PROTOCOL TITLE: A Multicenter Trial of Contrast-Enhanced Ultrasound in the Evaluation of Abdominal Solid Organ Injuries in Pediatric Trauma Patients

NCT NUMBER: NCT03147690

DOCUMENT DATE: 10/16/2019

PROTOCOL

PROTOCOL TITLE	A Multicenter Trial of Contrast-Enhanced Ultrasound in the Evaluation of Abdominal Solid Organ Injuries in Pediatric Trauma Patients
PROTOCOL NUMBER	P00025242
STUDY DESIGN (PHASE)	Prospective non-randomized cohort
PROTOCOL VERSION	Version 4
IND NUMBER	N/A
INVESTIGATIONAL PRODUCT	Lumason
INDICATION	To enhance visualization of abdominal solid organ injuries in children
SPONSOR	David P. Mooney, MD, MPH 300 Longwood Avenue Boston, MA 02115 617 355-0535
PRINCIPAL INVESTIGATOR	David P. Mooney, MD, MPH

Good Clinical Practices

This study was conducted under Good Clinical Practices, in accordance with the Declaration of Helsinki, in compliance with the International Conference on Harmonisation (ICH) guidelines.

Confidentiality Statement

This document contains confidential information of the Sponsor. This information is to be disclosed only to the recipient study staff and the Institutional Review Board or Institutional Ethics Committee reviewing this protocol. This information can be used for no other purpose than evaluation or conduct of this study without prior written consent from the Sponsor.

SYNOPSIS

Name of Sponsor	David P. Mooney, MD, MPH
Coordinating Center	Boston Children's Hospital
Investigational Product	Lumason
Indication (phase)	To improve ultrasound visualization of pediatric abdominal solid organ injuries
Title of Study	A Multicenter Trial of Contrast-Enhanced Ultrasound in the Evaluation of Abdominal Solid Organ Injuries in Pediatric Trauma Patients
Protocol Date	October 16, 2019
Protocol Version	Version 4
OBJECTIVES	
● To improve the ultrasonic visualization of abdominal solid organ injuries in children	
METHODOLOGY	
Study Design	Prospective non-randomized cohort
Treatments	Lumason intravenous contrast
Treatment Duration	50 to 90 minutes
Study Drug and Formulation	Lumason is provided by the manufacturer (Bracco Diagnostics) in single use 5 mL vials.
Dose and Route of Administration	Dosing and Administration information can be found in section 3.4.
Concomitant and Excluded Therapy	There are no concomitant or excluded therapies.
SUBJECT POPULATION	
Number Planned	132 subjects are to complete this study: all in the treatment arm. Each patient will have at least one injured organ and their other organs will serve as controls compared to the other study subjects. Any subjects who do no complete the study will not count toward this number.
Major Inclusion Criteria	● Age 8 to 17 years inclusive ● Abdominal solid organ injury visualized on computerized tomography ● Hemodynamically stable

Name of Sponsor	David P. Mooney, MD, MPH
Coordinating Center	Boston Children's Hospital
Investigational Product	Lumason
ASSESSMENTS	
Efficacy	Lumason will be considered efficacious if ≥90% of the abdominal solid organ injuries identified on computerized tomography are able to be visualized on contrast enhanced ultrasound.
Pharmacokinetics and Pharmacodynamics	No pharmacokinetics or pharmacodynamics will be performed.
Safety	Study subjects will be monitored for the development of any adverse reactions for 30 minutes following the final infusion of Lumason. No laboratory or other imaging studies are planned.
STATISTICAL METHODS AND ANALYSIS	
Efficacy	Lumason will be considered efficacious if ≥90% of the abdominal solid organ injuries identified on computerized tomography are able to be visualized on contrast enhanced ultrasound.
Pharmacokinetics and Pharmacodynamics	No pharmacokinetics or pharmacodynamics will be performed.
Safety	Study personnel will monitor subjects for 30 minutes following the last infusion of Lumason. Patients will be maintained on a cardiopulmonary monitor through this time period. All adverse events will be graded according to the criteria specified by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (NIH, June 14, 2010).

