CONFIDENTIAL

A Multicenter Clinical Evaluation of Safety and Efficacy of Lumason™/ as a Contrast Agent in Pediatric Echocardiography

LUMASON™

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Table of Contents

List of Tables

List of Figures

List of Appendices

Abbreviations and Definition of Terms

AE	Adverse Event
ALT	Alanine aminotransferase
AHA	American Heart Association
APTT	Activated Partial Prothrombin Time
ASE	American Society of Echocardiography
ASD	Atrial Septal Defect
AST	Aspartate aminotransferase
BMI	Body Mass Index
C°	Centigrade
CEUS	Contrast Enhanced Ultrasound
CFR	Code of Federal Regulation
CI	Confidence Interval
CMR	Cardiac Magnetic Resonance
CRF	Case Report Form
CRM	Clinical Research Manager
CRO	Contract Research Organization
CTR	Clinical Trial Report
DICOM	Digital Imaging and Communications in Medicine
DPPG.Na	Dipalmitoyl phosphatidylglycerol sodium
DSPC	Distearoylphosphatidylcholine
EBD	Endocardial Border Delineation
ECG	Electrocardiogram
F°	Farenheit
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GC-MS	Gas Chromatography-Mass Spectrophotometry
GGT	Gamma-Glutamyl Transpeptidase
h	Hour
HLHS	Hypoplastic Left Heart Syndrome
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
ITD	Intention to Diagnose
Kg	Kilogram
LDH	Lactic Acid Dehydrogenase
LV	Left Ventricle / Left Ventricular
LV EBD	Left Ventricular Endocardial Border Delineation
LVEF	Left Ventricular Ejection Fraction

MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
MI	Mechanical Index
min	Minute
mL	Milliliter
ng	Nanogram
PEG	Polyethylene Glycol
PT	Prothrombin Time
QRS	Q−wave — depolarization of the ventricular septum R−wave — activation of most of the ventricle S−wave — last stage of ventricular depolarization
QT	measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTc	QT corrected for heart rate
RBC	Red Blood cell Count (total erythrocyte count)
ROW	Rest of the World
RR	Time between beats used to calculate heart rate
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SF_6	Sulfur hexafluoride
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOF	Tetralogy of Fallot
2D	Two dimensional
WBC	White Blood Cell Count (total leukocyte count)
WHO	World Health Organization
UEUS	Unenhanced Ultrasound
ul	Microliter
USA	United States of America
VSD	Ventricular Septal Defect
w/v	Weight/ Volume
w/w	Weight/ Weight
βHCG	Beta Human Chorionic Gonadotropin

1 Title of Study

A MULTICENTER CLINICAL EVALUATION OF SAFETY AND EFFICACY OF LUMASON™ AS A CONTRAST AGENT IN PEDIATRIC ECHOCARDIOGRAPHY

2 Protocol Number

This study is being conducted under protocol number: BR1-140.
IND number: 46,958.

3 Introduction

Echocardiography has become the primary imaging tool in the diagnosis and assessment of congenital and acquired heart disease in children and adolescents.[1] Even though technically challenging echocardiographic windows are a more common challenge in adults, there is also a similar subset of children who do have limited, technically suboptimal echocardiographic windows secondary to obesity, congenital heart disease, chest wall abnormalities or prior surgical interventions resulting in suboptimal echocardiograms.[2]

Clinical trials have shown that suboptimal echocardiograms defined as non-visualization of at least 2 or more contiguous segments in the standard echocardiographic views [3] can be converted to a diagnostic study with the use of contrast in 75% to 90% of patients with fundamental and harmonic imaging modalities used.[4-8] For more than a decade, intravenous contrast echocardiography has been demonstrated to be a very useful tool in optimizing endocardial border delineation (EBD) in the adult population.[9-16] Ultrasound contrast is not approved by the Food and Drug Administration (FDA) for use during pediatric echocardiography because the safety and efficacy of contrast agents have not been established definitively in children.[17] Although the reported clinical use of transpulmonary contrast agents in the pediatric population is limited, the utility of these agents in pediatric population can be quite valuable.[18,19] Contrary to general belief, children do not always have diagnostic echocardiographic studies. Furthermore, dealing with children, especially sick children, presents a unique challenge. Children are frequently frightened and need coaxing and distractions to cooperate with the study. In addition, pediatric cardiologists have less training in regional wall-motion interpretation during stress echocardiography than their counterparts in adult cardiology.[17] These factors make contrast agents valuable in evaluating pediatric patients, particularly those who routinely undergo serial echocardiographic studies including stress echocardiograms (such as patients with Kawasaki disease,[20] those who have undergone the arterial switch operation, other coronary reimplantation surgery or cardiac transplantation), because contrast agents facilitate EBD.[17, 18, 19, 21]

Congenital heart defects are serious and common conditions that have significant impact on morbidity, mortality, and healthcare costs in children. The most commonly reported incidence of congenital heart disease is between 4 and 10 per 1000, averaging 8 per 1000 live births. An

estimated minimum of 32,000 infants are expected to be affected each year in the United States of America (USA). Of those, an approximate 25%, or 2.4 per 1000 live births require invasive treatment in the first year of life. Furthermore, congenital cardiovascular defects are the most common cause of infant death resulting from birth defects; >24% of infants who die of a birth defect have a heart defect. The 2008 death rate attributable to congenital cardiovascular defects was 1.1. Infant mortality rates under one year of age were ranging from 34.9 to 46.5.[22] Congenital heart defects range in severity from small holes between chambers that may resolve spontaneously to major malformations that can require multiple surgical procedures before school age and may result in death in infancy or in childhood. The most common complex defects include Tetralogy of Fallot (TOF), Transposition of the great arteries (TGA), atrial and ventricular septal defects (ASD/VSD), Coarctation of the aorta, Hypoplastic left heart syndrome (HLHS). [22] In children born with complex congenital heart disease, functional evaluation of the ventricle is often necessary and becomes challenging.[17] Poor acoustic windows in pediatric population often limit transthoracic images particularly for those have undergone surgical repair. Ultrasound contrast agents can be helpful in EBD of these geometrically unusual chambers, thereby aiding in functional assessment. These pediatric population groups include patients after procedures to repair TOF and after the Senning and Mustard procedures.[17, 18, 19, 21] The growing population of patients with congenital heart disease has led to a markedly increased use of non-invasive cardiac imaging in their care.[23] These patients often have residual structural and hemodynamic abnormalities, which necessitate serial comprehensive assessment, but transthoracic echocardiography is often challenging due to acoustic window limitations secondary to previous cardiac operations, implanted pacemaker leads, and alterations in the geometry due to shunts and baffles. Furthermore, these patients undergo cardiac magnetic resonance (CMR) imaging due to limitation of echo without contrast enhancement, if they do not have pacemaker, but the artifacts produced by implanted metal prevent assessment of imaging of cardiac structures within several centimeters of the stainless coils which are often used to occlude undesirable collateral vessels.[24]

In a recently published study,[21] efficacy of ultrasound contrast both at rest and stress has been prospectively studied in 51 patients with congenital heart disease some of which were children. Results of this particular study indicated that ultrasound contrast at rest and stress echo enabled safe and comprehensive assessment of anatomy and biventricular function by improving EBD in all patients with an increased number of wall segments visualized.[21]

Similarly, McMahon et al [19] demonstrated the safety of using contrast echocardiography in 20 children ranging in age from 9 to 18 for the evaluation of congenital heart disease, or cardiac function in children with arrhythmia or undergoing chemotherapy. Significant improvement in border delineation in all myocardial segments with the concomitant use of contrast and harmonic imaging has been reported. More importantly, significant reduction in interobserver variability of ejection fraction using harmonic imaging and contrast echocardiography versus fundamental or harmonic imaging alone without contrast was shown.[19] McMahon et al provided further evidence that the use of contrast agents is safe, improves accuracy of evaluation of cardiac function, and helps to better delineate the endocardial border of specific segments in children.[19] Given the ever increasing demand for acquisition of high quality echocardiographic data in children with cardiac

problems, contrast echocardiography plays an important role as a useful non-invasive diagnostic tool in obtaining accurate evaluation of anatomic and functional assessment.[19]

McMahon et al studied series of 70 children with a median age of 10.3 with suboptimal echocardiographic windows. The use of harmonic imaging and fundamental imaging without contrast usage has been compared. Scores for harmonic imaging were significantly higher for all views in comparison to fundamental imaging in children.[2] The improvement of the image quality obtained with harmonic imaging in children [2] is similar to the results of studies in adults.[13, 25-28]

Recent studies indicate that contrast enhanced two dimensional (2D) echocardiography with harmonic imaging had excellent correlation with radionuclide, magnetic resonance and computed tomographic measurements of Left ventricular (LV) volumes and left ventricle ejection fraction (LVEF) with improved interobserver agreement and physician interpretation confidence compared to unenhanced harmonic imaging in adult population.[29, 30] Furthermore, increasing accuracy of LVEF measurements have been associated with contrast-enhanced harmonic imaging over unenhanced harmonic imaging and fundamental imaging, respectively.[31,32]

Due to the increased use of transthoracic echocardiography as a non-invasive cardiac imaging tool in children and an increasing number of suboptimal, non-diagnostic quality studies mostly due to previous cardiac operations and alterations to cardiac anatomy especially in congenital heart disease patients, we aim to study the safety and efficacy of Lumason™ (sulfur hexafluoride lipid-type A microspheres) for injectable suspension in children 9-17 years with suboptimal images undergoing resting transthoracic echocardiogram with harmonic imaging modality. Lumason™ (under the trade name SonoVue®) is approved for use in echocardiography both at rest and stress in the European Union (EU) for use in the adult population. No Ultrasound Contrast Agent is currently approved in the pediatric population for use in rest echocardiography to improve LV EBD in patients with suboptimal unenhanced imaging.

4 Study Objectives

Primary Objective:

- To assess the efficacy of LUMASON enhanced transthoracic echocardiography [CEUS] in pediatric subjects with suboptimal Left Ventricular Endocardial Border Delineation (LV EBD) at unenhanced transthoracic echocardiography [UEUS] in terms of:
    - Change from baseline [UEUS] versus [CEUS] in total LV EBD score.
    - Proportion of subjects with adequate LV opacification

Secondary Objective:

- To obtain safety data in pediatric subjects administered LUMASON for LV EBD improvement during echocardiography.
- To obtain pharmacokinetic data in a subset of pediatric subjects undergoing analysis of SF_6 concentration in blood.

