Title:	Evaluation of Bowel Perfusion with Contrast-Enhanced Ultrasound in Necrotizing Enterocolitis
Short Title	Contrast-Enhanced Ultrasound Evaluation of Bowel Perfusion in Necrotizing Enterocolitis

Drug Name(s):	Sulfur hexafluoride lipid-type A microspheres (Lumason™)
FDA IND	137874
Regulatory Sponsor:	Misun Hwang, MD
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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
Category B Drug	Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women
CEUS	Contrast-Enhanced Ultrasound
CFR	Code of Federal Regulations
CHOP	Children's Hospital of Philadelphia
CT	Computed Tomography
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
HIPAA	Health Insurance Portability and Accountability Act
ICU	Intensive Care Unit
IDS	CHOP Investigational Drug Service
IND	Investigational New Drug
IV	Intravenous
IRB	Institutional Review Board
Lumason™	FDA-approved for contrast-enhanced ultrasound; the investigational drug
MRI	Magnetic Resonance Imaging
NEC	Necrotizing Enterocolitis
ORC	The CHOP Office of Research Compliance
PI	Principal Investigator
Pneumatosis intestinalis	Gas cysts in the intestinal wall, sometimes indicative of necrotizing enterocolitis
PHI	Protected Health Information
SAE	Serious Adverse Event
US	Conventional Ultrasound Scan
XR	Abdominal Radiography
PACS	Picture Archiving and Communication System

ABSTRACT

Context:

There is no bedside imaging technique that can quantify dynamic bowel perfusion with high soft tissue contrast and sensitivity. Our goal is to assess the feasibility of utilizing contrast-enhanced ultrasound (CEUS) in bedside monitoring of bowel perfusion in neonatal necrotizing enterocolitis.

Objectives:

- Primary: Assess bowel perfusion characteristics in necrotizing enterocolitis using CEUS.
- Secondary: Optimize the CEUS scanning technique and quantification methods for clinical translation.

Study Design:

Single site, open-label clinical study.

Setting/Participants:

Subjects in the neonatal or pediatric intensive care unit aged 1.5 years or younger with suspected or diagnosed necrotizing enterocolitis.

The study will be performed at Children's Hospital of Philadelphia (CHOP). CHOP will be the only site of the study.

Study Interventions and Measures:

Contrast-enhanced ultrasound scan with a duration of approximately 20 minutes. Qualitative analysis with visual assessment and quantitative analysis of the acquired scans will be performed by the sponsor-investigator. The scans will be assessed for diagnostic quality of images, artifacts encountered, and the presence of additional contributory diagnostic information.

PROTOCOL SYNOPSIS

Study Title	Evaluation of Bowel Perfusion with Contrast-Enhanced Ultrasound in Necrotizing Enterocolitis
Funder	Children's Hospital of Philadelphia
Clinical Phase	Phase II
Study Rationale	Necrotizing enterocolitis (NEC) is a devastating disease of the newborn affecting 1-5% of all neonatal Intensive care unit (NICU) admissions and up to 10% of neonates under 1500 g with mortality rates of 50% or higher depending on severity [1] Bowel wall perforation occurs in 12%-31% of patients with NEC, increasing the mortality rate from 30% to 64% [2]. Therefore, early detection of ischemia and necrotic bowel which leads to perforation is vital in improving morbidity and mortality associated with NEC. Abdominal radiography, the standard imaging algorithm for monitoring of NEC, has a low sensitivity of 40% in the diagnosis of severe NEC with necrotic bowel [1]. Currently, there is no bedside tool with high soft tissue contrast to accurately monitor and quantify bowel perfusion in pediatric patients. Bowel perfusion is altered in developing ischemia which is important for early diagnosis and intervention in neonatal necrotizing enterocolitis, a disease of high mortality and morbidity if diagnosed late. Note that the current standard algorithm for diagnosis of necrotizing enterocolitis is abdominal radiography, which misses up to 60% of advanced necrotizing enterocolitis. If the disease is missed at an early stage, highly morbid surgeries consist of extensive surgical resection of bowel which are not without serious potential complications. Abdominal ultrasound can be obtained to assess for gross complications of advanced necrotizing enterocolitis such as pneumatosis intestinalis or complex ascites but its diagnostic sensitivity for early necrotizing enterocolitis is limited. As compared to CEUS, color Doppler quantification of bowel perfusion offers suboptimal soft tissue contrast and inaccuracies related to artifact. As compared to CEUS, MRI is not easily obtainable in neonates and infants due to cost, support staff, transport and/or sedation requirements. CEUS on the other hand permits serial monitoring of bowel perfusion at the bedside with high soft tissue contrast and at lower cost than MRI. Further validation of this technique and standardization of

	quantification methods will prove to be of significant clinical value There is a dire need to introduce better imaging tools such as contrast-enhanced ultrasound to the clinical setting that can detect NEC at an early stage and prompt therapeutic implementation. In this regard, contrast-enhanced ultrasound (CEUS) enables safe, serial monitoring of dynamic quantification of bowel perfusion at the bedside. Safety of intravenous use of Sulfur hexafluoride lipid-type A microspheres was based on evaluation of published literature involving use of Lumason™ in over 900 pediatric patients, as noted on the 2016 FDA product label. Non-fatal anaphylaxis was reported in one pediatric patient, but none in a neonate. Animal data of daily intravenous administration of Sulfur hexafluoride lipid-type A microspheres to rats (administered up to 10 times the recommended maximum human dose) and rabbits (administered up to 20 times the recommended maximum human dose) for 30 consecutive days and 14 consecutive days, respectively, resulted in no toxicity to the fetus in animal studies, as noted on the 2016 FDA product label. Specifically pertaining to the use of Sulfur hexafluoride lipid-type A microspheres in bowel imaging in pediatric patients, we expect a similar risk of adverse events. For the proposed study, the same pediatric dosage, route of administration, safety monitoring guidelines, and low mechanical index used for contrast ultrasound settings will be used.
Study Objective(s)	**Primary** • Assess bowel perfusion characteristics in necrotizing enterocolitis using CEUS. **Secondary** • Optimize the CEUS scanning technique and quantification methods for clinical translation.
Test Article(s)	Sulfur hexafluoride lipid-type A microspheres (Lumason™, Bracco Inc) is an FDA-approved ultrasound contrast agent which consists of active ingredients including Sulfur hexafluoride (strength 60.7 mg in 1 mg), Distearoylphosphatidylcholine, DL- (strength 0.19 mg in 1 mg), 1,2-Dipalmitoyl-Sn-Glycero-3-Phospho-(1'-Rac-