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ABBREVIATIONS AND DEFINITIONS

Abbreviation	Term
AE	Adverse event
BCH	Boston Children's Hospital
BP	Blood pressure
CEUS	Contrast Enhanced Ultrasound
CFR	*Code of Federal Regulations*
CNS	Central Nervous System
CRA	Clinical Research Associate
CRF	Case report form
CT	Computerized Tomography
CTC	Common Toxicity Criteria
EKG	Electrocardiography
FAST	Focused Abdominal Sonography for Trauma
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous(ly)
Kg	Kilogram
MA	Massachusetts
Min	Minute
mL	milliliter
NPV	Negative Predictive Value
PPV	Positive Predictive Value
RR	Respiratory Rate
SAE	Serious adverse event

1. BACKGROUND

Injury is the most common cause of morbidity and mortality among children. Children are more susceptible to multisystem trauma than adults given their anatomic and physiologic characteristics. Computed tomography (CT) with intravenous (IV) contrast is the gold standard test for evaluating the abdomen in children with suspected injury. CT is used liberally since abdominal injuries are often suspected and difficult to rule out with history and physical examination alone. There is a 60-75% rate of normal abdominal CTs for trauma in the pediatric population reported in the literature (1). Literature suggests that children may have an increased risk of cancer related to the radiation exposure required to perform an abdominal CT. This risk may be as high as 1 in 300 in children under 5 years of age (2). In addition, there is a 1 in 200 chance of a reaction to the IV contrast agent used (3).

To date, CT is the only reliable and accurate diagnostic modality to evaluate for intra-abdominal injuries in children. Although FAST (Focused Abdominal Sonography for Trauma) exams are used frequently in adults, this test has not been demonstrated to have sufficient sensitivity to detect abdominal organ injury in the pediatric patient (4, 5). If contrast enhanced ultrasound (CEUS) improves the sensitivity and specificity of sonography in diagnosing an abdominal solid organ injury, it may become a valuable diagnostic tool, supplanting CT.

In Europe, CEUS using SonoVue, the European brand-name for Lumason, has been used clinically for more than a decade, reported mainly in adults. Valentino, et al. published results of a prospective study performed in Italy comparing the sensitivity and specificity of ultrasound and CEUS to abdominal/pelvis CT with IV contrast. A total of 27 patients were prospectively studied and they found CEUS to have a 92.9% sensitivity, 100% specificity, 100% positive predictive value (PPV) and 93.8% negative predictive value (NPV) compared to CT (6). Valentino published a subsequent study of 133 adult trauma patients with blunt abdominal solid organ injury on CT who underwent CEUS and found 96.4% sensitivity, 98% specificity, 98.8% PPV and 94% NPV (7) compared to CT.

Clinical experience with the use of CEUS has been limited in the United States. An as yet unpublished pilot study conducted by Zalieckas et al enrolled 18 children with 21 abdominal solid organ injuries identified on CT. Children underwent greyscale and Doppler ultrasound studies, then CEUS using the contrast agent Optison. They found that greyscale/Doppler ultrasound had a 45.2% sensitivity, 96.4% specificity, a 79.2% PPV, and an 85.3% NPV compared to CT. CEUS had an 85.7% sensitivity, 98.6% specificity, 94.7% PPV and 95.8% NPV. Two of the 3 missed injuries were in one 100 kilogram pediatric patient, suggesting that further trials be limited to patients otherwise considered to be good candidates for abdominal sonography.

Optison and Lumason are both contrast agents used to perform CEUS. Lumason is now FDA-approved for use in the characterization of focal liver lesions in adult and pediatric patients. It has been studied and used used in Europe, under the brand name SonoVue, to identify abdominal solid organ injuries. Because Lumason is FDA-approved for a closely related indication in a pediatric population, we have chosen to use Lumason for this study.

1.1 NAME AND DESCRIPTION OF INVESTIGATIONAL PRODUCT

Lumason (sulfur hexafluoride lipid-type A microspheres) for injectable suspension is used to prepare the ultrasound contrast agent. The single use kit contains the following three items:

1) one clear glass 10 mL vial containing 25 mg of lyophilized powder lipid-type A, 60.7 mg of sulfur hexafluoride gas and capped with a blue flip-cap

2) one prefilled syringe containing 5 mL Sodium Chloride 0.9% Injection, USP (Diluent)

3) one Mini-Spike

Each vial is formulated as a 25 mg sterile, pyrogen-free lyophilized powder containing 24.56 mg of polyethylene glycol 4000, 0.19 mg of distearoylphosphatidyl-choline (DSPC), 0.19 mg of dipalmitoylphosphatidylglycerol sodium (DPPG-Na) and 0.04 mg of palmitic acid. The headspace of each vial contains 6.07 mg/mL (± 2 %) sulfur hexafluoride, SF6, or 60.7 mg per vial.

Each prefilled syringe with 5 mL of diluent 0.9% Sodium Chloride Injection is sterile, nonpyrogenic, preservative free containing 9 mg sodium chloride per mL.