5 Investigational Plan

5.1 Overall Study Design Description

This is a Phase III multicenter, open-label study that will be conducted at approximately 7-12 sites in the United States in pediatric subjects with suboptimal LV EBD on non-contrast 2D transthoracic echocardiography with harmonic imaging within 30 days of the LUMASON administration. It is estimated that 92 subjects will be enrolled to provide 73 evaluable subjects.

Three cardiologists unaffiliated with enrolling centers (blinded readers), blinded to the subject's identity and clinical profile will independently evaluate the echocardiograms. The efficacy analysis will be primarily based on the blinded reader evaluations.

Imaging conditions will be representative of those used in routine clinical practice and will include LV EBD with harmonic imaging modality.

One or two of the sites participating in this study will also be requested to consent a subset of subjects for additional blood sampling for analysis of SF_6 concentration (Pharmacokinetic sub-set) in blood from a total of 6 subjects (3 males and 3 females) in the age group 9 up to 12 years of age and 6 subjects (3 males and 3 females) in the age of >12 up to and including 17 years.

5.2 Discussion of Study Design

The current study is designed to assess the efficacy of LUMASON enhanced echocardiography vs. unenhanced echocardiographic imaging in pediatric population (age 9 to 17 years) with suboptimal images and assess the proportion of subjects with "adequate" LV opacification. The subjects to be enrolled in the current study are representatives of those who could benefit most from contrast-enhanced echocardiography.

Echocardiography has become the primary and ideal tool for cardiac assessment and serial follow up of ventricular size and function as it is non-invasive, portable and efficacious in providing detailed anatomic, hemodynamic and physiologic information about the pediatric heart.[2] There has been an increasing number of sub-optimal, non-diagnostic quality studies mostly due to previous cardiac operations and alterations to cardiac anatomy due to shunts and baffles especially in congenital heart disease patients, which necessitate serial comprehensive assessment of their residual structural and hemodynamic abnormalities.[1, 3, 17, 21, 23]

The current guidelines of American Heart Association (AHA) [3] and American Society of Echocardiography (ASE) [17] suggest application for ultrasound contrast use in difficult to image patients with suboptimal image quality at their presenting rest echocardiography exams. Both guidelines define suboptimal studies when two contiguous segments are not visualized in any three of the apical views. The same criteria will be applied in children and representative of those used in routine clinical practice and will include assessment of LV EBD using harmonic imaging modality. Commercially available echocardiographic equipment and transducers will be used for all patients. Patients must have undergone a previous transthoracic echocardiogram with harmonic imaging modality within the one month period prior to enrollment resulting in suboptimal LV EBD in order to be included in the study. Subjects who are enrolled in the study

will then undergo a pre-injection (baseline) echocardiogram with the standard apical 4-, 2-, and 3-chamber views obtained with harmonic imaging. A recording of 2D transthoracic harmonic echocardiography images will be performed from 30 seconds prior to injection of LUMASON and then continue until all contrast enhanced images are acquired.

The first primary efficacy endpoint of LUMASON studies (change from baseline in total of LV EBD score) is the one prospectively defined and agreed upon with the FDA for the adult studies.

A LV 17-segment model will be utilized as recommended by ASE guidelines.[17, 3] The standard views of a 2D echocardiogram as defined by the ASE are all used as part of pediatric echocardiogram.[1, 17] The clinical utility of improvement in LV EBD resulting from opacification of LV as an aid in determination of more clinically relevant parameters is recognized by the 2008 ASE consensus statement as leading to improvement in feasibility, accuracy, and reproducibility of echocardiography for the qualitative and quantitative assessment of LV structure and function.[17]

The co-primary efficacy endpoint (adequacy of LV opacification following injection) will be graded according to the 4-point rating scale (see section 7.7). Useful appearance of contrast within the LV including ventricular opacification rating of +2 (non-homogeneous) or +3 (complete and homogeneous) will be considered as adequate LV opacification.

A subset of 12 subjects is considered to be sufficient for the estimation of the pharmacokinetic parameters, based on the findings of a prior study in healthy adult subjects.[33]

In adults, the dose for LV opacification is a fixed dose of 2 mL. Assuming a notional adult body weight of 70 kg, this represents a dose of 0.03 mL/kg body weight. This dose will be used for the study in pediatric population.

Most or all of contrast agent dose rapidly dissolves in the blood and subsequently eliminates by the lungs. The cumulative recovery of SF_6 in expired air averaged 86% to 94% of the administered dose in healthy subjects.[33] The results of this study published by Morel et al [36] had shown that LUMASON rapidly removed from the blood by the pulmonary route with 40% to 50% of the injected dose eliminated within the first minute after administration and 80% to 90% eliminated by 11 minutes after administration. Furthermore, the recovery of SF_6 in expired air in subjects with impaired lungs averaged 102%.[34] This finding indicates that the patients eliminate all of the SF_6 from LUMASON via their lungs rather than an alternate elimination route, despite the impairment of lung function. Most alveolar maturation occurs in the first 2 years of life, so that no differences in the elimination of the gas between adults and pediatric patients 9-17 years-old should be expected.[35] No studies ever reported the use of ultrasound contrast agents during rest echocardiography in patients below 9 years of age.

5.2.1 Risk-Benefit Considerations

A benefit from contrast-enhanced LV EBD resulting in improved [36] diagnostic quality of echocardiographic examinations should be expected. Given the fact that the subjects to be enrolled in the present clinical trial will be already scheduled for echocardiographic examination but with suboptimal images and have no evidence of intracardiac shunts, inclusion in the study is not likely to add any risk for the subjects, since no particular safety concerns can be expected

when administering LUMASON to children with respect to the safety profile of LUMASON. Furthermore, contrast enhanced transthoracic echocardiogram supposed to prevent the risk of those subjects being exposed to radiation through other imaging modalities in the down streaming of appropriate assessment of cardiac structures.

5.3 Study Duration

The procedure associated with the investigational echo exam prior to and after the administration of the investigational product will be completed within approximately 15-30 minutes.

Safety monitoring will begin at the time of signing Informed Consent (and subject's "assent," according to requirements by local regulations), and will continue for 72 hours post dose.

5.4 Study Population

The study will be conducted in subjects who have sub-optimal images from transthoracic harmonic echocardiography. It is expected that approximately 7-12 study centers in the United States will participate in the study. Each site may enroll approximately 20 subjects in this trial.

Approximately 92 subjects will be enrolled in order to obtain 73 evaluable subjects.

5.4.1 Inclusion Criteria

Enroll a subject in the study if they meet the following inclusion criteria:

- Is male or female, 9 to 17 years of age;
- Written informed consent is obtained from the subject's parent(s) or legal acceptable representative(s) (according to local regulations);
- Assent from the subject is obtained when applicable, that is, when it is required according to local regulations (IRB requirements) (see section 10.2);
- Is suspected of having cardiac disease or undergoing evaluation of cardiac anatomy for congenital heart disease;
- Has undergone a previous transthoracic echocardiogram within a one month period prior to enrollment in this study resulting in suboptimal LV EBD defined as $\geq$2 contiguous segments in any given view that cannot be visualized.

5.4.2 Exclusion Criteria

Exclude a subject from this study if the subject does not fulfill the inclusion criteria, or if any of the following conditions are observed:

- Children < 9 years of age;
- Has previously been enrolled in this study;
- Has been administered any other contrast agent either intravascularly or orally within 48 hours of the first LUMASON administration;

- Has known right-to-left, bidirectional or transient cardiac shunt, (ruled out with agitated saline study performed before administration of LUMASON) ;
- Has any known hypersensitivity to one or more of the ingredients of the investigational product (sulfur hexafluoride or to any components of LUMASON);
- Has received an investigational compound within 30 days before enrolling into this study;
- Is a pregnant or lactating female. Exclude the possibility of pregnancy in subjects who have started their menses (by testing on site at the institution (urine βHCG) within 24 hours prior to the start of investigational product administration);
- Is determined by the Investigator that the subject is clinically unsuitable for the study.

5.4.3 Discontinuation Criteria

Clearly document the reason for the subject's discontinuation on the Case Report Form. Discontinued subjects are not replaced. Discontinue a subject from the study if the subject:

- Withdraws consent/or assent if applicable;
- No longer meets the Inclusion Criteria;
- Experiences any of the Exclusion Criteria;
- Has an adverse event that, in the opinion of the Investigator, requires the subject's discontinuation. Perform patient follow-up in accordance with Section 8.1.5.

6 Investigational Product

The study investigational product (IP) is named LUMASON in the United States. The investigational product is supplied as a 3-part kit containing the following:

- clear glass vial labeled as LUMASON (sulfur hexafluoride lipid-type A microspheres) for injectable suspension,
- prefilled 5 mL syringe labeled as Sodium Chloride Injection, USP, 0.9% Sodium Chloride and its plunger rod
- Mini-spike

6.1 Description and Labeling

LUMASON is formulated as a 25 mg sterile, non-pyrogenic lyophilized powder in a septum-sealed vial. The gas phase in the vial is SF_6, an innocuous gas. The lyophilized powder is made of a combination of pharmaceutical grade polyethylene glycol (PEG) molecular weight 4000, phospholipids and palmitic acid. Phospholipids from chemical synthesis were selected for their higher chemical purity and lower pyrogenic potential.

A mixture of distearoylphosphatidylcholine (DSPC) and dipalmitoyl phosphatidylglycerol sodium (DPPG.Na) is used in the IP.

Following reconstitution, the injectable suspension contains 1.5 to 5.6 x 10^8 microspheres/mL.

Table A: Description of Investigational Product

Ingredient	Concentration / Amount per Unit
Polyethylene glycol (PEG) 4000	4.91 mg/mL
Phospholipids (DSPC/DPPG 1:1 w/w)	0.075 mg/mL
Palmitic Acid	0.008 mg/mL
Sulfur Hexafluoride (SF_6)	8 µL/mL

Investigational product will be supplied by Bracco. Labeling will be done according to the National Legal requirements of each country where the study may be conducted. Each vial will bear a two-part label, the second part of which once used will then need to be affixed to the appropriate page of the Case Report Form (Appendix A).