	Glycerol), Sodium Salt (0.19 mg in 1 mg). Inactive ingredients include Polyethylene Glycol 4000 (strength 24.56 mg in 1 mg) and Palmitic Acid (0.04 mg in 1 mg). The Sulfur hexafluoride lipid microspheres are composed of SF6 (molecular weight 145.9) gas in the core surrounded by an outer shell monolayer of phospholipids consisting of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt (DPPG-Na) with palmitic acid as stabilizer. Sulfur hexafluoride lipid-type A microspheres fall under Category B, that is, animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), with empirical formula C44H88NO8P, has a molecular weight of 790.6. 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-glycerol sodium (DPPG-Na), with empirical formula C38H74 NaO10P, has a molecular weight of 745.
Study Design	Single site, open-label clinical study.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Males and females aged 1.5 years or younger 2. Post menstrual age of 29 weeks or older 3. Patients with suspected or diagnosed necrotizing enterocolitis 4. Patient in the CHOP NICU or PICU 5. Parental permission **Exclusion Criteria** 1. Medical history of Lumason hypersensitivity 2. Hemodynamic instability as defined by rapid escalation of cardiopulmonary support in the past 12-24 hours, as defined by the clinical care team including ≥1 intensive care physician not part of the study team

	3. Pulmonary insufficiency as defined by FiO2 requirements of >40% and/or subjects with pulmonary hypertension requiring nitric oxide
Number Of Subjects	200; CHOP will be the only site of the study.
Study Duration	The study duration per subject will be approximately 20 minutes including the time to prepare Lumason™ contrast agent and perform the pre-contrast imaging and the CEUS, as well as the 60 minute monitoring period after the first and second injection of Lumason™. A second CEUS may be performed at the time of suspected or diagnosed NEC, and at the time of surgery (for subjects undergoing surgery as part of clinical care) or at short-term follow-up of clinical condition (approximately within 1-2 weeks from the first scan) for a total of two CEUS exams of 1 hour and 15-minute duration each. Study participation will be complete when the 60-minute monitoring period after the last CEUS performed is complete (after the first CEUS in patients who undergo one exam, or after the second CEUS is complete in patients who undergo two exams).
Study Treatment	CEUS has a total duration of 1 hour and 20 minutes: CEUS has a duration of approximately 20 minutes for the CEUS and 60 minutes for post-examination monitoring of potential adverse events.
Efficacy Evaluations	There are no efficacy evaluations for this diagnostic study. The endpoints of this study are: **Primary Endpoint** • The primary objective of this study is to evaluate whether CEUS technique can be utilized to detect early alterations in bowel perfusion in NEC. The differences in bowel perfusion between normal subjects (those with suspected or at risk of necrotizing enterocolitis but turn out to be normal on imaging and clinical evaluation) versus NEC patients will be assessed qualitatively and quantitatively using the CEUS technique, as detailed above.

	Secondary Endpoint • Diagnostic quality of the CEUS exams will be evaluated with the scoring system of non-diagnostic (1), moderate artifacts degrading diagnostic quality (2), mild artifacts without degradation of diagnostic quality (3), and diagnostic (4).
Pharmacokinetic Evaluations	There are no pharmacokinetic evaluations.
Safety Evaluations	All subjects entered into the study and receiving at least one injection of investigational drug will be included in the safety analysis. The frequencies of AEs by type, severity, and temporal relationship to the CEUS scan will be summarized. SAEs (if any) will be described in detail.
Statistical And Analytic Plan	Baseline and demographic characteristics will be summarized by standard descriptive summaries. All subjects entered into the study and receiving at least one injection of investigational drug will be included in the safety analysis. The frequencies of AEs by type, severity, and temporal relationship to the CEUS scan will be summarized. SAEs (if any) will be described in detail. Details of sample size and power calculations for this study are described in Section 6 of the protocol.
DATA AND SAFETY MONITORING PLAN	The safety monitoring for this study is the primary responsibility of the sponsor-investigator. Monitoring the safety outcomes following IV administration of the investigational drug will be conducted primarily by the Principal Investigator and/or specifically designated study personnel. An independent safety monitor will be designated to oversee the safety reports, and help adjudicate attribution of serious adverse events, should they occur. Regular meetings to discuss the outcomes of the study, and of the safety events, will be conducted by the study team. The occurrence of adverse events, serious adverse events and unanticipated events will be reported by the study team in accordance with federal and institutional guidelines, as outlined in Section 8 of this clinical study. Prior to study initiation, the Office of Research Compliance (ORC) will conduct a pre-trial monitoring visit, to assess trial readiness of the study staff. Once the pre-trial monitoring visit has been successfully completed, the ORC will also monitor the IND study on at least an annual basis.

1 Background Information and Rationale

1.1 Introduction

Necrotizing enterocolitis (NEC) is a devastating disease of the newborn affecting 1-5% of all neonatal Intensive care unit (NICU) admissions [1] and up to 10% of neonates under 1500 g. Bowel wall perforation occurs in 12%-31% of patients with NEC, increasing mortality rate from 30% to 64% [2]. Therefore, early detection of ischemia and necrotic bowel which leads to perforation is vital in improving morbidity and mortality associated with NEC. Despite extensive research and improvements in the field of neonatal care, the morbidity and mortality associated with NEC have remained unchanged over the last 3 decades [3]. Strategies and sensitive/specific tests for predicting and preventing NEC are lacking.