Upon reconstitution with 5mL diluent, Lumason is a milky white, homogeneous suspension containing sulfur hexafluoride lipid-type A microspheres. The suspension is isotonic and has a pH of 4.5 to 7.5; it is only for intravenous administration.

The sulfur hexafluoride lipid microspheres are composed of SF6 gas in the core surrounded by an outer shell monolayer of phospholipids consisting DSPC and DPPG-Na with palmitic acid as a stabilizer. Sulfur hexafluoride has a molecular weight of 145.9. Each milliliter of reconstituted Lumason suspension contains 1.5 to 5.6 x108 microspheres, 68 mcg SF6 (12 mcL), 0.038 mg DSPC, 0.038 mg DPPG-Na, 4.91 mg polyethylene glycol 4000 and 0.008 mg palmitic acid. The sulfur hexafluoride associated with the microspheres suspension is 45 mcg/mL. Fifteen to twenty three percent of the total lipids in the suspension are associated with the microspheres. The sulfur hexafluoride lipid microsphere characteristics are listed in the following table:

Microsphere Characteristics	
Mean diameter range	1.5 – 2.5 μm
Percent of microspheres	≤ 10 μm ≥ 99%
Upper size limit	100.0% ≤ 20 μm

1.2 RESULTS OF NONCLINICAL STUDIES

In a study reported by Tang et al using a dog model of induced liver and spleen injuries, CEUS using SonoVue showed a high level of concordance with CT (8).

1.3 RESULTS OF CLINICAL STUDIES

Preliminary Studies

Several European trials have investigated the use of CEUS to identify abdominal solid organ injuries seen on CT in children. These studies have used SonoVue, which is the European brand-name of Lumason. Sessa reported a retrospective series of 254 adults and children aged from 7

to 82 who underwent ultrasound, CEUS using SonoVue and CT to evaluate their abdomen after injury. 84 abdominal injuries were noted on CT, 50 of which were seen on ultrasound. The use of CEUS allowed the visualization of 81 of 84 injuries with no adverse events related to contrast administration (9).

Valentino et al reported a prospective study of the sensitivity and specificity of ultrasound and CEUS (CEUS) versus CT. 27 children were enrolled, with a mean age of 9 years. SonoVue was injected as a bolus followed by a normal saline bolus. 12 patients had 14 abdominal solid organ injuries seen on CT. 13 injuries were identified on CEUS (sensitivity 92.9%, specificity 100%, PPV 100%, NPV 93.8%). No adverse events were reported from contrast administration (10).

Menichini et al reported a retrospective series of 73 children who presented to a single emergency department with concern for an abdominal injury. 67 of the 73 children had an abdominal solid organ injury, only 26 of which were seen on standard ultrasound. Using CEUS with two 1.2 mL doses of SonoVue all 67 solid organ injuries seen on CT were able to be seen for a sensitivity, specificity, PPV and NPV of 100%. No adverse events were noted related to the SonoVue administration (11).

Rosado and Riccabona reviewed the off-label use of intravenous ultrasound contrast in 502 children distributed over 19 published studies (12). The most common agent used was SonoVue, in 447 children, and the second most common reason was to identify abdominal solid organ injuries. Ten patients (2%) were reported to have an adverse event, with 9 of the events being minor and transient. One patient (0.2%) suffered an anaphylactic reaction to the contrast agent and this resolved with proper treatment (12). This adverse event profile compared favorably to that seen with CT contrast (13).

2. STUDY OBJECTIVES

The primary goal of the study is to evaluate whether the use of ultrasound contrast can improve the sensitivity and specificity of ultrasound in diagnosing abdominal solid organ injuries in pediatric patients, thereby making it a valuable diagnostic tool.

Since all patients entered on the study will have CT positive results, the primary endpoint is the proportion of patients for whom all organs identified by CT with injuries are also identified by CEUS, regardless of injury grade. This will provide an estimate of the sensitivity assuming CT as the gold standard.

The secondary objectives are to determine: 1) for each organ, the proportion of patients for whom the organ is identified by CT and CEUS as injured, 2) proportion of injuries identified by CEUS which are within 1 grade of the injury identified by CT, 3) the proportion of patients where the absence or presence of peritoneal fluid identified by CT is also identified by CEUS, and 4) the comparison between 'real-time' and centralized interpretation of CEUS images.

3. INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN

To address the primary objective, we propose a multi-center study with participation from 7-10 sites to enroll 132 subjects who complete the study aged 8-17 over 2-3 years. Dr. David Mooney is the Sponsor Investigator, with Boston Children's Hospital acting as the coordinating center. Prior to enrollment, each subject will have already undergone an abdominal CT that identified at least one solid organ abdominal injury. As part of the study, subjects will undergo a greyscale/Doppler ultrasound and CEUS within 48 hours of injury. The study will measure the number of organ injuries identified, and the severity of organ injury. The ultrasound studies will be performed by a radiologist who is blinded to the results of the CT. The findings on greyscale/Doppler ultrasound and CEUS will be compared to the CT, using the CT findings as the standard.

3.1.1 Study Endpoints

- Primary endpoint is the proportion of patients for whom all organs identified by CT with injuries are also identified by CEUS, regardless of injury grade.
- Secondary endpoints:
    - For each organ, the proportion of patients for whom the organ is identified by CT and CEUS as injured.

- The proportion of injuries identified by CEUS which are within 1 grade of the injury identified by CT.
- The proportion of patients where the absence or presence of peritoneal fluid identified by CT is also identified by CEUS.
- The proportion of patients with agreement between 'real-time' and centralized interpretation of CEUS images.

3.2 DISCUSSION OF STUDY DESIGN, INCLUDING CHOICE OF CONTROL GROUPS

Children who have an abdominal solid organ injury visualized on abdominal computerized tomography form the study group. Computerized tomography is the current gold standard for the diagnosis of these injuries in children. The purpose for choosing children who have already undergone abdominal computerized tomography is to be able to compare the new imaging technique: contrast enhanced ultrasound, to the gold standard. Children from age 8 through 17 years have been chosen to assure that they are able to assent to the study. At least 1 of the 5 abdominal solid organs (liver, spleen, pancreas, left kidney, right kidney) will be injured per patient and each subject will serve as their own control. CTs will be interpreted by radiologists at the participating institution. Sonographers will be blinded to the results of the computerized tomography.

This study will take place at 7-10 participating institutions. Based on prior data, approximately 30 potentially eligible patients are expected to present at each institution per year. We estimate enrollment will take 2-3 years to enroll 132 subjects who complete the study.

3.3 SELECTION OF STUDY POPULATION

Subjects who fulfill the following eligibility criteria will be recruited regardless of their race or gender. Prior to receiving a CT scan, all menarchal females will have a urine pregnancy test as per clinical care. The results of this test can be found in the medical record. If the pregnancy test is positive, the subject will not receive a CT scan and will therefore not be eligible for the study. No pregnancy tests will be administered for research purposes.

3.3.1 Inclusion Criteria

To be eligible to participate in this trial, subjects must meet all of the following criteria:

All study participants must be:

- Hemodynamically stable, as determined by the trauma team
- Age 8 through 17 years
- Interpretable CT of the abdomen and pelvis that demonstrates at least one abdominal solid organ injury among the liver, spleen, pancreas, and kidneys
- Plan for observation or admission to the hospital
- Candidate for abdominal ultrasound based on body habitus
- Have a Glasgow Coma Score of 15
- Able to complete the study procedures within 48 hours of injury

3.3.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

- Known cardiac abnormality
- Pulmonary hypertension
- Known sensitivity to sulfur hexafluoride, polyethylene glycol 4000, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylglycerol sodium (DPPG-Na), or palmitic acid
- Unable to roll over
- Unable to assent
- Pregnant
- Lactating
- CT images not available for transmission to central image repository

3.3.3 Removal of Subjects from Therapy or Assessment

Stopping Point

The study will be suspended immediately for any two Serious Adverse Events and will not resume until an investigation by the DSMB has concluded that the study should resume.

3.3.3.1 Subject Withdrawal or Early Termination

Subjects could choose to discontinue from the study at any time, for any reason, specified or unspecified, and without prejudice. Subjects could be discontinued from the study for any of the following reasons:

- At the subject's or their parent/guardian's request
- At the discretion of the site Investigator, if deemed appropriate, for any reason, such as the subject's medical status worsening.

3.3.3.2 Planned Follow-up for Discontinued Subjects

The study has a single phase which lasts 50-90 minutes. We do not anticipate any discontinued subjects.

3.4 STUDY DRUG

3.4.1 Identity of Investigational Product

Lumason (sulfur hexafluoride lipid-type A microspheres) is the investigational product.

3.4.2 Packaging and Labeling

Lumason (sulfur hexafluoride lipid-type A microspheres) is available in a carton of five 5 mL single use vials. (NDC 0270-7099-16)

DOSAGE AND ADMINISTRATION

The recommended dose of Lumason for children is 0.03 mL/kg up to a maximum dose of 2.4mL injected into a peripheral intravenous catheter. This will be repeated once to ensure all organs are visualized. See individualization of dose below.