6.2 Storage

Store unused LUMASON vials in a secure area with limited access at controlled room temperature at 25°C (77°F), excursions are permitted to 15-30 °C (59-86°F)

6.3 Blinding and Randomization

This is an open-label, non-randomized study.

6.4 Handling and Preparation

Prior to administration, the lyophilized powder should be reconstituted with 5 mL of sterile 0.9% w/v sodium chloride solution to give a final concentration of 8µL of SF_6 lipid microspheres per mL. After adding the sodium chloride solution, the vial should be shaken vigorously for 20 seconds, after which a homogeneous white milky liquid is obtained. A detailed instruction for the reconstitution of LUMASON is presented in (Appendix B).

Once reconstituted, LUMASON can be left at room temperature. The suspension is usable for up to 3 hours, upon standing for more than 15 minutes, buoyancy causes some of the larger microspheres to rise to the surface. Therefore, gently agitate the reconstituted LUMASON vial in a top-to-bottom manner to resuspend the sulfur hexafluoride lipid microspheres before administration. The desired volume of the product is then withdrawn into a syringe for administration to the subject.

In the case of incomplete use of vial contents, the product cannot be reused.

6.5 Administration

Once reconstituted, LUMASON will be administered intravenously as a single 0.03 mL/kg bolus injection during echocardiography. The LUMASON injection will be followed immediately with 5 mL of saline to flush the intravenous line of any remaining contrast agent.

6.6 Accountability

In accordance with International Conference of Harmonization (ICH) and United States FDA requirements, the Investigator and/or Drug Dispenser must at all times be able to account for all IP furnished to the institution. The appropriate site personnel must sign, date and immediately forward to the Sponsor the IP receipt form included with each shipment.

No IP is to be used outside of this study. Record the use of the IP on the appropriate Drug Accountability Record. All containers of IP must be accounted for, whether used or unused, during the course of and at the conclusion of the study. At the conclusion of the study, all shipments of unused IP to the Investigator must be returned to the Sponsor accompanied by a completed, signed copy of the appropriate IP inventory reconciliation form.

7 Methodology

7.1 Study Schedule

Table B: Study Schedule

	Pre dose			Post dose				
	Within -24 h	Within -30 min [e]	Prior to IP administration	0 min	5 min	30 min	24 h	72 h
Written Informed Consent [a]	×							
Adverse Events Monitoring [b]	×	⇒	⇒	⇒	⇒	⇒	⇒	×
Concomitant Medications [c]	×	⇒	⇒	⇒	⇒	⇒	×	× [f]
Medical History	×							
Physical Examination	×						×	
Vital Signs		×			×	×	×	
Pregnancy Test (if necessary)	×							
Pulse Oximetry/ ECG monitoring [d]		×	⇒	⇒	⇒	×		
Laboratory Evaluations	×						×	
12-Lead Electrocardiogram		×				×	×	
IP Administration				×				
Unenhanced Echo			x					
Contrast Enhanced Echo				×				

a Obtain prior to implementation of any study procedure.

b Start monitoring from the time Informed Consent is given up to 24 h post dose and continue monitoring up to 72 hrs post dose through a telephone call placed at Day 3. Only post dose events will be tabulated as adverse events.

c For any subject where sedation is administered, record the sedation medication on the concomitant page of the CRF and indicate for sedation.

d Continuous pulse oximetry and ECG monitoring will be performed 30 min prior through 30 minutes following IP administration for all subjects suffering from pulmonary hypertension or unstable cardiopulmonary conditions.

e For any subject where sedation is administered, obtain a second vital sign series and ECG immediately before the echocardiography exam and after the sedation drugs have been administered.

f Record any new medication taken for treatment of an adverse event that occurred after the signed informed consent was obtained through 72 hours following IP administration.

Note: A subset of 12 subjects will undergo additional blood draws for analysis of SF_6 concentrations, (see Section 7.6).

7.2 Written Informed Consent

Obtain written Informed Consent from the subject's parent(s) or legal acceptable representative(s) (according to local regulations) prior to the implementation of study procedures required by the protocol. Obtain written Assent from the subject, when it is required by local IRB. Give to the subject's parent (s) or legal acceptable representative(s) a copy of the signed

and dated written informed consent form including any other written information regarding the study. Document Informed Consent process (Assent when applicable) in detail, in the source record at the site.

Separate written Informed Consent (Assent when applicable) is needed for those subjects who participate in the Pharmacokinetic subset.

7.3 Subject Numbering

Assign a 4-digit subject number for each subject who qualifies for the study and signs the written Informed Consent. The first two digits of the subject number will be the investigational site number assigned by Sponsor. For site numbers less than ten use a leading zero. The third and fourth digits will be a number starting with 01 for the first subject enrolled and incrementing by "1" for each sequential subject.

For example, at site 01, the investigative site will assign the first subject 0101; the second subject 0102, etc.

Never reassign subject numbers. In the event that a subject withdraws from the study, the number assigned to that subject is retired and the next subject receives the next sequential number.

7.4 Subject Evaluations

7.4.1 Medical History

Obtain a complete medical history after the subject has signed the Informed Consent and within 24 hours prior to IP administration. Record the subject's medical history on the Medical History section of the Case Report Form.

7.4.2 Pregnancy Test

If the subject is female, and of child bearing potential, exclude the possibility of pregnancy by testing (urine βHCG) within 24 hours prior to the start of IP administration.

7.4.3 Concomitant Medications

Record all medications (prescription and over-the-counter) taken within 24 hours prior to IP administration in the Concomitant Medication section of the Case Report Form. Record newly prescribed pharmacological treatments in this section from within 30 minutes prior to IP administration up through 24 hours post investigational product administration. Additionally, any new medication taken for treatment of an adverse event that occurred after the subject signed informed consent through 72 hours following IP administration should be recorded.

7.4.4 Safety Assessments

7.4.4.1 Adverse Events

Monitor subjects for any untoward medical events from the time of signed Informed Consent through 72 hours after investigational product administration.

Subjects will be evaluated at up to 72 hours post dose either by telephone call made by a healthcare professional, or by a scheduled, follow up visit.

Record all untoward medical events in the Adverse Event section of the Case Report Form as specified in Section 8. Only post dose untoward medical occurrences will be tabulated as adverse events.

7.4.4.2 Physical Examination

Perform a physical examination within 24 hours prior to IP and at 24 hours after IP administration.

7.4.4.3 Vital Signs

Collect the following vital signs within 30 minutes pre dose and at 5 minutes, 30 minutes and 24 hours post dose. Obtain the following vital signs in a position that is consistent for all time points for each subject:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Heart rate (beats/minute)
- Respiratory rate (breaths/min)

7.4.4.4 Laboratory Evaluations

Collect blood samples within 24 hours prior to the investigational product administration and 24 hours after the IP administration. A central laboratory will perform evaluations for the analytes listed in Table C.

Table C: Laboratory Analytes

Hematology	Clinical Chemistry	
Hematocrit	Sodium	AST/SGOT
Hemoglobin	Potassium	ALT/SGPT
RBC count	Chloride	Alkaline Phosphatase
WBC count	Glucose	GGT
differential WBC count	Urea Nitrogen	Uric Acid
Platelets	Creatinine	Total Protein
PT	Total Bilirubin	Albumin
APTT	LDH	Calcium

Prepare blood and ship via overnight courier per the Instruction Manual provided by the central laboratory.

7.4.4.5 Electrocardiograms

A 12-Lead Electrocardiogram (ECG) will be performed in all subjects within 30 minutes prior to IP administration, and at 30 minutes and 24 hours after IP administration and a copy of the ECG report will be affixed to the CRF.

The ECG examinations will be evaluated by a central ECG laboratory that will provide the ECG equipment to the site and will employ a cardiologist to perform the centralized ECG evaluation containing the following information:

- RR interval
- PR interval
- QRS interval
- QT and QTc interval (Bazett's and Fridericia's)
- Overall diagnostic assessment
- Comparison to Baseline

7.4.4.6 Pulse Oximetry and ECG Monitoring

Continuous pulse oximetry and ECG monitoring will be performed starting 30 min prior and continuing through 30 minutes following IP administration for all subjects suffering from pulmonary hypertension or unstable cardiopulmonary conditions.

7.4.4.7 Sedation

When children are unable to cooperate, sedation may become necessary to ensure that images of diagnostic quality are obtained. Whether or not sedation is necessary depends on several variables, including age and maturity of the subject, prior sedation history, duration of the procedure, and any associated discomfort. Subjects will be sedated or anesthetized only for

procedural purposes and then according to standard hospital routines by qualified individuals (e.g., nurses, physicians, or physician assistants) who are experienced in the management of sedated children and have the knowledge to perform careful subject screening so as not to put any subject at unacceptable risk of serious complications.

All drugs administered for sedation will be recorded on the Case Report Form on the Concomitant Medications page. Monitoring of the subject will start with documentation of pre-sedation vital signs, and ECG recordings on the Case Report Form. Continuous subject monitoring should be conducted according to routine clinical practice. When sedation is required, additional vital signs and ECG data will be performed after sedation and before the echocardiography examination and recorded on the CRF.

Whenever adverse events are recorded, the Investigators will be asked to prospectively report in the CRF their causality assessment including an evaluation of whether the event may be related to the sedation drug(s) or the IP.

7.5 Imaging Procedures

At each site, the same commercially available echocardiographic equipment and transducers will be used for all subjects. Subjects will be examined in either the supine or lateral decubitus position. The transmit power (≤ 0.8 MI) and the gain setting will be adjusted at the beginning of each procedure to obtain optimal endocardial visualization. Both settings must be kept constant throughout each subject's evaluation. Every effort must be made to keep the optimal transducer position for standard views. At each site, image acquisitions can be performed by the same sonographer throughout the study.

Prior to administration of IP, further evaluation for intracardiac shunt will be demonstrated with agitated saline study to assess the interatrial septum. If no evidence of intracardiac shunt is confirmed by the pediatric cardiologist, the subject will further undergo CEUS.

