms will be eletronically signed and dated by the study personnel who completed the form. In some instances data for which there is no prior written or electronic record will be recorded on electronic CRFs and these will be considered source documents. This will be limited to the following CRFs:

1. *IP05 (Physical Exam)*
2. *IP06 (LaRh Collections) (with supplementary attachments)*
3. *IP07 (Specimen Collection)*
4. *IP10 (Adverse Events) (with supplementary attachments)*
5. *IP11 (Serious Adverse Events) (with supplementary attachments)*
6. *IP12 (Protocol Deviation Form)*
7. *IP13 (Early Termination)*

10.8 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed by the site and implemented promptly.

11 QUALITY CONTROL AND QUALITY ASSURANCE

This section summarizes the separate written quality management plan for quality control and quality assurance at the investigative site (site).

Each site will have a delegation of responsibility log. The log will list the staff involved in the trial and their responsibilities. The PIs will ensure that all staff are adequately trained for their responsibilities. A log of the trainings completed by the staff will be maintained with the regulatory documents.

A checklist will be created for key quality indicators such as eligibility criteria and consent. All records will be reviewed for these indicators. In addition, eligibility criteria will be reviewed and signed by the site PI. Informed consent and HIPAA forms will be reviewed by site staff for completeness, including date and any additional checks or signatures required. Data collected from protocol procedures will be reviewed monthly for compliance with procedures and accuracy with source documents.

Specimen collection and storage documentation will be compared to the Manual of Procedures. Chain of custody documentation and laboratory storing and processing procedures will also be reviewed. Deviations from the Manual of Procedures may require a corrective action plan.

A summary of review findings will be filed with regulatory documents.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

The investigator(s) will ensure that this study is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 312, and/or ICH E6; 62 Federal Regulations 25691 (1997). The PI/Institution will hold a current FWA issued by OHRP for federally funded research.

12.2 Institutional Review Board

Each participating institution IRB must review and approve this protocol, associated informed consent documents, and recruitment material. Any amendments to the protocol or consent materials must also be approved before they are implemented. Only institutions holding a current US FWA issued by OHRP are authorized to review and approve these documents (*http://www.hhs.gov/ohrp/assurances/)*.

13 INFORMED CONSENT PROCESS

The informed consent process will be initiated before a volunteer agrees to participate in the study and should continue throughout the individual's study participation. The parent/LAR will sign the informed consent document before any procedures are undertaken for the study. Extensive explanation and discussion of risks and possible benefits of this investigation will be provided to the subjects in understandable language. Adequate time will be provided to ensure that the subject has time to consider and discuss participation in the protocol.

Drs. Viscardi, Sundararajan, or research coordinators will review all UMMC NICU admissions to determine eligibility. For those babies who meet criteria, study personnel will approach the parents for consent. The content of the consent form and the purpose of the study will be explained. The risks, alternative to not participate and right to withdraw without penalty will be explained. The study team member will offer to answer any questions. Consent will be documented by parent/LAR signature on the IRB approved consent form. The original signed consent form will be filed for study records and the consent process documented in the infant's medical record. The parent/LAR will be given a copy of the consent form for their records.

13.1 Assent Process (Minor)/Parental Permission/Consent (If applicable)

Not applicable

13.2 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study.

No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the sponsor and the subject.

The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

13.3 PI Responsibility When Subject Withdraws or Is Discontinued

Subjects may be withdrawn from the study for the following reasons: 1) at the request of the subject's parent(s) or guardian(s), or at the request of other legally authorized representative; 2)

if, in the investigator's opinion, continuation in the study would be detrimental to the subject's well being.

13.4 Future Use of Stored Specimens

Specimens will be stored until the conclusion of the research. The consent form will specify if specimens may be stored indefinitely for future research, destroyed at the end of the research, or if the participants must be contacted to obtain permission for specimens to be used for other research. Future use may only include IRB approved protocols. Specimens will be labeled with a code indicating such.

14 PUBLICATION POLICY

Final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication at the National Library of Medicine's PubMed Central website. The clinical trial is listed at the ClinicaTrials.gov website.

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Supplements/Appendices

APPENDIX A: SCHEDULE OF EVENTS

Study procedure	DOL 0-5	DOL 7-10	DOL 21
Visit	1	2	3
Screen for eligibility	R		
Obtain consent	R		
Physical exam		*R*	
Review of Medical History	R		
Review of Concomitant Medications	R	*R*	*R*
Adverse events		*R*	*R*
Perform lactulose/rhamnose (La/Rh) intestinal permeability test Collect urine		R	
Collect stool	R	R	R

APPENDIX B-MODIFIED BELL STAGING OF NEC

Modified Bell staging NEC

Stage	Classification of NEC	Systemic signs	Abdominal signs	Radiographic signs
IA	Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus
IB	Suspected	Same as above	Grossly bloody stool	Same as above
IIA	Definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
IIB	Definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus ascites
IIIA	Advanced, severely ill, intact bowel	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites
IIIB	Advanced, severely ill, perforated bowel	Same as IIIA	Same as IIIA	Same as above, plus pneumoperitoneum

DIC: disseminated intravascular coagulation. *Adapted from Neu J. Pediatr Clin North Am 1996; 43:411, 1996.*

APPENDIX C: QUALITY MANAGEMENT PLAN

Quality Management Plan

The combined processes of quality control (QC) and quality assurance (QA) form the basis of quality management and will be applied to this clinical research in accordance with a CCHI Quality Management Plan. This plan identifies the protocol, details the frequency of quality control checks, quality assurance reviews, priority of review, and the percentage of records selected for review.