1. Follow the Lumason injection with a flush of 5 ml of 0.9% Sodium Chloride Injection, USP.

2. The maximum total dose for children as listed in the package insert should not exceed 2.4 mL in any 10-minute period. For this study, over any 10-minute period we intend to administer the approved dose of 0.03 mL/kg up to a maximum of 2.4 mL.

3. The maximum total dose for children as listed in the package insert should not exceed 4.8 mL in any one patient during the full time on study. In this study, we intend to administer a

total dose in any one patient during the full time on study of 0.06 mL/kg with a maximal dose of 4.8 mL.

3.4.3 Dosage Administered

Licensed study personnel will administer Lumason 0.03 mL/kg in two separate injections to a maximum of 2.4 mL per injection:
Injection 1: Image right side: right kidney and then liver (transverse and sagittal sweeps) and pancreas (transverse sweeps).
The second injection will be administered a minimum of 10 minutes after the first injection.
Injection 2: Image left side: left kidney and then spleen (transverse and sagittal sweeps) and pancreas (transverse sweeps).

The maximum dose will not exceed 0.03 mL/kg up to a maximum of 2.4 mL in any 10 minute period. The total allowable dose for any one patient is 0.06mL/kg up to a maximum of 4.8mL.

Following each Lumason contrast push injection, a flush of 5 mL of 0.9% sodium chloride will be administered intravenously.

3.4.4 Selection of Doses in the Study

The doses to be used have been chosen because they have been approved by the FDA for the visualization of focal liver lesions in children. The liver is one of the most common organs injured in trauma and it is expected that an ultrasound contrast dose that results in good visualization of the liver will also result in good visualization of the other abdominal organs. In addition, the selected dose, which may be repeated twice, is in line with the dose used in European studies which safely performed CEUS for abdominal solid injuries in children using SonoVue, the European brand-name for Lumason.

3.4.5 Investigational Product Accountability

The Sponsor will be responsible for Drug Accountability requirements for this trial. Lumason is an FDA approved agent available for sale for patient use in the United States. It will be purchased directly from the market, subject to the same manufacturing safety standards as other FDA approved agents on the market.

The agent will be stored in the research pharmacy equivalent at each institution and will be provided to study personnel once confirmation of consent has been obtained, per each participating hospital's research pharmaceuticals protocol. A drug accountability log will be

maintained by each sites' appropriately designated research pharmacy, including the study subject number alongside the product number of the vial used for that subject.

One sealed vial of agent will be used for each subject. The contents will be drawn up in sterile fashion by licensed study personnel and will be administered into an existing peripheral intravenous line. The dose used will vary by subject body weight as previously stated. At the completion of each subject's CEUS, all vials, including any residual agent left over, will be returned to the Research Pharmacy equivalent where it will be handled according to proper drug accountability procedures.

3.4.6 Prior and Concomitant Treatments

3.4.6.1 Prior and Concomitant Therapies

There are no prior or concomitant therapies in this study.

3.4.6.2 Prior and Concomitant Medications

There are no prior or concomitant medications in this study.

3.5 ASSESSMENTS

3.5.1 Schedule of Study Activities

Study Timeline

- Potential subjects who meet inclusion criteria, including having undergone a CT that demonstrates an abdominal solid organ injury, will be identified in the participating institutions' Emergency Departments. CTs may have been performed by a participating institution or a referring institution, in which case the CT has been delivered to the participating institution. These CTs will be read by the study staff at the participating institution and eligibility criteria will be assessed by reviewing medical records.

- Eligible subjects and their guardians will be approached and invited to participate. After sufficient time to allow thoughtful consideration of participation, appropriately designated study personnel will obtain consent from the guardian and assent from the

subject. Subjects may be consented and enrolled at any time during their hospital stay as long as the study procedures can be performed within 48 hours of injury.

- After evaluation and diagnostic studies are completed for the medical care of the subject, he/she will be taken to the Radiology department. If the subject is in the ICU, bedside ultrasounds may be performed instead of going to the Radiology department.
- Ultrasound personnel, blinded to the results of the CT and the medical records of the subject, will perform a greyscale/Doppler abdominal ultrasound and record the results.
- Licensed study personnel will then administer Lumason via an existing peripheral IV, and the sonographer will perform a contrast-enhanced abdominal ultrasound and record the results. Any physical findings on the subject's abdomen will also be recorded.
- The subject will be monitored for 30 minutes after the final Lumason injection as detailed in the Assessments sections.
- After completion of the ultrasounds, subsequent patient care will be as medically indicated.