Contrast enhanced ultrasound (CEUS) is a novel technique in pediatrics which has proven itself as a safe and effective modality with benefits over conventional ultrasound (US) by its improved real-time evaluation of the micro- and macro-vascularity of normally and abnormally perfused tissue [4]. CEUS does not depend on ionizing radiation, can be performed bedside and the ultrasound contrast agent used is not associated with renal toxicity. There have been no studies on the use of CEUS to evaluate NEC. Despite the favorable safety profile and diagnostic benefits, the paucity of CEUS studies for NEC in the U.S. resulted from the off-label usage of this novel contrast agent.

Abdominal radiography (XR) and computed tomography (CT) have been the diagnostic gold standard for NEC but are far from flawless [5, 6]. Pneumoperitoneum is the only radiologic sign that has been universally agreed upon as an indication for surgical intervention (i.e. laparotomy or peritoneal drainage) for patients with NEC, however not all neonates with bowel necrosis and perforation have free air on XR [1, 7]. Bowel dilation is a nonspecific finding in NEC that remains best appreciated on XR and is sometimes the only sign present in NEC [8]. Additional findings suggestive of NEC on XR include intramural air and portal venous air. Abdominal XR remains a necessary, primary imaging modality for the evaluation of NEC. However, signs of NEC on XR may be subtle, can easily be missed, especially in the early stages of NEC and consequently provide limited information that could lead to clinical interventions that may prevent or predict the onset of NEC [6].

Conventional abdominal ultrasound (US) has been implemented as a useful adjunct to XR for the early detection of intramural air, bowel wall edema, decreased peristalsis, and decreased or absent perfusion that suggests bowel wall ischemia/necrosis. Furthermore, hyperechogenic air bubbles may be seen within the diseased bowel wall. US may show pathology before XR becomes abnormal. Near-infrared spectroscopy (NIRS) is another non-invasive technique that has shown promise in improving early detection of NEC but suffers from limited depth penetration and poor soft tissue contrast [6]. CT and magnetic resonance imaging (MRI) have a very limited role in the diagnostic work up of NEC because the critically sick children have to be transported to either the CT or MRI suite. Furthermore, CT uses ionizing radiation which should be avoided

when possible in these very young, radiation sensitive neonates and MRI studies suffer from the longer acquisition times and image degradation due to the susceptibility artifacts from the air inclusions and motion. The use of contrast enhanced ultrasound is an exciting new modality that shows promise in early and reliable prediction of NEC. These imaging modalities all leave something to be desired when it comes to the evaluation, prediction and prevention of NEC.

In summary, the current imaging algorithm is not sensitive enough to detect early NEC and prevent high mortality and morbidity associated with the disease. There is therefore a dire need to introduce better imaging tools to the clinical setting that can detect NEC at early stage and prompt therapeutic implementation.

1.2 Name and Description of Investigational Product or Intervention

Sulfur hexafluoride lipid-type A microspheres (Lumason™, Bracco Inc) is FDA-approved ultrasound contrast agent which consist of active ingredients including Sulfur hexafluoride (strength 60.7 mg in 1 mg), Distearoylphosphatidylcholine, DL- (strength 0.19 mg in 1 mg), 1,2-Dipalmitoyl-Sn-Glycero-3-Phospho-(1'-Rac-Glycerol), Sodium Salt (0.19 mg in 1 mg). Inactive ingredients include Polyethylene Glycol 4000 (strength 24.56 mg in 1 mg) and Palmitic Acid (0.04 mg in 1 mg). The sulfur hexafluoride lipid microspheres are composed of SF6 (molecular weight 145.9) gas in the core surrounded by an outer shell monolayer of phospholipids consisting of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt (DPPG-Na) with palmitic acid as stabilizer. Sulfur hexafluoride lipid-type A microspheres fall under Category B, that is, animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), with empirical formula C44H88NO8P, has a molecular weight of 790.6. 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-glycerol sodium (DPPG-Na), with empirical formula C38H74 NaO10P, has a molecular weight of 745. In pediatric patients, after reconstitution 0.03 mL per kg is administered intravenously. The weight-based dose of 0.03 mL per kg will be repeated twice during a single examination. Following each injection, an intravenous flush of 0.9% Sodium Chloride is injected.

1.3 Compliance Statement

This study will be conducted in full accordance of all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable federal and state laws and regulations including 45 CFR 46. All episodes of noncompliance will be documented. Research will also be conducted in full accordance with Food and Drug Administration (FDA) regulations 21 CFR 50 (Protection of Human Subjects), 21 CFR 56 (Institutional Review Boards) and 21 CFR 312 (Investigational New Drug).

The investigators will perform the study in accordance with this protocol, will obtain consent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be

accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

The purpose of the study is to evaluate bowel perfusion in neonatal necrotizing enterocolitis using the CEUS technique and optimize the technique for clinical use.

2.1 Primary Objective (or Aim)

The primary objective of this study is to evaluate the altered bowel perfusion in neonatal necrotizing enterocolitis using the CEUS technique.

2.2 Secondary Objective (or Aim)

The secondary objective is to optimize and standardize the CEUS scanning technique and quantification method.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

Patients with suspected or diagnosed necrotizing enterocolitis will be recruited for the study. Following parental consent, the subject will undergo an investigational CEUS exam, which will be performed separately from any clinically indicated conventional ultrasound. CEUS duration of approximately 20 minutes, followed by 60 minutes of monitoring. A second CEUS may be performed at the time of surgery (for subjects undergoing surgery as part of clinical care) or at short-term follow-up of clinical condition (approximately within 1-2 weeks from the first scan) for a total of two CEUS exams of 1 hour and 20-minute duration each. The CEUS exam includes a pre-contrast ultrasound evaluation with FDA-approved technologies (e.g. gray-scale ultrasound, Doppler ultrasound, microvascular imaging).

3.1.1 Screening Phase

Potential subjects will be identified by: 1) Identification by neonatologists of patients requiring abdominal ultrasound during the neonatal or pediatric intensive care unit stay for suspected, at risk of or diagnosed necrotizing enterocolitis.