The Protocol-specific Quality Management Plan and written quality assurance communication (e.g., audit reports, problem resolution worksheets, regulatory checklists, audit summaries, QC/QA trend reports, quarterly and annual reports, etc.) will be kept in a separate Protocol-specific file Quality Management file.
Quality Management Plan Audit Tools include as appropriate

- ∞ Quality Assurance Audit Tool (RedCap)-
- ∞ Consent Form Quality Assurance Tool
- ∞ Regulatory File Review Tool
- ∞ Quality Assurance Audit Report (RedCap)
- ∞ Quality Assurance Annual Progress Report

Quality Control (QC) and Quality Assurance (QA)
Designated research staff will prospectively review and verify 100% of all data collected and entered into the participant's study record. This review will be performed as close to the time of data collection as possible ("real-time") to ensure the collection and accurate documentation of all key data points. Data will undergo quality control "checks" prior to transfer from source document(s) to paper or electronic case report forms (CRFs) when applicable, however, where this is not feasible, the data will undergo quality control checks within 2-3 business days.
Quality control checks or "QC checks" will begin upon the initiation of screening and recruitment activities and will continue through to study completion. These checks will entail the review of 100% of all study-specific consent forms, privacy authorizations, source documents and/or case report forms. Consent forms (screening, study-specific, stored specimen, and eligibility checklists will be reviewed at time of screening, on the day of enrollment and prior to study product administration (initial and subsequent administration). "All" other source documents and study-related forms will undergo quality control checks on an ongoing basis throughout data collection. *Twenty-five percent of all CRFs will be reviewed every 3 months for accuracy verified by review of source documents.*

The quality control procedure requires that data collected and recorded by one member of the research staff be "checked" for logic and edits by another member of the research staff. Communication of errors or problems identified during "QC checks" may be communicated verbally to the person who either collected and/or recorded the data and/or via written communication (i.e., post-it note which will be removed once the error has been corrected).

All data corrections will be made by drawing a single line through the error, entering the correct information, initials, and the date on which the correction was made. This method of data correction is in accordance with the Good Clinical Practice Guideline (GCP) and is important for maintaining an audit trail. The date of corrections must reflect the date on which the correction was made and late entries dated the day the late "entry" is made. Corrections to data will be made by authorized staff only. Data and/or clerical errors noted in the source document that require a change to a database will be corrected by the designated data entry staff. Once the data has been corrected, the data entry staff will provide the coordinator written documentation of the change. Completion of "QC checks" will be indicated by entering "Qc'd by" in the lower right hand corner of the document followed by the initials of the person verifying completion of the "QC check" and the date. In cases where documentation of the QC check is not permissible by the sponsor, another method of documentation may be implemented.

Quality control "checks" will verify the following as applicable to the study:

- HIPAA authorization obtained and documentation that study participant or authorized representative have been given a copy
- Consent Form(s) are current, valid, IRB approved and are appropriately signed, dated and witnessed by the study participant or authorized representative and by the person obtaining consent
- Consent process is documented by the person(s) obtaining consent.
- Date and time of consent is documented
- Eligibility checklist, medical history, physical exam forms have been signed and dated by the principal investigator or appropriate medical clinician as required by the sponsor
- All study forms, source documents, and CRFs are complete (e.g., all data collection fields and header information is complete; check boxes are appropriately checked, etc.) and are signed by persons who obtained the data
- All visits, screening through termination, are documented
- Screening tests and visit procedures are completed for all visits
- Missed visits and attempts to contact participant or authorized representative are documented
- Data recorded on medical history and adverse event forms is consistent with concomitant medications forms
- All concomitant medications as reported by the participant or authorized representative are recorded
- Prohibited medications have been identified and documented
- All forms are correctly dated and participant identifiers (PIDs) correctly recorded
- Data quality is good (ALCOA - attributable, legible, contemporaneous, original, and accurate)
- All adverse events are documented and serious adverse events (SAEs) reported to sponsor/ IRB
- All required safety labs obtained and clinical significance assigned by the investigator to abnormal values
- Study product temperature appropriately monitored and temperatures documented. Cold Chain maintained
- Verify that study samples have been collected, processed, and/or stored as described in the protocol, MOP and/or SOP.