A pre-injection (baseline) UEUS with the standard apical 4, 2, and 3 views with harmonic imaging will be performed. Contrast-enhanced echocardiography will be performed with administration of the IP. A recording of 2D transthoracic harmonic echocardiography images will be performed from 30 seconds prior to injection of the IP and continued until all images are acquired. For CEUS images, the apical 4-chamber view will be acquired first followed by the apical 2- and 3-chamber views.

Both the UEUS and CEUS examinations must be identified by subject number and stored as DICOM clips for transfer to the central imaging lab according to the instructions provided in the Imaging Manual. The investigational sites are also required to maintain a copy of the subject's examination as a record of the study.

7.6 Pharmacokinetic Evaluation

A subset of subjects at one or two sites will undergo additional blood sampling for the determination of SF_6 concentration in blood. A total of 6 subjects (3 males and 3 females) age 9 and up to 12 years of age, and 6 subjects (3 males and 3 females) in the age >12 and up to and

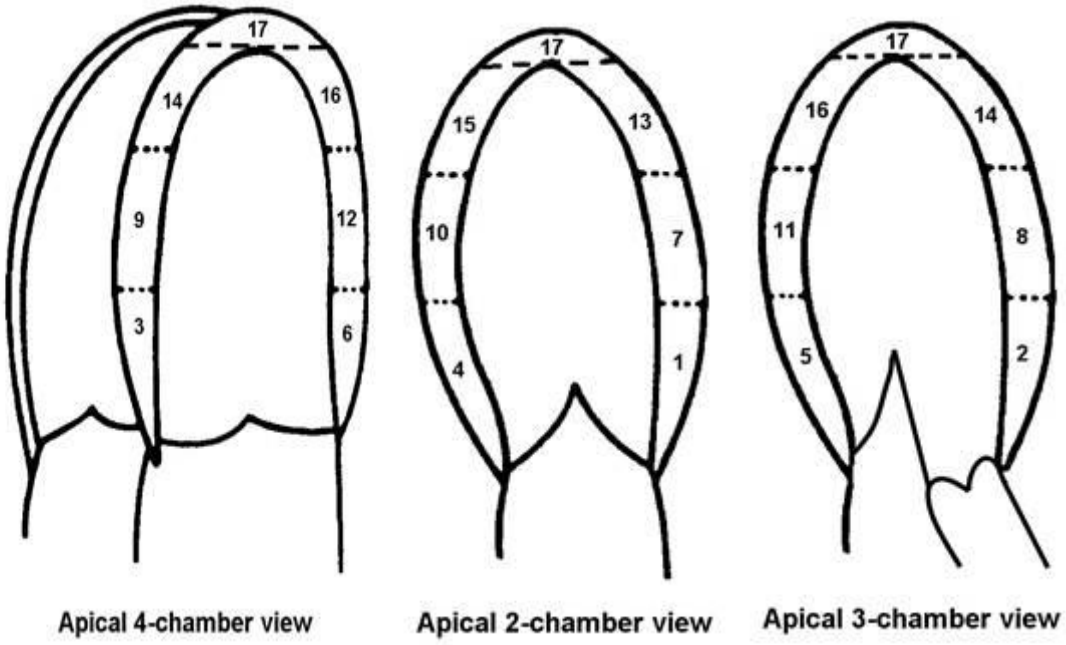
including 17 years will have additional blood draws of 1 mL each immediately pre-dose, and at 1, 2, 3, 5, 10 and 15 minutes post-dose for the purposes of analyzing SF_6 concentration in blood. Blood samples will be taken from the arm contralateral to that used for the injection of LUMASON and analyzed by gas chromatography-mass spectrometry (GC-MS) or other acceptable method to obtain SF_6 concentration in blood. A designated laboratory will perform evaluations of SF_6.

7.7 Efficacy Evaluations

For all subjects, UEUS and CEUS images will be randomized and independently evaluated off-site by 3 blinded readers unaffiliated with the enrollment centers. No on-site evaluation of efficacy will be performed for this study.

From the apical 4-chamber, apical 2-chamber and apical long (3-chamber) views, the heart will be divided into 17 segments according to the guidelines of the American Society of Echocardiography[17] as seen in Figure 1.

Figure 1 Three echocardiographic apical views used in the study



EBD for each segment will be graded by each off-site reader as:

0 = Inadequate (endocardial border not visible)

+1 = Sufficient (endocardial border barely visible)

+2 = Good (endocardial border clearly visible).

A separate off-site methodology document will be prepared and will contain the details regarding how the images will be anonymized, randomized and presented to the readers. No clinical information will be provided to the readers.

Both the UEUS and CEUS examinations must be identified by subject study identification number and stored as DICOM clips for transfer to the central imaging lab according to the instructions provided. Under Good Clinical Practices guidelines, the investigational sites are also required to maintain the subject's examination as a record of the study.

The timing of LV EBD assessment should start when LUMASON reaches from the apex to the mitral valve plane level.

The degree of LV opacification following injection will be graded according to the following 4-point rating scale:

0 = none, i.e., no visible contrast within the left ventricular cavity
+1 = faint, i.e., weak or trace effect of contrast within the left ventricle
+2 = non-homogeneous, i.e., some areas of the left ventricle fully opacified but without a time when the whole cavity is filled with contrast to the same high intensity
+3 = complete, homogeneous and high intensity effect.

Useful appearance of contrast within the left ventricle including ventricular opacification rating of +2 (non-homogeneous) or +3 (complete and homogeneous) will be considered as adequate LV opacification.

8 Reporting Safety Information

The Investigator is responsible for the detection, documentation and reporting of adverse events.

Adverse event (AE) collection begins when a subject signs the Informed Consent and continues through the follow-up period defined in the Section 7.4.4.1 "Adverse Events" of the protocol. In addition, an investigator should report any serious AEs that occur after this time period that he/she believes may be related to the IP.

Any untoward medical occurrence that occurs from the time of signed Informed Consent to the time immediately prior to investigational product administration will be tabulated in the Clinical Trial Report as a "pre dose event."

8.1 Adverse Events

8.1.1 Definitions

An AE is any untoward medical occurrence in a subject or a clinical trial subject administered a medicinal product and which does not necessarily have to have a causal relationship with the use of the product.

Any AE that occurs after the follow-up period defined in the protocol is not required to be collected in the AE section of the CRF.

An existing condition, which is detected by the diagnostic procedure conducted to test the efficacy of an investigational contrast agent, is not considered an AE.

Symptoms or medically significant laboratory or instrumental (e.g., electrocardiographic) abnormalities of a pre-existing condition, such as cancer or other disease, should not be considered an AE. However, new symptoms and laboratory or instrumental abnormalities, as well as worsening of pre-existing ones are considered AEs.

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (i.e. the subject was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity (where disability is defined as a permanent or substantial disruption of ability to carry out normal life functions, either reported or defined as per clinical judgment),

- is a congenital anomaly/birth defect,
- is an important medical event that may not result in death, be life-threatening, or require hospitalization but may be considered serious when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed in the definition above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Any suspected transmission via a medicinal product of an infectious agent is also considered an SAE.

A non-serious AE is any AE that does not meet the criteria listed above for a SAE.

The reference safety document will be the same for the whole clinical trial.

An unexpected AE is one where the nature, severity, specificity or outcome is not consistent with the applicable product reference document.

In particular, the Sponsor will determine the expectedness of the AEs reported during this clinical trial according to the following reference document(s):

LUMASON: latest version of the Investigator's Brochure (IB).This will be provided as a separate document.

If an updated version of the reference safety document becomes available during the conduction of the study and before the last subject out, the Sponsor will provide the new version to all investigators. The new version will become the reference safety document for assessing the expectedness of AEs'.

An AE with fatal outcome should be considered unexpected unless the reference safety document specifically states that the event might be associated with a fatal outcome.

When it is uncertain whether an AE is expected, the AE should be treated as unexpected.

Upon notification of a serious AE, the Sponsor reviews all of the information provided by the Investigator and will urgently seek additional information when needed. The Sponsor will determine expectedness of the serious AE according to the latest version of the IB.

Reporting of serious AEs to the FDA will be done by the Sponsor according to the applicable and current Code of Federal Regulations (CFR). Additionally, any unexpected related serious adverse events will be reported to the investigators and Institutional Review Boards (IRBs) participating in the study as required by the applicable FDA CFR.

Whenever a serious AE is reported which is considered to be related to IP **and** unexpected based on the current IB, in addition to reporting to FDA, IRBs/Ethics Committees and Investigators as stated above, the Sponsor will review all available safety data to determine whether or not the study should be placed on hold and/or additional urgent safety measures added to the protocol. Any action taken in response to a safety concern will be communicated to FDA.

8.1.2 Expedited Reporting a Serious Adverse Event to Sponsor

The Investigator must report all serious adverse events **within 24 hours**, by telephone or by fax, to both the Drug Safety group and the Clinical Manager of the Sponsor as listed in Table D.

A Serious Adverse Event Report (SAER), see (Appendix C) must be completed by the Investigator and faxed to the Sponsor (both the Drug Safety group and the Clinical Manager) within 3 calendar days after the Investigator first became aware of the serious event. The top two copies of the completed SAER should also be sent to the Sponsor by traceable mail. The bottom copy remains on-site with the CRF.

In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to both the Drug Safety group and the Clinical Manager of the Sponsor by traceable mail together with the SAER, with a copy retained on-site with the CRF. If an autopsy is performed, a copy of the autopsy report should be actively sought by the Investigator and sent to the Sponsor as soon as available with a copy retained on-site with the CRF.

A follow-up SAER should be filled in by the Investigator if important follow-up information (e.g., diagnosis, outcome, causality assessment, results of specific investigations) becomes available after submission of the initial SAER. The follow-up SAER should be sent to the Sponsor in the same manner and following the same timeline as described for the initial.

If the Investigator becomes aware of any serious adverse events within the follow-up window established in the protocol following investigational product administration, they will be reported to both the Drug Safety group and the Clinical Manager of the Sponsor as described above.