3.5.2 Assessment of Efficacy

The presence or absence of an injury as well as the grade of the injury will be determined for the liver, spleen, pancreas, and each kidney in each patient. In addition, the presence or absence of peritoneal fluid as well as a rough estimate of the amount of fluid will be determined for each patient. These data will be determined by a sonographer for each of the study subjects. Inter-rater reliability will be determined by comparing the "real-time" read to a central read. These data will then be compared to the findings on computerized tomography, using the tomography as the standard. Sensitivity and specificity of the sonograms and the CEUS to identify injuries diagnosed on computerized tomography will be determined. Lumason will be considered effective if ≥90% of the injuries identified by CT are visualized by CEUS.

3.5.2.1 Screening Assessments

All subjects will have undergone a CT identifying at least one abdominal solid organ injury prior to being approached for the study. If the CT was performed at a referring institution, the CT will

be delivered to the participating institution and will be re-read at the participating institution by study staff. Study personnel will assess all inclusion and exclusion criteria for each potential subject. Only subjects who meet all criteria will be consented into the study.

Subjects may be consented and enrolled at any time during their hospital stay as long as the study procedures can be performed within 48 hours of injury.

The de-identified CTs for all enrolled subjects will be sent to a repository at Boston Children's Hospital, the coordinating center, for a central read.

3.5.2.2 Assessments during Study Procedure

Subjects will be transported to the Radiology department for the study treatment. If the subject is in the ICU, bedside ultrasounds may be performed instead of moving to the Radiology department. The sonographer will first perform a greyscale/doppler ultrasound and record findings, including presence or absence of injury for each organ, grade of injury if applicable, presence or absence of peritoneal fluid, and approximate amount of fluid if applicable. Licensed study personnel will then inject the contrast agent and the sonographer will perform a CEUS and record the same findings as listed above. A second injection will be given a minimum of ten minutes after the first injection to complete the CEUS procedure. Any physical findings on the abdomen will also be recorded.

The ultrasounds for all enrolled subjects will be sent to a repository at Boston Children's Hospital, the coordinating center, for a central read.

3.5.2.3 Follow-up Assessments

All patients will be maintained on a cardiopulmonary monitor as a standard part of the clinical care of their injury. Vital signs, including blood pressure, heart rate, respiratory rate, and oxygen saturation will be monitored prior to the injection of the contrast agent and continuing for 30 minutes after the final injection. The vital signs will be recorded at 5 minute intervals by the study personnel. The study personnel will ask the patient about any side effects he/she experiences pre-contrast injection, after each contrast bolus, and at the completion of the 30 minute observation period.

After the completion of the ultrasounds, subsequent patient care will be as otherwise indicated per the trauma team for the management of their injuries.

3.5.3 Assessment of Safety

Patients will be monitored for adverse events for 30 minutes after completion of the final contrast injection.

3.5.3.1 Adverse Events

3.5.3.1.1 Definition of Adverse Events

For purposes of this study, an adverse event will be defined as any unfavorable and unintended diagnosis, symptom, sign, syndrome or disease which either occurs during the study, having been absent at baseline, or, if present at baseline, appears to worsen.

An unexpected adverse event will be defined as any adverse event that is not listed as a risk in the current version of the Protocol or Package Insert.

An adverse reaction will be defined as any adverse event caused by the Investigational Product. A suspected adverse reaction will be defined as any adverse event for which there is a reasonable possibility that the Investigational Product caused the event.

All adverse events will be graded by the site investigator according to the criteria specified by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (NIH, June 14, 2010).

Adverse events that have been reported as possibly related to the study procedure include the following: headache, nausea, dysgeusia, injection site warmth or pain, feeling hot, chest discomfort or pain, dizziness, cardiopulmonary reactions, and hypersensitivity reactions.

3.5.3.1.2 Definition of Serious Adverse Events

An AE occurring with any study treatment regimen should be classified as “Serious” if it meets one of the following criteria:

- It results in death (ie, the AE caused or led to death).

- It is life threatening (ie, the AE placed the subject at immediate risk of death). This classification does not apply to an AE that hypothetically might cause death if it is more severe.
- Event severe enough to require airway management, ICU admission, hypotension requiring fluid or IV vasopressor administration.
- It requires or prolongs inpatient hospitalization (ie, the AE requires at least a 24-hour inpatient hospitalization or prolongs a hospitalization beyond the expected length of stay). Hospitalizations for elective medical or surgical procedures, scheduled treatments, or routine checkups are not SAEs by this criterion.
- It is disabling or incapacitating (ie, the AE results in a substantial disruption of the subject's ability to carry out normal life functions).
- It is a congenital anomaly or birth defect (ie, an adverse outcome in a child or fetus of a subject exposed the molecule or study treatment regimen before conception or during pregnancy).
- It does meet any of the above criteria, but could jeopardize the subject and might require medical or surgical intervention to prevent one of the outcomes listed above.
- Any other CTCAE event of grade 3 or greater deemed possibly or definitely related to the study drug.