Before discussing participation in the study, we will confirm eligibility by reviewing the subjects' medical records. Participation will be discussed between the PI and the referring neonatologist. Participation will be discussed with the parents/guardian by the neonatologist and/or radiologist after identification and confirmation of eligibility. Consent of the parents/guardian will be obtained prior to the exam by the PI, co-investigator, or neonatologist co-investigator in a private setting. Questions will be answered by the PI, co-investigator, or referring neonatologist co-investigator.

3.1.2 Study Treatment Phase

Investigational CEUS scan will be performed separately from any clinically indicated conventional US, in the ICU. A second CEUS may be performed at the time of suspected or diagnosed NEC, and at the time of surgery (for subjects undergoing surgery as part of clinical care) or at short-term follow-up of clinical condition (approximately within 1-2 weeks from the first scan) for a total of two CEUS exams of 1 hour and 20-minute duration each. Injection of Lumason™ contrast agent will be performed via the existing peripheral intravenous line or central line using the FDA-recommended dose of up to 0.03 mg/kg. Contrast-agent injection will be performed twice per CEUS scan to ensure image quality and test reproducibility. In the case of more stable patients without an IV line, a peripheral IV line will be started to conduct the investigational CEUS. Two bolus injections will performed to evaluate for dynamic bowel perfusion and several 2-minute cine clips as well as static images will be acquired during the exam.

3.2 Allocation to Treatment Groups and Blinding

Not applicable. The CEUS scan will be interpreted by the sponsor-investigator only.

3.3 Duration of Study Participation

The study duration per subject will be approximately 20 minutes including the time to prepare Lumason™ contrast agent, perform the pre-contrast imaging, and perform the CEUS, as well as the 60 minute monitoring period after the first and second injection of Lumason™. CEUS will be performed at the time of suspected or diagnosed NEC, a second CEUS may be performed, and at the time of surgery (for subjects undergoing surgery as part of clinical care) or at short-term follow-up of clinical condition (approximately within 1-2 weeks from the first scan) for a total of two CEUS exams of 1 hour and 20-minute duration each.

Study participation will be complete when the 60 minute monitoring period after the last CEUS performed is complete (after the first CEUS in patients who undergo one exam, or after the second CEUS is complete in patients who undergo two exams).

3.4 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at one site, The Children's Hospital of Philadelphia. It is expected 200 subjects will be enrolled to produce 100 evaluable subjects.

3.5 Study Population

3.5.1 Inclusion Criteria

1. Males and females aged 1.5 years or younger
2. Post menstrual age of 29 weeks or older

3. Patients with suspected or diagnosed necrotizing enterocolitis
4. Patient in the CHOP NICU or PICU
5. Parental permission

3.5.2 Exclusion Criteria

1. Medical history of Lumason hypersensitivity
2. Hemodynamic instability as defined by rapid escalation of cardiopulmonary support in the past 12-24 hours, as defined by the clinical care team including ≥1 intensive care physician not part of the study team
3. Pulmonary insufficiency as defined by FiO2 requirements of >40% and/or subjects with pulmonary hypertension requiring nitric oxide

In this regard, there is a published report of infusion of the investigational drug in twelve neonates, ranging from 26.9 to 41 weeks gestational age, including four premature infants from 26.9 to 29.5 weeks gestational age [9] as well as 3 additional premature subjects from unpublished data of the sponsor. The infusion of the investigational drug in these neonates and premature infants was safe, and imaging results of good quality.

Subjects who do not meet all of the enrollment criteria may not be enrolled. Subjects will be excluded from the study, if in the judgement of the primary clinical team, they are too unstable to tolerate the procedure.

Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Screening

- Identify patients scheduled for abdominal ultrasound for suspected or diagnosed necrotizing enterocolitis
- Review of medical records (for inclusion and exclusion criteria)
- Discussion of the case with neonatologists and study team to determine eligibility
- If subject is considered eligible, written or electronic consent can be obtained at this stage by PI, co-investigator, or neonatologist co-investigator
- Coordinate abdominal ultrasound schedule

4.2 Study Treatment Phase

Subjects enrolled to this clinical trial are anticipated to receive the study drug and have CEUS imaging conducted in the ICU setting. In this case, the study drug and US imaging unit will be brought to the ICU for study drug administration and imaging. Any clinically indicated imaging must be performed prior to contrast-enhanced abdominal ultrasound so as not to delay clinical care.

Pre-injection documentation

Prior to injection, vital signs and baseline assessment will be recorded and documented from the medical record and clinically in-place monitoring. Baseline assessment will consist on neurological status as described in the clinical evaluations recorded in the medical chart prior to intervention.

Pre-injection evaluation

The exam will include a pre-contrast injection evaluation with FDA-approved technologies (e.g. gray-scale ultrasound, Doppler ultrasound, microvascular imaging) to assess anatomical structures and guide contrast evaluation. This will last approximately 5 minutes.

CEUS scan

CEUS duration is of approximately 20 minutes. The CEUS examination will be terminated if there is a deterioration in the subject's clinical status during imaging.

60-minute monitoring period

The study team, who are composed of personnel trained in recognizing signs of infusion reaction, will conduct the 60-minute monitoring period and record any untoward reaction that may be related to the infusion of the contrast drug. The 60 minute monitoring post-CEUS will be conducted by study team members.

Vital signs will be recorded and documented from the medical record and clinically in-place monitoring at 1) 30 minutes post-scanning, and 2) 60 minutes post-scanning. During monitoring, subjects will be assessed for rash, allergic reactions, anaphylaxis, and abrupt deviations from the subject's baseline hemodynamic parameters trend not related to medical intervention.

Adverse event assessment and documentation

Adverse events will be recorded at 1) 60 minutes post-scanning when the monitoring period is completed, and 2) through 48 hours post-scanning, with documentation at 48 hours post-scanning if no adverse event presents until this point. During the 48-hour AE assessment period, the following AEs of special interest will also be assessed:

a. worsening cardiopulmonary status,
b. worsening pulmonary hypertension, which may be suggested by new requirement of nitric oxide use, elevated pulmonary artery pressures on echocardiography, or differential limb pulse oximetry measurements,
c. worsening neurological status, and
d. serious gastrointestinal complications

4.2.1 CEUS #1

- Obtain consent prior to scheduled CEUS exam (if not previously obtained by, PI, co-investigator, or neonatologist co-investigator)
- Record vital signs and baseline assessment prior to injection of contrast
- Perform pre-contrast injection evaluation.