If outside the follow-up window established in the protocol, any serious adverse events are reported to the Investigator, which he/she believes are related to the administration of the IP it is the Investigator's responsibility to report this serious adverse event to both the Drug Safety group and the Clinical Manager of the Sponsor. Such serious adverse events will be reported using a SAER or any other way chosen by the Investigator. Do not use the CRF.

The names, telephone, fax numbers, and email address of the contact persons are given below in Table D:

Table D: Adverse Events Reporting – Sponsor Contact Personnel

Name/Title	Office Telephone Number	Alternate Telephone Number	Fax Number / E-mail
North America			
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

* *To be used outside the normal business hours.*

For additional SAE questions call the Medical Expert whose contact details are on the cover page of the protocol.

8.1.3 Breaking the study blind

This is an open-label, non-randomized study

8.1.4 Data Collection

The Investigator will collect adverse events through non-leading questions and examination of the subjects.

For each event, record the following information in the Adverse Event section of the Case Report Form:

- **Classification of the Event:** Classify the event as either serious or non-serious (see definitions in Section 8.1.1).
- **Description of Signs or Symptoms:** Whenever possible, record a specific diagnosis for the event. If a diagnosis cannot be made, then record each sign or symptom separately. If multiple episodes of an event occur, separated by an appropriate time interval to justify considering the subsequent episodes as a repeat occurrence, record each episode separately on the Case Report Form. Indicate if the event is local (i.e., occurring at the site of administration). For serious AEs only: provide a detailed chronological description of the clinical course of the event(s) and of all relevant signs and symptoms.
- **Onset Date and Time:** Record the date and time the event started. If a change from pre dose in a laboratory test is reported as an adverse event, record the start date as the date of collection of the first lab sample that shows the change.
- **Stop Date and Time:** Record the date and time the event resolved. If a change from pre dose in a laboratory test is reported as an adverse event, record the stop date as the date of collection of the first post dose sample that shows a return to the pre dose level.
- **Intensity:**
  1. **Mild:** Event not resulting in disability/incapacity, which resolves without treatment.
  2. **Moderate:** Event not resulting in disability/incapacity, which requires treatment.
  3. **Severe:** Event resulting in temporary and/or mild disability/incapacity, which requires treatment.
- **Relationship to the IP:** Make every effort to determine the cause of each adverse event. Classify the correlation between the IP and the adverse event as follows:

1. **Reasonable Possibility**

The event falls into one of the two following categories:

1) a) The event follows a reasonable temporal sequence from administration of the IP;

AND

b) The event follows a known response pattern to the IP but could have been produced by any of the following features:

- the subject's clinical state, or
- other therapy administered, or
- the diagnostic/interventional procedure;

OR

c) The event cannot be reasonably explained by any of the following features:

- the subject's clinical state, or
- other therapy administered, or
- the diagnostic/interventional procedure;

OR

d) There is evidence of partial or complete disappearance of the event after withdrawal of the product (positive dechallenge).

2) The report of the event contains:
a) conflicting data
AND/OR
b) dubious or insufficient/poor evidence

2. **No reasonable Possibility**

The event is either a pre dose event or is definitely due to causes separate from the administration of the IP, i.e.,

- documented pre-existing condition
- technical and manual procedural problems
- concomitant medication
- the subject's clinical state
- the event is judged as not related and does not fall under either of the categories for "reasonable possibility"
- erroneous administration of treatment

- Action Taken:
  0. None
  1. Change in the investigational product administration (including brief interruption of administration of total dose and early termination of administration, i.e., dose reduction)
  2. Drug treatment required (a medication was prescribed or changed; record in the Concomitant Medication section of the Case Report Form)
  3. Non-drug treatment required (a non-drug treatment was prescribed or changed, record under "Comments" in the Adverse Event section of the Case Report Form)
  4. Hospitalization or prolonged hospitalization
  5. Diagnostic or clinical test(s) conducted (attach a copy of the results to the Case Report Form)
  6. Subject discontinued from the study
- Subject Outcome:
  1. Recovered without sequelae
  2. Recovered with sequelae (describe the sequelae under "Comments" in the Adverse Event section of the Case Report Form)
  3. Not Recovered, event on-going (follow the subject until a definite outcome can be determined. When follow-up data are collected, report follow-up information under "Comments" in the Adverse Event section of the Case Report Form; if the event is serious, fill in a follow-up Serious Adverse Event Report)
  4. Died (list primary cause of death under "Event Description" in the Adverse Event section of the Case Report Form; if available, attach a copy of the autopsy report to the Case Report Form and send a copy to Sponsor)
- Comments:
  Provide other pertinent clinical information and observations, and the rationale for the provided causality under "Comments" in the Adverse Event section of the Case Report Form. For example, record predisposing or contributing conditions, such as previous history, concomitant diseases or medications, and/or procedural risks and explain the reasoning for attributing the event(s) to the cause chosen.
  If the investigator states that there is no reasonable possibility that the event is related, he/she should provide details of an alternative explanation for the event.

8.1.5 Subject Follow-up

Make every attempt to follow the subject until the adverse event is resolved, stabilized, returned to baseline or deemed irreversible.

8.2 Special Situations

The Investigator must report all special situations **within 24 hours** by telephone or by fax using the form supplied by the Sponsor, to both the Drug Safety group and the Clinical Manager of the Sponsor as listed in Table D.

Special situations include but are not limited to the following: accidental overdose of IP, medication error with IP (such as administration of the wrong drug or wrong dose, wrong administration rate or wrong technique in drug usage process, administration via the wrong route, radiation under-dose, labeled drug-drug or drug-disease or drug-food interaction). In addition, any pregnancy of a subject or partner must be reported by the Investigator to the Sponsor within 24 hours using the Pregnancy Report form; any associated SAEs must be reported concurrently using the SAER.

In case urgent safety measures are taken during the conduct of the study these will be timely communicated by the Sponsor to the applicable regulatory authorities and also to concerned investigators and subjects.

8.3 Laboratory Evaluations

8.3.1 Reporting and Evaluation of Central Laboratory Test Results

The central laboratory will send a report of the laboratory results to the Investigator within 48 hours after the sample is picked up from the investigational site. Attach a copy of the laboratory report to the Case Report Form.

The Investigator or sub-investigator must review laboratory values within 24 hours of receipt of the laboratory report. After the review is completed, the Investigator must sign and date each laboratory report.

The central laboratory will provide normal reference ranges for the laboratory tests on the laboratory results report. A value is **normal** when it falls on or within the upper and lower limits of the reference range. A value is **abnormal** when it falls outside the upper or lower limit of the reference range. The central laboratory will flag all abnormal values on the laboratory report and will verify that the result is not due to pre-analytical problems (e.g., sample taken improperly, sample stored incorrectly, sample labeled incorrectly) or to analytical problems (e.g., machine not accurately calibrated, technical problems with equipment or reagents, deterioration of analyte).

The central laboratory has established 'alert criteria' for some laboratory tests and will make an **immediate telephone call** to the Investigator in the event that a laboratory result meets these criteria.

The Investigator must evaluate any change from pre dose to post dose in a laboratory test which represents a worsening of the subject's clinical state as to whether it meets the definition of an adverse event. **Record all changes determined to meet the definition of an adverse event in the Adverse Event section of the Case Report Form**.

8.3.2 Repeat Testing

Collect additional samples to repeat the lab test that represents a worsening of the subject's clinical state and meets the definition of an adverse event, until the value returns to the pre dose level or clinically stabilizes, or until the Investigator or physician of record determines that further follow-up is unnecessary.

8.3.3 Emergency Laboratory Analysis

If a laboratory result needs to be obtained immediately, split the sample and send one-half to the local laboratory for immediate analysis and the other half to the central laboratory. Attach results from both laboratories to the Case Report Form.

8.4 Electrocardiograms

If a worsening from pre dose to post dose is observed on an ECG, repeat the ECG at the discretion of the Investigator, in addition to obtaining an ECG at the 30 min and 24 hours post dose time points required by the protocol. The Investigator will assess any worsening from pre dose to post dose in the ECG for clinical relevance (whether it meets the definition of an adverse event). Record all changes determined to meet the definition of an adverse event in the Adverse Event section of the Case Report Form.

8.5 Physical Examinations

If a worsening from pre dose to post dose is observed, repeat the physical examination at the discretion of the Investigator, in addition to obtaining a physical examination at the 24 hour post dose time point required by the protocol. The Investigator will evaluate any worsening at the post dose physical examination for their clinical relevance and to determine whether they meet the definition of an adverse event. Record all changes determined to meet the definition of an adverse event in the Adverse Event section of the Case Report Form. Record specific signs, symptoms, and/or laboratory information supporting these changes.

8.6 Vital Signs

If a worsening from pre dose to post dose is observed in vital signs, repeat the vital sign measurement at the discretion of the Investigator, in addition to obtaining vital sign measurements at 5 minutes, 30 minutes and the 24 hour post dose time points as required by the protocol. The Investigator will evaluate any worsening in vital signs for its clinical relevance as to whether it meets the definition of an adverse event. Record all changes determined to meet the definition of an adverse event in the Adverse Event section of the Case Report Form.

9 Statistical Methods

In general, summary statistics (mean, median, standard deviation, minimum, and maximum) will be provided for continuous variables, and the number and percentage of each category will be provided for categorical data. Any changes in the original statistical methodology will be documented in the statistical analysis plan (SAP).

All statistical analyses will be performed using SAS® software.

9.1 Subject Disposition and Demographic and Baseline Characteristics

Summary tables will be provided for the number of subjects who have been enrolled, dosed and completed according to the protocol. The number of subjects who prematurely discontinued the study and the reasons for their discontinuation will be summarized.

Summary statistics will be presented for demographic and baseline characteristics, including age, sex, race, height, and weight, BMI, other relevant study entry criteria.

9.2 Analysis Population

Safety Analysis Population – all subjects who received IP will be included in the safety analysis population.

Efficacy Analysis Population – all subjects who received IP and have data available for both non-contrast echo exam and contrast echo exam will be included in modified Intent-to-Diagnose population (ITD population).

9.3 Concomitant Medications

Frequencies of concomitant medication use will be tabulated by World Health Organization (WHO) class, level I and level II.

9.4 Extent of Exposure

Descriptive statistics will be presented to summarize the volume (mL) of IP administered. Dose administration for IP will be listed for each injection by subject.