3.5.3.1.3 *Documentation and Reporting of Adverse Events*

The severity of toxicities will be graded in accordance with the Common Terminology Criteria for AE (CTCAE) version 4.0.3. Grade 2 AEs deemed possibly, probably, or definitely related to study medication and all Grade 3 and higher AEs, regardless of suspected causal relationship to study drug, will be recorded as AEs in the CRFs.

All SAEs must be reported to the Sponsor or designee within 24 hours of site awareness, regardless of the relationship of the event to the study treatment regimen. Refer to the previous section for the definition of a serious adverse event. The completed SAE form will be scanned and emailed securely within 24 hours to the Sponsor at david.mooney@childrens.harvard.edu

and Leah Cheng at leah.cheng@childrens.harvard.edu. Relevant follow-up information will be submitted to the Sponsor or its designee as soon as it becomes available.

Boston Children's Hospital PI will inform the DSMB, as well as the Boston Children's Hospital IRB according to Institutional guidelines for reporting. The occurrence of 2 or more serious adverse events will result in a pause in the study while an investigation is conducted by the DSMB to determine if the study should resume.

3.6 SAFETY ISSUES RELATED TO INVESTIGATIONAL PRODUCT

Anticipated Risks

All patients will have at least one functioning intravenous catheter in place as a part of standard medical care and no new IV access will be required. We expect that there may be some mild discomfort related to the performance of the ultrasound study itself and will monitor for this. Other than the discomfort from the ultrasound probe, we do not expect any other ultrasound-related adverse events. Patients will be monitored during the study interval and for 30 minutes after final contrast injection by licensed study personnel, and only hemodynamically stable patients will be selected, making it very unlikely that there will be a hemodynamic issue during the brief time of the study.

Licensed study personnel will administer Lumason and monitor the patient during injection and for 30 minutes afterward for any adverse effects. A questionnaire will be filled out during the study to record any potential reactions to Lumason. Resuscitation equipment and trained personnel will be available during the administration of Lumason and the follow-up period.

Reported risks related to Lumason administration include:

Adverse Reactions in 6,856 patients receiving Lumason		
Adverse Reaction	Number	(%)
Any Adverse Reaction	340	5
Headache	65	1
Nausea	37	0.5
Dysgeusia	29	0.4

Injection site pain	23	0.3
Feeling hot	18	0.3
Chest discomfort	17	0.2
Chest pain	12	0.2
Dizziness	11	0.2
Injection site warmth	11	0.2

Serious cardiopulmonary reactions, including fatalities, have occurred uncommonly in adults during or shortly following administration of ultrasound contrast agents, including Lumason. These reactions typically occurred within 30 minutes of administration. Hypersensitivity reactions such as skin erythema, rash, urticaria, flushing, throat tightness, dyspnea, or anaphylactic shock have uncommonly been observed following the injection of Lumason. The exclusion criteria eliminate any subjects with a known cardiac abnormality or a known sensitivity to sulfur hexafluoride, so we do not anticipate that these reactions will occur. As stated above, resuscitation personnel and equipment will be available during the study treatment and follow-up period.

3.7 DATA QUALITY ASSURANCE

Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures. These are described in the following sections.

3.7.1 Data Collection, Monitoring, and Transfer

All clinical research data will be recorded and maintained on study specific case report forms (CRFs). These CRFs will be stored within individual subject binders in accordance with Good Clinical Practice Standards. Study materials will be kept in designated locked file cabinets and access will be restricted to authorized study staff only. Subject confidentiality will be maintained by recording subject-specific data using a unique confidential study identifier.

All study data will be entered into a REDCap database and monitored for data accuracy. Data and source documents will be maintained in compliance with ICH-GCP and local and national regulatory requirements.

The coordinating center will maintain a central repository of all de-identified CT and US scans for all subjects across all sites in order to provide the scans to the central reviewers.

3.7.2 Data and Safety Monitoring Plan

A Data and Safety Monitoring Board (DSMB) will be created with members with expertise in ultrasonography, statistics, and trauma surgery. The DSMB will meet at a minimum of twice per year. Details of the reporting procedures to the DSMB are included in the DSMB Charter. The DMSB will provide recommendations to change the study or to continue the study.