- CEUS scan performed, at the time of suspected or diagnosed necrotizing enterocolitis
- Monitor patients for 60 minutes following the CEUS scan for documentation and treatment of potential adverse events
- Documentation of any adverse events through 48 hours post-scanning

Subjects may undergo surgery as part of clinical care. A second CEUS may be performed at the time of surgery for subjects undergoing surgery as part of clinical care, or at short-term follow-up of clinical condition (approximately within 1-2 weeks after the first scan) for a total of two CEUS exams of 1 hour and 20-minute duration each.

4.2.2 CEUS #2

- Record vital signs and baseline assessment prior to injection of contrast
- Perform pre-contrast injection evaluation.

- CEUS scan performed at the time of surgery for subjects undergoing surgery as part of clinical care, or at short-term follow-up of clinical condition (approximately within 1-2 weeks from the first scan)
- Monitor patients for 60 minutes following the scan for documentation and treatment of potential adverse events
- Documentation of any adverse events through 48 hours post-scanning

Study participation will be complete when the 60-minute monitoring period of the last CEUS performed is complete (after the first CEUS in patients who undergo one exam or after the second CEUS is complete in patients who undergo two exams).

The study team will not use sedation or general anesthesia to conduct the research CEUS scans.

The results of the CEUS imaging will be collected for research purposes only. The results of the CEUS will not be used to direct clinical care decisions, without confirmation of diagnosis by another medically established diagnostic product or procedure.

4.3 Concomitant Medication

No concomitant medications will be recorded, with the exception of rescue medications, as noted below.

4.4 Rescue Medication Administration

All the rapid response equipment and resuscitation staff are readily available 24/7 in the intensive care unit setting at Children's Hospital of Philadelphia. In the rare event that a significant allergic reaction occurs, or anaphylaxis results following injection of Lumason™, standard clinical medication to treat the subject will be administered. For presumed allergic reactions, medications may include intravenous diphenhydramine and bolus corticosteroids (prednisolone), based on clinical care. For anaphylaxis, medications may include epinephrine as well as fluid and oxygen administration for emergency treatment, based on clinical care decision making and on the severity of symptoms. Any severe allergic or anaphylactic reaction will be reported to both the IRB and the FDA.

4.5 Subject Completion/Withdrawal

Subjects' families may withdraw their child from the study at any time without prejudice to their child's care. A study investigator may withdraw a subject to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, the adverse events will be recorded and reported.

4.5.1 Early Termination Study Visit

A subject may be withdrawn by a parent prior to or during a CEUS scan, provided the 60 minute monitoring is completed after injection of the investigational drug. The CEUS examination will be terminated if there is a deterioration in the subject's clinical status during imaging.

4.5.2 Review of medical records from EPIC and/or other sources

- Date of birth
- Weight
- Clinical diagnosis
- Treatment history (medications, chemotherapy, antibiotics, steroids)
- Surgical history
- Pathology report

4.5.3 Review of diagnostic images from the PACS (iSite Radiology or iSite Enterprise)

- Review of abdominal ultrasound and CEUS images (iSite)

5 STUDY EVALUATIONS AND MEASUREMENTS

The results of the pre-contrast injection and CEUS imaging will be collected for research purposes only. The results of the CEUS will not be used to direct clinical care decisions,

without confirmation of diagnosis by another medically established diagnostic product or procedure.

Qualitative analysis
Visual rating by 2 teams consisting of primary investigator and second radiologist (co-investigator). Each scan will be rated for diagnostic quality and qualitative rating of bowel perfusion. The visual rating scale used will be: 0 (absent flow), 1 (decreased flow), 2 (normal flow), 3 (increased flow).

Quantitative analysis
Qontrast (Bracco Diag Inc., 510K regulatory status granted in 2004) contrast quantification software or similar software will be used to analyze the obtained CEUS scans. For each scan, wash-in and wash-out curves will be generated to quantify the rate of wash-in, time to peak intensity, peak intensity, and area under the curve.

The CEUS scans will be interpreted by the sponsor-investigator, and a second interpretation by a "second reader" in Radiology, who is part of a group of radiologists with sufficient training and expertise to read CEUS scans.

Monitoring After Investigational Drug Administration:

The monitoring post-administration will encompass 60 minutes, during which time the subject will be observed for the occurrence of infusion reactions. Any concern by the investigative team or attending staff for severe allergic reaction or anaphylaxis will be managed as all similar reactions are managed as part of clinical care, with close observation and treatment as clinically indicated, which may include diphenhydramine, corticosteroids, fluids, oxygen and epinephrine. Post-infusion reactions will be recorded and reported following established IRB and FDA reporting guidelines.

6 STATISTICAL CONSIDERATIONS

Up to 100 evaluable patients should be adequate for assessment of clinical feasibility and optimization of protocol. Qualitative analysis will be used as detailed above. Quantitative analysis will be performed by drawing a region of interest in bowel segments and compared to control subjects (those with suspected or at risk of necrotizing enterocolitis but turns out to be normal on imaging and clinical evaluation) using non-parametric Wilcoxon testing.

6.1 Primary Endpoint

The primary objective of this study is to evaluate whether CEUS technique can be utilized to detect early alterations in bowel perfusion in NEC. The differences in bowel perfusion between normal subjects (those with suspected or at risk of necrotizing enterocolitis but turn out to be normal on imaging and clinical evaluation) versus NEC patients will be assessed qualitatively and quantitatively using the CEUS technique, as detailed above.

6.2 Secondary Endpoints

Diagnostic quality of the CEUS exams will be evaluated with the scoring system of non-diagnostic (1), moderate artifacts degrading diagnostic quality (2), mild artifacts without degradation of diagnostic quality (3), and diagnostic (4). As a gold standard for NEC diagnosis, surgical findings (in those subjects undergoing surgery as part of clinical care) or Bell's staging criteria for NEC in combination with radiographic and clinical signs (for subjects not undergoing surgery) will be used.