9.5 Safety Analysis

The safety data will be summarized for all subjects dosed. Summary tables, including change from UEUS to CEUS or clinically significant changes where applicable, will be presented for the following safety endpoints:

- Adverse Events
- Clinical Laboratory Evaluations
- Electrocardiograms
- Pulse Oximetry
- Vital Signs

All adverse events will be coded by MedDRA and summarized by system organ class and preferred term, by intensity and by causal relationship to the investigational product.

Only those occurrences which occur from the start of IP administration through the follow-up period defined in the protocol will be tabulated in the Clinical Trial Report as “adverse events” See Section 8.1.1.

Physical examination results, concomitant medication data will be presented in the data listings.

Summary statistics will be presented for volume of investigational product administered.

9.6 Pharmacokinetics Analysis

Pharmacokinetic parameters will be calculated from blood data. The actual times (i.e., not the nominal sampling times) at which blood samples obtained will be used in all calculations. Blood concentrations below the limits of quantification will be replaced with zero for the calculation of all pharmacokinetic parameters. The blood concentration-time data will be analyzed using appropriate software.

A listing of all raw blood concentration data and tables containing standard descriptive statistics will be reported. Profiles of blood concentrations will be represented graphically for the mean and individual data to show the inter-subject variability. The pharmacokinetic parameters will be normalized by subject body weight if applicable. Appropriate pharmacokinetic compartment/ non-compartment modeling will be applied based on the performance of goodness of fit criteria and the inter-subject variability of the subject pharmacokinetic profiles. Pharmacokinetic analysis approach will be similar to the methodology utilized in the adult study [redacted] to be able to compare the results between pediatric and adult pharmacokinetic data.

9.7 Efficacy Analysis

The primary analysis will be based on the off-site reader evaluations. The analysis will be performed and presented separately for each reader. There will be no adjustment for multiple readers. There will be 2 primary endpoints for the study: the change from baseline (UEUS to CEUS) in total LV EBD score and proportion of subjects with adequate LV opacification. As we have to achieve success for both endpoints there will be no multiplicity adjustment for the analysis and power consideration.

9.7.1 Primary Efficacy Endpoints - Anatomical Characteristics

The first primary efficacy endpoint will be the change from baseline in total LV EBD scores (UEUS vs. CEUS). Total LV EBD score is calculated as the sum of the individual scores (0, 1, or 2) assigned to each of the 17 segment images with a total score range 0-34.

The co-primary endpoint will be the proportion of subjects with adequate LV opacification. Left ventricular opacification will be graded as 0 to +3, 4-point rating scale. Left ventricular opacification rating of +2 (non-homogeneous) or +3 (complete and homogeneous) will be considered as adequate LV opacification.

9.7.2 Analysis Methods

9.7.2.1 Analysis of LV EBD Score

The null hypothesis of the primary test is that there is no difference between the CEUS and UEUS in the mean total LV EBD score in the pediatric population. The alternative hypothesis is that the CEUS is superior to UEUS in term of the mean total LV EBD score in pediatric population.

H_0: $\mu_{CE} - \mu_{UE} = 0$

H_1: $\mu_{CE} - \mu_{UE} > 0$

where μ_{UE} represents the mean of the total LV EBD score for the UEUS; and μ_{CE} represents the mean of the total LV EBD score for the CEUS.

For each blinded reader assessment, paired t-test will be used to compare total LV EBD score between the CEUS and UEUS. In addition, the 2-sided 95% confidence intervals (CIs) will be calculated for the mean total LV EBD scores for each exam as well as for the change from baseline (UEUS to CEUS). The distribution of the individual scores (0, 1, or 2) assigned to each of the 17 ventricle segments will be presented.

The study will be considered as a success for the primary efficacy endpoint if: 2 of the 3 readers achieve superiority of the CEUS over the UEUS in terms of the mean total LV EBD score. The satisfaction of superiority is met if the alternative hypothesis is concluded, i.e., $\mu CE - \mu UE > 0$ based on paired t-test at significance level of 0.05.

Inter-reader agreement of the assessment for each segment among 3 off-site readers will be evaluated (for UEUS and CEUS separately) by kappa statistic.

The number and percentage of subjects with reduction of inadequate LV EBD in at least one pair of adjacent segments (combined 4-chamber, 2-chamber, and 3-chamber view) following administration of IP will be summarized for each reader.

9.7.2.2 Analysis of LV Opacification

The proportion and its 95% CI of subjects with adequate LV opacification scores at the CEUS will be estimated using binomial proportion estimate method. Assuming expected proportion of subjects with adequate LV opacification scores at the CEUS is 80%. The success criteria for the LV opacification score will be 2 out of 3 blinded readers reach >70% as the lower limit of 95% confidence limit for the proportion of subjects with adequate LV opacification score.

Inter-reader agreement of the assessment of adequate LV opacification for each subject among 3 off-site readers will be evaluated by kappa statistic.

9.8 Sample Size

Sample size determination for this study was based on the 2-tailed paired t-test using software nQuery Advisor 7.0. The primary objective of this trial is to show a significant intra-subject change in total of LV EBD scores for each subject (Hypothesis specified above) from UEUS to LUMASON -enhanced echocardiography. Sample size determination for this study was based on the 2-tailed paired t-test.

A peer-reviewed publication showed [37] that the percentage of endocardial border visualization at unenhanced harmonic images (74%) was significantly lower comparing to a contrast enhanced images (88%) in the suboptimal subjects non-diagnostic imaging group, which translated to the difference of 4 in total LV EBD scores. A previously conducted Bracco-sponsored study [redacted] had the same endpoint of the total LV EBD score range 0-32 assessed on harmonic

images on adult subjects with known or suspected coronary artery disease. The results from the analysis of the suboptimal subjects in this study showed that the average difference of the total LV EBD score between CEUS and UEUS was 3.2; the standard deviation of the difference was 8.3. Based on these results as the assumptions for current study and considering the probability of a Type I error (α) of 0.05 and the probability of a Type II error (β) of 0.10, 73 subjects will be needed to show a difference between CEUS and UEUS for 90% of power.

When the sample size is 73, a 2-sided 95% CI for a single proportion using the large sample normal approximation will extend 9% from the observed proportion for an expected proportion of 80% subjects with adequate LV opacification score. This can be translated as lower limit of 95% CI >70% for the observed proportion of subjects with adequate LV opacification score.

Based on the above considerations, for both endpoints (Total LV EBD score and LV opacification score), total of 73 evaluable subjects are needed. Considering 20% of early dropout, 92 subjects will need to be enrolled.

A subset of 12 subjects is considered to be sufficient for the estimation of the pharmacokinetic parameters, based on the findings of a prior study in healthy subjects.

9.9 Data Handling

All data collected will be entered into the database and displayed in the data listings and tables. Detail of the data handling procedures will be specified in the SAP. Baseline values will be defined as the last measurement prior to administration of the IP.

9.10 Interim Analyses

No interim analysis is planned.

10 Ethics and Good Clinical Practice

10.1 Ethical and Regulatory Compliance

The study will be conducted in accordance with the protocol, ICH, Good Clinical Practice, FDA regulations, ethical principles that have their origin on the Declaration of Helsinki and all applicable local regulations, whichever offers greatest protection for the subject.

10.2 Informed Consent and Subject Information

Written informed consent and permission must be obtained from the subject's parent(s) or legal acceptable representative(s) prior to the implementation of study procedures required in the protocol. This must be done according to the guidelines provided in the Declaration of Helsinki, ICH E6 Guideline for Good Clinical Practice, and as applicable, the requirements of Title 21 CFR 50.20 through 50.27 and related guidance. If the subject is capable, the subject should assent to having the investigational procedures, according to local regulations. This may be

determined by the IRB. The board takes into account age, maturity, and psychological state. Assent may be site specific, as the IRB also determines how the assent is obtained.

Give the parent(s) or the subject's legally acceptable representative a copy of the Informed Consent Form and Assent if applicable. The subject's parent(s), or legal as applicable, must be made aware and agree that personal information may be scrutinized during inspection or audit by authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available.

10.3 Institutional Review Board/Ethics Committee Approval

The protocol, Informed Consent Form, Subject Information Sheet, if applicable, and any advertisement for the recruitment of subjects must be reviewed and approved by an appropriately constituted IRB/EC, as required in chapter 3 of the ICH E6 Guideline, and as applicable, Title 21 CFR 56.107 through 56.115, and related guidance. Written IRB approval must be obtained by Sponsor prior to shipment of investigational product or subject enrollment.

The investigator is committed in accordance with local requirements to inform the IRB/EC of any emergency problem, serious adverse events, and/or protocol amendments.

10.4 Financial Disclosure

Financial support to Investigators/Sub-Investigators other than the cost of conducting the clinical study or other clinical studies will be disclosed where applicable in accordance with Title 21 CFR 54.2 and related guidance.

11 Administrative Considerations

11.1 Regulatory Requirements–Sponsor/Investigator Obligations

This study will be conducted in accordance with the Declaration of Helsinki, ICH E6 Guideline, and as applicable, Title 21 CFR 312.50 through 312.70 and related guidance. To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation by authorized individuals, and competent and regulatory authorities.

11.2 Sponsor Regulatory Obligations for SUSARs Reporting

The Sponsor shall ensure that all relevant information about suspected unexpected serious adverse reaction (SUSAR) which occurs during the course of the clinical trial, whether or not this event is fatal or life-threatening, will be reported to the applicable Regulatory Authority including but not limited to the FDA.

11.3 Protocol Amendment

No change to the protocol may be made without the joint agreement of both the Investigator and Sponsor. Any amendment to the original protocol will be signed by both parties and submitted to the IRB for approval or notification prior to implementation except in circumstances when it is necessary to implement urgent safety measures to remove an immediate hazard to the study subject(s).

11.4 Curriculum Vitae

The Investigator and any sub-investigator(s) must provide Sponsor with current copies of their own signed and dated curriculum vitae.

11.5 Administrative Structure

The administrative structure of the study (e.g., monitoring and vendor personnel, statistician, laboratory facilities, and clinical trial supply management) is presented in (Appendix E). A complete and controlled list of the Investigators, study sites, and IRB's involved in this study can be found in the Trial Master File maintained by the Sponsor.