3.7.3 Study Monitoring and Auditing

This study will be monitored in accordance with the BCH Program Data Safety Monitoring Plan. Monitoring and auditing procedures as outlined in the BCH Program Data Safety Monitoring Plan will be followed to ensure that the entire study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements of Boston Children's Hospital.

Monitoring of timeliness of Adverse Event and Serious Adverse Event reporting will be done as events are reported to the coordinating center. Case report forms for each subject enrolled into the study will be monitored for completeness and quality.

3.8 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

3.8.1 Sample Size and Power Considerations

Sample size for the study was determined based on the precision of the confidence interval for the primary endpoint. This approach will be considered promising if the true proportion of subjects with all injuries identified by CT also identified by CEUS is greater than or equal to 90%. Assuming we observe 90% of subjects with all injuries identified by CT and by CEUS, a sample size of 132 patients will attain a two-sided 95% confidence interval with a width equal to 11%, with exact Clopper-Pearson lower and upper confidence limits of 83.6% and 94.5% respectively. For secondary endpoints, a sample size of 132 patients will produce 95% confidence intervals with a maximum width of 17.6%.

3.8.2 Statistical Analysis Plan

We will summarize patient demographic and clinical characteristics using descriptive statistics: means, standard deviations, medians, and ranges for continuous measures; frequencies and proportions for categorical measures. For the primary objective, we will calculate the proportion and 95% confidence interval. For the secondary objectives, we will calculate the proportion and 95% confidence interval. We will also report the sensitivity and specificity of the CEUS relative to CT in identifying individual organ abnormalities in separate models and combined models. In the combined analysis, sensitivity and specificity will be estimated using random effects model or generalized estimating equations to adjust for potential correlation between organs within an individual. Statistical analysis will be performed using standardized packages (SPSS version 19.0 from SPSS Inc./IBM, Chicago, IL or SAS version 9.4 from SAS Institute Inc., Cary, NC).)

3.8.3 Study Duration

To attain the target sample size of 132, we expect to enroll 1-2 patients/month/site across 7-10 sites for enrollment over 2-3 years. The total study duration, including analysis, will be 3-5 years.

3.8.4 Interim Analysis

An interim analysis will be conducted after the first 73 patients. At this time, the conditional power will be calculated. If the probability of obtaining a point estimate ≥ 90% for the primary endpoint is less than 10% at the interim analysis, we will consider stopping the study for futility.

3.8.5 Safety Analysis

All subjects who receive study drug will be analyzed for safety. The proportion of patients with specific adverse events will be reported as well as the worst grade observed across all adverse events. The maximum confidence interval width for these estimates is 17.6%. If the observed estimated adverse event proportion is 10%, the 95% confidence interval is (5.5,16.4%).

3.9 COMPLIANCE STATEMENT

The study will be conducted in accordance with the protocol, Good Clinical Practices, the relevant ICH guidelines, the applicable regulatory requirements, and the ethical principles that have their origins in the Declaration of Helsinki. As required by United States Food and Drug

Administration (FDA) Code of Federal Regulations (CFR) (21 CFR 56) and the Declaration of Helsinki, the study protocol, amendments, and Informed Consent form will be reviewed and approved, according to 21 CFR §50 and §56, respectively, by our center's IRB.

3.10 SUBJECT INFORMATION AND CONSENT

The study will be explained to each subject and their guardian and before enrollment in the study, an Informed Consent form will be signed by each subject's guardian and assent will be obtained. A copy of the signed consent form will be given to each guardian.

For any sites that choose to execute an IRB reliance agreement, Boston Children's Hospital Investigational Review Board, as part of the reliance agreement, will provide each site with the informed consent template with prompted areas for each institution's required language. The Sponsor will review the written Informed Consent forms for each study center and submit to BCH IRB (as well as any sites whose IRB maintains local review) for final approval. These documents will meet requirements for subject information, as outlined in FDA regulations (21 CFR 50), ICH Guideline E6, and the Declaration of Helsinki. The Sponsor requires that all subjects be given this information before study participation.

4. COMPENSATION, INSURANCE AND INDEMNITY

There will be no compensation provided for participation in this study.

5. REFERENCE LIST

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6. APPENDIX I

Study Title:	**A Multicenter Trial of Contrast-Enhanced Ultrasound in the Evaluation of Abdominal Solid Organ Injuries in Pediatric Trauma Patients**
Protocol Number:	P00025242
Protocol Version:	Version 4: 10/16/2019

I have read the protocol and agree that it contains all the details necessary for carrying out this study, and I will conduct the study as described herein.

Investigator's Signature: _____

Investigator (Printed name): _____

Date: _____

Institution: _____