6.3 Statistical Methods

6.3.1 Baseline Data

Baseline and demographic characteristics will be summarized by standard descriptive summaries.

6.3.2 Safety Analysis

All subjects entered into the study, who receive at least one dose of investigational drug per CEUS scan will be included in the safety analysis. The frequencies of AEs by type, severity, and temporal relationship to the CEUS scan will be summarized. SAEs (if any) will be described in detail.

Adverse events will be recorded at 1) 60 minutes post-scanning when the monitoring period is completed, and 2) through 48 hours post-scanning, with documentation at 48 hours post-scanning if no adverse event presents until this point.

6.4 Sample Size and Power

As noted in the main text, no previous study has used contrast ultrasound to examine bowel wall perfusion. For power analyses, estimates of group differences were obtained from a study that provided mean perfusion values for a control group using Doppler ultrasound. Although contrast ultrasound and Doppler ultrasound rely on different methods to quantify perfusion (contrast quantifies by absolute intensity and Doppler quantifies by counting the color signal) the methods are similar enough that Doppler findings can inform the present study (and of note, given that contrast ultrasound is more sensitive, power analysis based on the Doppler findings are considered to be conservative). Given the reported bowel perfusion mean and standard deviation value for controls of 3.78 (SD = 1.1) [1], assuming a moderate effect (Cohen's D = 0.71), a sample size of 40 per group would provide power = 0.88 (alpha = 0.05). Since each subject has a clinical care US (control group) followed by a research CEUS (experimental group), each subject will serve as its own control. Since the CEUS method has not been applied to NEC imaging before, an interim statistical analysis may be considered to review image quality and comparisons between the results of each subject's CEUS scans and any available clinical US exams, to better inform the number of subjects required for statistical significance.

7 STUDY DRUG

7.1 Description

Lumason™ is currently FDA approved for use in the pediatric population for echocardiography and evaluation of focal hepatic lesions, and very recently (Jan 2017) approved for use in children for the evaluation of the urinary tract in pediatric patients with known or suspected vesicoureteral reflux. Previously, the presence of cardiac shunts was a contraindication for its use, but this was recently cancelled by the FDA as of December 2016. However, for this clinical study, the FDA has recommended that subjects with hemodynamically significant PDA and right-to-left cardiac shunts be excluded from study participation. Ultrasound contrast agents have been approved for use in Europe for almost two decades. In the case of SonoVue (now called Lumason™), a second-generation lipid/sulfur hexafluoride US contrast agent (Bracco, Milan, Italy), the European Union approved its intravenous use in adults in 2001. The FDA in the United States just recently approved the use of Lumason™ for evaluation of focal hepatic lesions in the pediatric population in 2016. Through decades of clinical utilization of ultrasound contrast agents, there are established recommended doses for the intravenous route of administration. Recommended intravenous dose for Lumason™ is weight-based, 0.03mL/kg as an intravenous injection. As an example, for the intended study population it may be roughly estimated that the maximum weight of an infant 1 year of age may be up to approximately 10 kg, for a maximum dose of 0.30 mL. Two injections per exam will be performed.

7.2 Dosing

Through decades of clinical utilization of ultrasound contrast agents, there are established recommended doses for the intravenous route of administration. Recommended intravenous dose for Lumason™ is weight-based, 0.03mL/kg as an intravenous injection, up to a maximum of 2.4mL per injection. As an example, for the intended study population it may be roughly estimated that the maximum weight of an infant 1 year of age may be up to approximately 10 kg, for a maximum dose of 0.30 mL. Two injections per exam will be performed. Since investigational findings are subject to pre-analytic variability, performing two contrast-agent injections facilitates validation of findings if reproducibility is found. No increased risk was found at initial experience at Johns Hopkins Hospital, where 5 of 10 patients required double injection of contrast-agent. Clinical cases at Johns Hopkins Hospital and The Children's Hospital of Philadelphia have required double injection due to variable factors such as microbubble trapping within intravenous tubing. Adverse effects are not dose-dependent, thus risk is not increased by modifying the timing of interventions or increasing the number of contrast-agent injections.

Even though the initial dosing of investigational drug for this study is 0.03 mL/kg, it is possible that the optimal dose for CEUS imaging of hypoxic ischemic injury may be less than 0.03 mL/kg. Since there would be no apparent safety concern regarding the administration of a lower dose of the investigational drug, the study team would proceed with a lower dose administration, if initial imaging studies suggest that a dose less than

0.03 mL/kg may provide more optimal imaging results. Therefore, the study team proposes that a dose range of 0.01-0.03 mL/kg be considered for dose optimization of the initial subjects, as indicated. Based on the published report of safe infusion of the investigational drug in four premature infants from 26.9 to 29.5 weeks gestational age [15] as well as 3 additional premature subjects from unpublished data of the sponsor, this dose range seems appropriate. For each subject, the dose per injection, as well as the total dose delivered, will be recorded in the study file for each administration of study drug. The expectation is that the intravenous injection of this ultrasound contrast agent will permit noninvasive, non-ionizing delineation of physiology and pathophysiology with higher resolution and accuracy than conventional ultrasound techniques. Since preliminary studies at Hopkins showed that half of neonates required two injections of contrast agent to achieve evaluable CEUS images, two injections will be performed per CEUS scan in all subjects to ensure adequate image quality and reproducibility.

7.3 Investigational Drug Handling and Accountability

Bulk supplies of drug will be directly shipped to IDS, by drug supplier. On an as-needed basis, only 1 box of either 5 or 20 vials, will be ordered from IDS by the principal investigator. The 1 box supply will be located in a storage unit, inside the Ultrasound Suite, with access limited to study personnel and dedicated Ultrasound personnel.

The investigational imaging drug, Lumason™, will be maintained as a separate supply from the Radiology Departments and central supply's Lumason™ that is used for clinical care purposes. Specifically, the investigational drug Lumason™ for this IND will be physically segregated from the clinical use Lumason™, and stored in a location that identifies the study drug as "Investigational Drug Lumason™, For IND Research Only" so that all Radiology personnel know to limit the use of the investigational drug Lumason™ for study in this particular IND research exclusively.