● Verify that sample storage temperatures have been monitored and recorded daily and if applicable, excursions have been reported as deviations to the sponsor and IRB as appropriate

● Protocol deviations and/or violations, if any, (e.g., eligibility, missed visits, labs, procedures, out-of-window visits, etc.) are documented and appropriately reported to sponsor and IRB Signatures have been obtained on all documents, as required

● Treatment and/or study discontinuation is adequately documented and study status form(s) completed.

● Reason for ineligibility is adequately documented and all study-specific documents are completed up to the point of ineligibility determination

● Study-specific logs are complete and accurate (e.g., questionnaires) are complete

● Laboratory equipment is maintained according to manufacturer's directions

● Calibration data is quality checked before entry the research record

● All laboratory data are checked by two people for entry in to the research record

● Study product management (i.e., accountability, qualified personnel, dispensing, administration, disposition, secure storage, and access by authorized study personnel) follows protocol.

Quality Assurance Process

Quality Assurance (QA) audits of the study will be performed by a Quality Management (QM) Coordinator. Ten to 20% of study charts will be audited for each study, greater if deemed necessary by the PI or if QA findings suggest the need to increase the number of charts to be reviewed. The minimum number of charts to be reviewed will be no less than 10%.
Quality assurance audits broadly assess investigator and site adherence to the IRB approved protocol, UMB-HRPO policies, sponsor guidelines, and other applicable regulatory authorities. Data and/or clerical errors or other problems identified during quality assurance audits will be documented on the appropriate Problem Resolution Worksheet and communicated to the PI and the Research Coordinator. Data errors and/or problems may be resolved during the audit, however, if unresolved at the end of audit visit, will be noted on the Problem Resolution Work-sheet, corrected by the appropriate staff and returned to the person performing the audit within 2 weeks of the audit completion date. The investigator will be provided with a summary report for each protocol that is audited.

"All" data and/or clerical errors identified during the quality assurance audit will be corrected in accordance with the Good Clinical Practice Guideline (GCP). A single line will be drawn through the error, then initialed and dated. The date of data correction must reflect the date of the correction and late entries dated the day the late "entry" is made. This method of correction is essential to maintaining an audit trail. Corrections to study data will be made by authorized staff only. The person performing the audit will only identify errors and/or problems and is not permitted to make changes to the study data, make entries into the study record (source, paper or electronic CRFs).

Trends, significant findings, data or quality management related issues identified during quality assurance audits will be communicated to the research staff and discussed during routine staff meetings and documented in quarterly and annual reports. Monitoring reports will be reviewed

by the PI and/or the Research Coordinator. Any findings unresolved upon completion of monitoring visits will be promptly addressed. Significant findings noted on monitoring reports will be considered for integration into quality improvement initiatives, as appropriate, re-training of staff, revision of existing MOPs and/or SOPs, development of new MOP and/or SOPs, and/or Corrective Action Preventive Action (CAPA) plans.

Quality assurance audit reports, summaries, worksheets, and other communications, are not part of the regulatory file therefore, will be maintained in a separate protocol-specific Quality Management file. Quality management plans for multiple year studies will be re-evaluated on annual basis by the PI and the protocol-specific research team.

Quality assurance audits will verify the following as applicable to the study:

- The regulatory file (essential documents, IRB approvals, continuing reviews, protocol exceptions, etc.)
- Informed consent(s) for inclusion of requirements for elements of informed consent
- Documentation of the informed consent process
- Eligibility (completion of screening procedures / requirements within protocol-specified timelines)
- Adherence to protocol procedures and parameters
- Documentation of adverse events (AEs) and concomitant medications
- Documentation and timely reporting of internal and external Serious Adverse Events (SAEs)
- Documentation of clinical evaluations
- Documentation of study endpoints (e.g., study specimens).
- Documentation of specimen collection, processing, and storage; shipping manifests, and freezer temperature logs.
- Documentation and reporting of protocol deviations (e.g., missed visits, study labs, study procedures, temperature excursions) within specified timelines
- Documentation and reporting of protocol violations (e.g., eligibility, deviations that impact participant safety, rights, well-being) within specified timelines
- Documentation of treatment and study discontinuation
- Signatures obtained as required
- Adherence to institutional, sponsor and GCP documentation standards
- Compare data consistency between source documents and electronic and/or paper CRFs
- Assess data quality (ALCOA - attributable, legible, contemporaneous, original, and accurate)

Data management

When an electronic data base is used study-data will undergo quality control checks prior to transfer from source documents to case report forms (paper or electronic), where feasible. Every effort will be made to enter data into the electronic database within the timeframe specified by the sponsor. Data and/or clerical errors received from the data management center(s) will be promptly reviewed by appropriate members of the research staff and corrected. Data will be corrected or amended by persons authorized to make such changes to paper or electronic CRFs. Persons entering data into electronic CRFs will be trained in the use of the computerized system.