11.6 Protocol Deviations, Violations and Exceptions

As a matter of policy, Bracco will not grant exceptions to protocol-specific entry criteria to allow subjects to enter a study. If investigative center personnel learn that a subject who did not meet protocol eligibility criteria was entered in a study they must immediately inform Bracco.

If a violation is serious, the CRM takes appropriate action according to the requirements and timelines stated the Code of Federal Regulations, and local regulations as applicable.

11.7 Monitoring Procedures

11.7.1 Study Monitoring

An appropriate representative of Sponsor (Study Monitor) will maintain contact with the Investigator and will visit the study site for the purpose of discussing and/or retrieving data.

An initiation visit will be made by the Study Monitor to discuss the protocol and the obligations of both the Sponsor and the Investigator. The Investigator must allow the Study Monitor to perform periodic, interim monitoring visits. The purposes of these visits are to:

- verify that written Informed Consent was obtained prior to each subject's participation in the study;
- assess the progress of the study;
- review the compliance with the study protocol;
- determine whether all adverse events were appropriately reported;

- determine whether the Investigator is maintaining the essential documents;
- discuss any emergent problem;
- check the Case Report Forms for legibility, accuracy and completeness;
- validate the contents of the Case Report Forms against source documents;
- assess the status of investigational product storage, dispensing and retrieval.

All data required by the protocol must be reported accurately on the Case Report Form and must be consistent with the source documents. Source documents are original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies, magnetic media, X-rays or other diagnostic images, subject files, laboratory records). The Investigator will make available the source documents for inspection. This information will be considered confidential.

The Study Monitor will perform a close-out visit at the conclusion of the Investigator's involvement in the study.

11.7.2 Case Report Form

Sponsor will provide a three-part Case Report Form for each subject. An appropriate Sponsor representative will collect the first and second parts of the form; the Investigator should archive the third part at the investigational site. Case Report Forms must be completed for all subjects who sign Informed Consent even if the subject fails to complete the study.

Case Report Forms must be clearly printed on with a black ball point pen; erasable ink, pencil, or free-hand writing is not acceptable. Any corrections or deletions are to be made by crossing out with a single line (so it is still legible), then initialing and dating by the Investigator or other authorized person. The use of correction fluids to "white-out" mistakes in data entry is not permitted.

If requested, copies of the Case Report Forms are to be made available to the appropriate and Regulatory Authorities.

11.7.3 Inspection and Auditing

The Investigator/Institution will make available for direct access all trial related records including source documentation for inspection by Regulatory Authorities, IRB/EC and for auditing by the Sponsor. This information will be considered confidential.

11.8 Archiving of Records

Essential documents (copies of the protocol, subject identification codes, CRF, source data, Informed Consent Form and other documents) pertaining to the study conduction must be kept for the maximum period of time as required by the study center. This time period must be at least two years after the last approval of the marketing application of the investigational product in an ICH region and until there is no pending or contemplated marketing application in an ICH region or at

least two years have elapsed since the formal discontinuation of clinical development of the investigational product.

No study document should be destroyed without prior written agreement between Sponsor and the Investigator.

Originals of all documentation and copies of outgoing correspondence concerning the study will be stored and retained in a safe area in the Trial Master File of Sponsor for the lifetime of the product. In particular, the final report must be retained by Sponsor, or the subsequent owner, for five years beyond the lifetime of the investigational product.

11.9 Study Results

A final report of the study results will be written by Sponsor or its designee. The Clinical trial report (CTR) will be written within one year of the end of the notification of the study. It will be reviewed and approved by the Investigator when required by local authorities.

11.10 Use and Publication of Study Results

All unpublished documentation (including the Protocol, Case Report Form and Investigator's Brochure) given to the Investigator is strictly confidential. All recipients must agree not to disclose the information herein contained to any person without the prior written authorization of Sponsor. The submission of these documents to the IRB is expressly permitted. The Investigator agrees that Sponsor maintains the right to use the results of this study in their original form and/or in a global report for submission to governmental, competent, and regulatory authorities of any country.

The results of the study may be presented during scientific symposia or published in a scientific journal only after review by Sponsor in accordance with the guidelines set forth in the applicable publication or financial agreement.

11.11 Financing

A financial agreement (separate from the protocol) will be made with the Investigator or designee. Such agreement will be archived in the relevant file.

11.12 Change in Investigator

In the event that the Investigator is unable to continue the study, another suitable person at the site will become the designated Investigator, and documentation testifying to this will be submitted to Sponsor. The new Investigator must be acceptable to both Sponsor and the IRB before the study can be continued.

11.13 Definition of the End of the Study

The end of the study is defined as the last subject's image review conducted by the off-site blinded assessor.

11.14 Premature Termination of the Study

If Sponsor, the Investigator, or the IRB should discover conditions arising during the study that indicate the study should be prematurely terminated, an appropriate schedule for termination will be instituted. If the Investigator prematurely terminates the study, an explanatory letter must be provided to Sponsor.

Sponsor also reserves the right to discontinue this study for administrative reasons at any time. The Investigator will be reimbursed for reasonable expenses incurred, if it is necessary to prematurely terminate the study or an individual subject's participation. Sponsor will not reimburse the Investigator for the evaluation of subjects if the evaluations are not conducted in compliance with the present protocol.

11.15 Information Material

Before the beginning of the study the Investigator will be given the current version of reference safety document, Investigator's Brochure. If the Investigator Brochure is revised during the study, the Investigator will receive a copy of the revised version. The Investigator's Brochure and the protocol are confidential communications of Sponsor. Acceptance constitutes the agreement by the recipient that no unpublished information herein contained will be published or disclosed without Sponsor's prior written approval except that this document may be disclosed to appropriate IRB as long as they are required to keep it confidential.

11.16 Insurance

Whenever applicable, the Sponsor will provide verification of insurance coverage for damages emerging from the study and involving test subjects treated with the investigational product. The Investigator will be supplied with all data concerning the insurance company and policy number for a maximum sum insurable as stated in the Subject Information Sheet and Informed Consent Form.

12 Confidentiality

All information provided to the Investigator dealing with the investigational product will be regarded as confidential. The members of the research team agree not to discuss such information in any way without prior written permission from Sponsor.

13 Protocol Acceptance

I agree to conduct this clinical study according to the above protocol and to make no additions or changes without prior consent of Sponsor and in accordance with good clinical practice and local requirements/regulations.

_____ _____

Investigator Date

_____

Investigator (Printed Name)

_____ _____

For Bracco Date

14 References

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Appendix A: Investigational Product Labeling

On the clinical supply box the following label will be applied:

Carton Label

For US Sites

LUMASON ™ **IND 46,958** **Protocol No. BR1-140** Contents: 1 vial of 25 mg lyophilized powder 1 pre-filled syringe with 5 mL NaCl 0.9% w/v 1 mini spike Store at 25 °C (77 °F) excursions permitted from 15-30°C (59-86°F) **CAUTION: New Drug - Limited by Federal Law to Investigational Use.** Batch No.: Exp. Date: Bracco Diagnostics Inc., Monroe Twp, NJ 08831

Vial Label

For US Sites

LUMASON™ 25mg **Protocol BR1-140** Store at 25° C (77 °F) excursions permitted from 15-30°C (59-86°F) For i.v. use after reconstitution with 5 mL of saline. **CAUTION: New Drug - Limited by Federal Law to Investigational Use.** **Subject No**.____ **Initials:**____ Batch No.: Exp. Date: Bracco Diagnostics Inc., Monroe Twp, NJ 08831	LUMASON™ 25mg **Protocol BR1-140** Store at 25° C (77 °F) excursions permitted from 15-30°C (59-86°F). For i.v. use after reconstitution with 5 mL of saline. **CAUTION: New Drug - Limited by Federal Law to Investigational Use**. **Subject No.____** **Initials:____** Batch No.: Exp. Date: Bracco Diagnostics Inc., Monroe Twp, NJ 08831

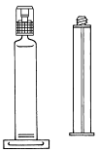
Once a vial is assigned to a subject and is reconstituted <u>one label</u> will remain on the vial while the <u>second one</u> will be removed and adhered to the CRF for that subject.

Appendix B: Investigational Product Preparation

LUMASON Reconstitution

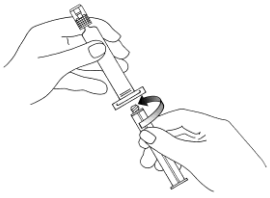
LUMASON is supplied within a kit containing the following:

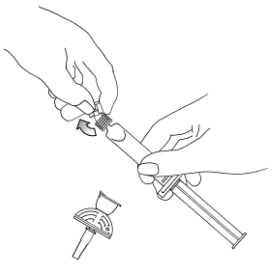
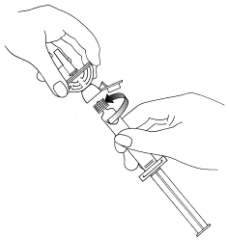
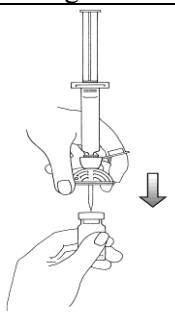
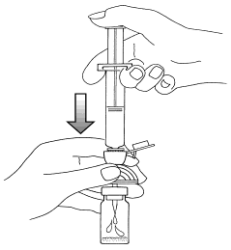
- a clear glass vial labeled as LUMASON (sulfur hexafluoride lipid-type A microspheres) for Injectable Suspension,
- a prefilled syringe labeled as Sodium Chloride Injection, USP, 0.9% Sodium Chloride and
- a Mini-Spike.