The investigational drug Lumason™ will be labeled according to FDA regulations, and identified as for IND research use only. The study team will use the investigational drug exclusively for the purposes of this IND study, and will not distribute or administer the investigational drug to persons not participating in the clinical study.

The study team will ensure that each Lumason™ vial used in the IND clinical trial is entered into an investigational drug log that contains at least the following details of each vial: Lot #number and expiration date. For each subject, the Lumason™ lot #number, expiration date, # of vials used, disposition of unused Lumason™, and discard procedure, will be recorded as part of the study record. Expired lots of Lumason™ will not be used in the clinical trial, and will be discarded.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study. Subjects enrolled to this clinical trial are anticipated to receive the study drug and have CEUS imaging conducted in the ICU setting. In this case, the study drug and US imaging unit will be brought to the ICU for study drug administration and imaging. CEUS has a duration of approximately 20 minutes. The 60 minute monitoring post-CEUS will be conducted by the study team members. The study team, who are composed of personnel trained in recognizing signs of infusion reaction (rash, allergic reactions, anaphylaxis) will conduct the 60 minute monitoring period and record any untoward reaction that may be related to the infusion of the contrast drug. Adverse events will be recorded at 1) 60 minutes post-scanning when the monitoring period is completed, and 2) through 48 hours post-scanning, with documentation at 48 hours post-scanning if no adverse event presents until this point. All adverse events suspected of being related to study drug infusion will be reported to the regulatory authorities.

In addition to assessing each patient after dosing, after 10 subjects, a complete safety analysis to determine the safety of continuing the study will be performed.

8.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

AEs will be recorded and graded per the International Neonatal Consortium (INC) Neonatal AE Terminology.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or 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.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event. Adverse events will be recorded at 1) 60 minutes post-scanning when the monitoring period is completed, and 2) through 48 hours post-scanning, with documentation at 48 hours post-scanning if no adverse event presents until this point.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening event (at risk of death at the time of the event), requires inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug 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. A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	**Initial Notification (Phone, Email, Fax)**	**Written Report**
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Notifications of SAEs/IND Safety Reports to the FDA

Unexpected fatal or life-threatening adverse events that are related to the study drug, will be reported to FDA as soon as possible but no later than 7 calendar days following the sponsor's initial receipt of the information.

Unexpected serious adverse events that are related to the study drug but not fatal or life-threatening, will be reported to FDA as soon as possible but no later than within 15 calendar days following the sponsor's initial receipt of the information.

Follow-up reporting: Any relevant additional information obtained by the sponsor that pertains to a previously submitted IND safety report will be submitted as a Follow-up IND Safety Report. Such report will be submitted as soon as the information is available, but no later than 15 calendar days after the sponsor receives the information.

All other adverse events, will be reported to the FDA at or by the time of the Annual Report.

8.7 Medical Emergencies

Any medical emergencies that develop following injection of the investigational drug will be managed according to clinical care. See protocol Section 4.4 for a more complete description of clinical care management for serious adverse events following infusion of the investigational drug.

8.8 Study Stopping Rules

The study will be stopped for image futility, if non-diagnostic imaging is obtained in the first 3 subjects.

There are some circumstances which potentially may arise, requiring temporary study stop. The FDA requires that the study be stopped for all patients (to allow for review of the protocol and procedures based on study related events) after one episode of anaphylaxis or death or other serious adverse event, regardless of relation to study drug. Therefore, anaphylaxis or death or another SAE will prompt a temporary stop to formally discuss the event with the FDA. As such an event (if deemed unrelated) may not meet the prompt reporting criteria for the IRB, the IRB will be notified as applicable (in accordance with CHOP IRB SOP 408). The study will proceed only with documented concurrence of the FDA (and the IRB, as applicable).

The occurrence of two non-fatal SAEs directly related to use of the study drug or one death directly attributed to use of the study drug will stop the study.

9 STUDY ADMINISTRATION

9.1 Data Collection and Management

Confidentiality: All subjects will be assigned a number unrelated to their medical record number and this will be kept in a master list. All of the data collected will be recorded using the respective “research number” to maintain anonymity. Only the master list will contain patient identifiers and a link to the research-specific code, and the data collection sheet will not contain patient identifiers.

Security: All files (master list and data collection sheet) will be password protected and will be stored on a password protected computer at CHOP on the secure storage network on the secure Hospital server. A password protected excel spreadsheets will be used for data collection. We will also store data in REDCap, a secure online tool for data collection and storage. The paper scoresheets will be identifiable only by the coded # of each subject and will be stored in a binder in a locked cabinet located in the study coordinator's office. The only way we will use to transfer information between co-investigators will be [send secure] emails using the study members @email.chop.edu account. These e-mails will be only accessed from CHOP network computers and will be erased after the download of the password-protected excel sheet. All computers will meet CHOP IT Policy A-3-6: Acceptable Use of Technology Resources.

Anonymization, de-identification, or destruction: After the study is finalized (results published in a scientific journal), the master list containing the reference to PHI will be archived in accordance with FDA and CHOP requirements. De-identified scoresheets and data gathered from the study will be archived in a password-protected folder on the primary computer of the P.I. These data will be destroyed only after a period of time compliant with federal and institutional guidelines.

9.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Data will be safeguarded by data entry into REDCap, a secure online data entry tool. Exported datasets will be stored on secure CHOP network drives. Patient names will be removed from images for use in the educational setting.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers (including others at CHOP) before sharing a limited dataset (PHI limited to dates and zip codes).

9.3 Data and Safety Monitoring Plan

The safety monitoring for this study is the primary responsibility of the sponsor-investigator. Monitoring the safety outcomes following IV administration of the investigational drug will be conducted primarily by the Principal Investigator. An independent safety monitor has been designated to oversee the safety reports, and help adjudicate attribution of adverse events, should they occur. Regular meetings to discuss the outcomes of the study, and of the safety events, will be conducted by the study team. The occurrence of adverse events, serious adverse events and unanticipated events will be reported by the study team in accordance with federal and institutional guidelines, as outlined in Section 8 of this clinical study.

Prior to study initiation, the Office of Research Compliance (ORC) will conduct a pre-trial monitoring visit, to assess trial readiness of the study staff. Once the pre-trial monitoring visit has been successfully completed, the ORC will also, at minimum, monitor the IND study on an annual basis.

9.4 Regulatory and Ethical Considerations

9.4.1 Risk Assessment

The pre-contrast scan is non-invasive and poses risk no greater than minimal. The potential risks associated with contrast-enhanced ultrasonography have been extensively studied, and the risk associated with the technique is less than that of CT or MRI contrast agents. The risk of adverse events is the lowest of all contrast agents available, with CT contrast being the highest (0.6%), followed by MR contrast (0.0088%) and ultrasound contrast (0.0086%) [10].

Several studies detail the safety profile of ultrasound contrast agents in children and have shown minor adverse events including nausea, tinnitus, lightheadedness, altered taste sensation [11-13]. Serious cardiopulmonary reactions, including fatalities, have occurred uncommonly in adults that had complex comorbidities. Risk for these reactions may be increased among patients with unstable cardiopulmonary conditions. One documented severe reaction in a child documented symptoms of generalized pruritus, nausea, hypotension with tachycardia initially then bradycardia [14]. Management in this instance consisted of oxygen, intravenous epinephrine, and fluids (0.9% normal saline) with resolution of symptoms in two hours. Treatment of both minor, mild, and severe adverse reactions post Lumason™ administration are the same as that of CT or MRI contrast agents.

In comparison to CT or MRI contrast agents, however, ultrasound contrast agents have proven to be much safer in children with only one serious adverse event over decades of its use to date (contrasting to approximately 15-20 adverse events per 2000 children if CT contrast agent were to be used). No serious adverse event has been reported in a neonate since its clinical use in this population. Animal studies on its toxicity profile also validate no fetal toxicity and the ultrasound contrast agent belongs to category B.

In the case of SonoVue (now called Lumason™), a second-generation lipid/sulfur hexafluoride US contrast agent (Bracco, Milan, Italy), the European Union approved its

intravenous use in adults in 2001. The FDA in the United States just recently approved the use of Lumason™ for evaluation of focal hepatic lesions and vesicoureteral reflux in pediatric population in 2016. For the remainder of clinical applications, the ultrasound contrast agents are being used off-label in both Europe and the United States.

Risks of the administration of the study drug are considered a minor increase above minimal risk, without the prospect of direct benefit. Adverse effects are not dose-dependent, thus risk is not increased by modifying the timing of interventions or increasing the number of contrast-agent injections.

Another risk of the study includes the insertion of a peripheral IV line. This is a no greater than minimal risk procedure, with the main risks of discomfort, bruising, and infection which are generally self-limited. There is a no greater than minimal risk of breach of confidentiality, which is minimized by having all of study personnel undergo HIPAA training.

Interference of CEUS with MRI is not expected since CEUS contrast-agent Lumason clears within minutes after injection. Elimination of Lumason (Sulfur Hexafluoride Lipid-Type A Microspheres) occurs via the lungs in the first minutes following contrast-agent injection (please see Package Insert's section 12.3 Pharmacokinetics for more detail, attached in Application's section 12.02 (3.0)). Additionally, Misun Hwang, the sponsor-investigator, has also previously performed brain CEUS before MRI in neonatal patients without adverse events.

Steps Taken to Minimize Risks

Parents and/or legal guardians of participants will be asked about contraindications to contrast enhanced ultrasonography examinations, as listed in the exclusion criteria. In order to appropriately treat potential rare adverse events, patients will be monitored by the study team for 60 minutes following contrast administration.

Vital signs will be recorded from the medical chart and in-place monitoring and documented at 1) 30 minutes post-scanning, and 2) 60 minutes post-scanning. During monitoring, subjects will be assessed for rash, allergic reactions, anaphylaxis, and abrupt deviations from the subject's baseline hemodynamic parameters trend not related to medical intervention.

Adverse events will be recorded at 1) 60 minutes post-scanning when the monitoring period is completed, and 2) through 48 hours post-scanning, with documentation at 48 hours post-scanning if no adverse event presents until this point.

9.4.2 Potential Benefits of Trial Participation

The patient will not receive a direct benefit as a result of participating in the study. Indirect benefits may include improvement in current diagnostic algorithm for detection

and monitoring of NEC and downstream reduction of high mortality and morbidity associated with NEC.

9.4.3 Risk-Benefit Assessment

The benefit to society outweighs the risks of this study.

9.5 Recruitment Strategy

Potential subjects will be identified by reviewing the subjects' medical charts to confirm eligibility by: 1) Neonatologists or 2) the radiologists to identify subjects with suspected or diagnosed necrotizing enterocolitis. Potential participation will be discussed by the PI with the referring neonatologist (co-investigator). Parents/legal guardians may be approached over phone/e-mail by a member of the study team to determine if they are interested in receiving more information about the study. Participation will be discussed with the parents/guardian by the neonatologist and/or radiologist. Parental/guardian permission (informed consent) will be obtained.

Additionally, the CHOP Research Discovery Finder, e-mails, and a tearpad flyer will be used in the recruitment strategy

9.6 Informed Consent and HIPAA Authorization

Approved members of the study team will obtain written or electronic (e-consent) parental/guardian informed consent prior to the proposed study in a private setting. The electronic consent form will be administered in REDCap. The investigators will assure that parents/guardian comprehend the nature of the study, the study procedures and the risks and benefits of participation, steps that will be taken to avoid coercion and documentation of consent. A combined HIPAA consent-authorization document will be used.

9.7 Payment to Subjects/Families

Families will be offered a gift card of 50 USD value for participation in the study.

10 PUBLICATION

The investigative team plans to publish the data collected in a scientific journal. Data may also be presented as abstract, podium presentation, or poster presentations at scientific meetings and conventions. No patient identifying information will be used in publications.

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