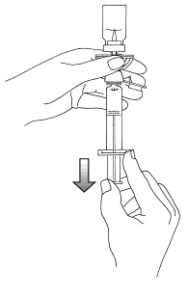
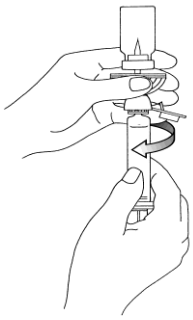
		
Glass vial	Prefilled syringe and its plunger rod	Mini-Spike

Reconstitution steps:

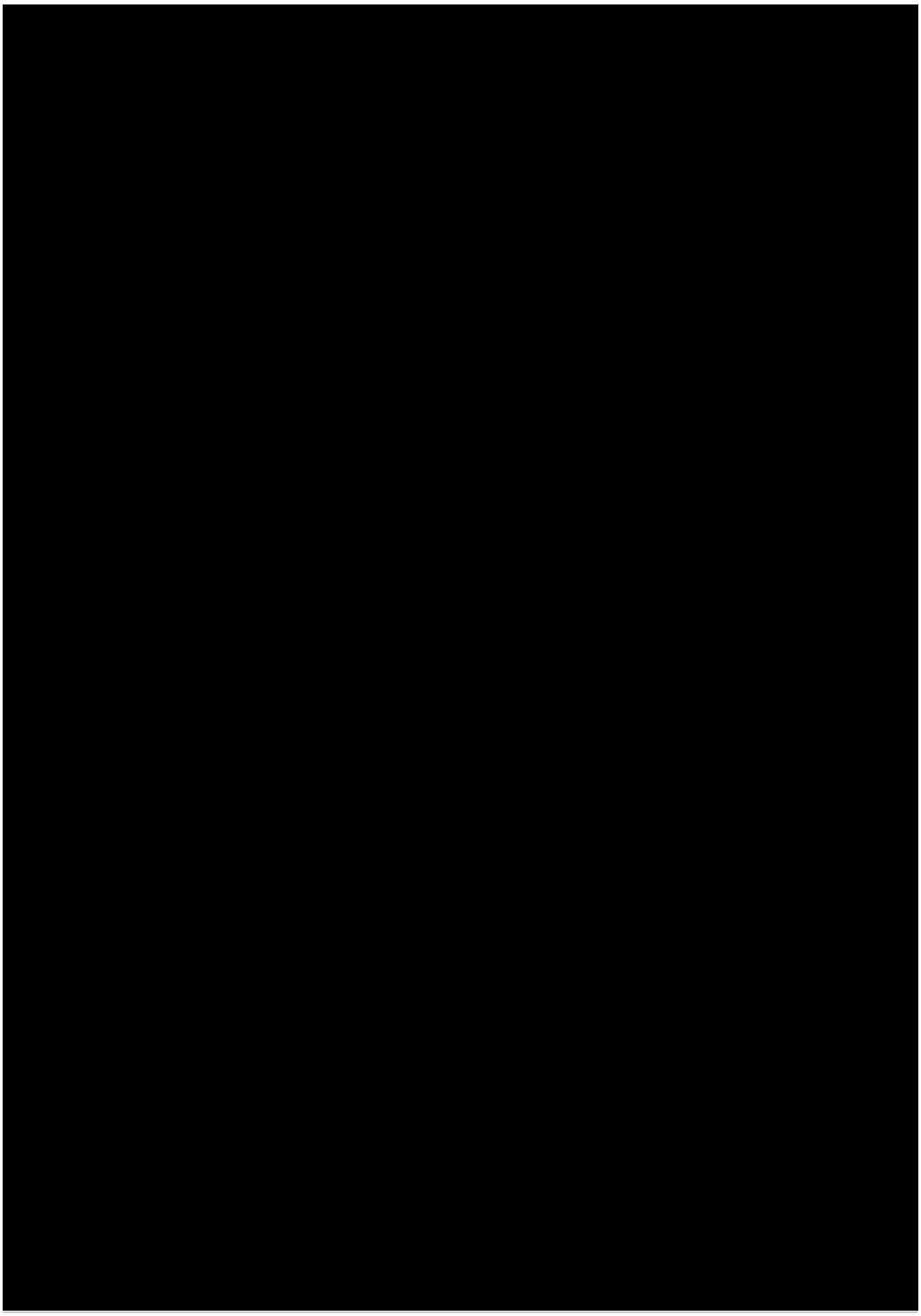
- Prior to LUMASON reconstitution, inspect the kit and its components for signs of damage. Do not use the kit if the protective caps on the vial and prefilled syringe are not intact or if the kit shows other signs of damage.
- Perform all LUMASON reconstitution steps under aseptic conditions. The LUMASON vial and the prefilled syringe do not contain a bacteriostatic preservative.
- LUMASON is reconstituted by injecting the prefilled syringe contents (5 mL saline) into the LUMASON vial using the following illustrated steps below.

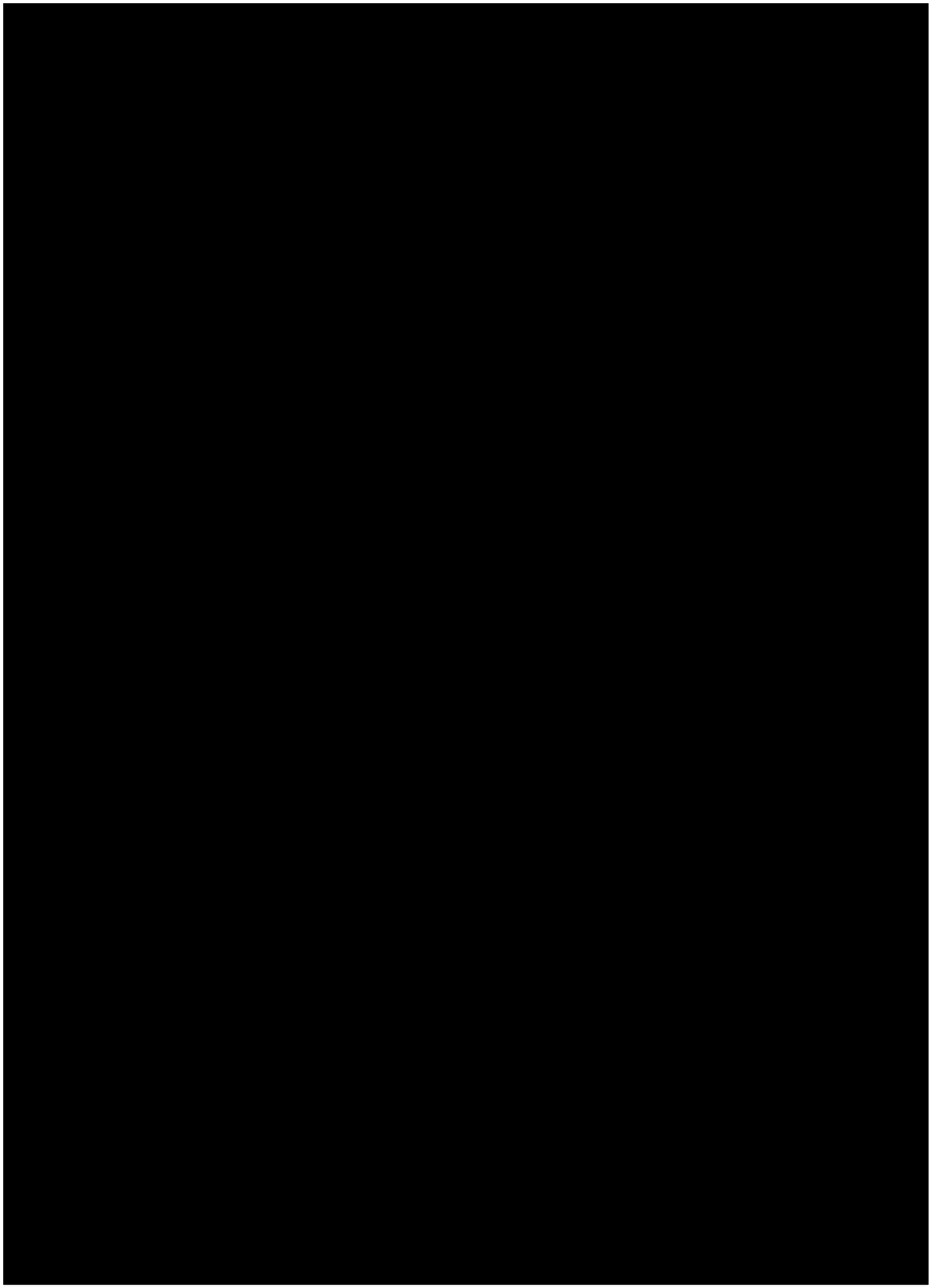
1. Connect the plunger rod to the prefilled syringe barrel by screwing it clockwise into the syringe (see Figure 1).	 Figure 1.

2. Open the Mini-Spike transfer system blister and remove the syringe tip cap (see Figure 2).	 Figure 2.
3. Open the Mini-Spike green cap and connect the syringe to the Mini-Spike by screwing it in clockwise (see Figure 3).	 Figure 3.
4. Remove the flip cap plastic protective cap from the vial, remove the Mini-Spike spike protection and position the spike in the center of the rubber stopper of the vial. Press firmly inward until the spike is fully inserted in the stopper (see Figure 4).	 Figure 4.
5. Empty the content of the syringe into the vial by pushing on the plunger rod (see Figure 5).	 Figure 5.
6. Shake vigorously for 20 seconds, mixing all the contents in the vial (see Figure 6). A homogeneous white milky liquid indicates formation of sulfur hexafluoride lipid microspheres.	 Figure 6.

7. Invert the system and slowly withdraw the needed volume of suspension into the syringe (see Figure 7).	 Figure 7.
8. Unscrew the syringe from the Mini-Spike (see Figure 8). Immediately connect the syringe to the dose administration line (20 G) and administer as directed under section 6.4.	 Figure 8.

Appendix C: Serious Adverse Event Report





Appendix D: Investigator Statement

Investigational Product	**LUMASON™**
Protocol No.	**BR1-140**
Protocol Title:	**A Multicenter Clinical Evaluation of Safety and Efficacy of Lumason™ as a Contrast Agent in Pediatric Echocardiography**
Investigator:	
Study Site:	

COMMITMENTS

By signing this document, I agree to conduct the study as outlined in the protocol and in accordance with: Title 21 CFR 56 - Institutional Review Boards and related guidance (if applicable), and the Declaration of Helsinki, as well as, all applicable government regulations, Good Clinical Practice and also

I declare:

1) I am well qualified by scientific training and experience to conduct investigational studies in the clinical area of the proposed study and I am affiliated with a recognized medical school or with an independent institution recognized for its excellence.
2) I have received and understand the information about pharmacology, toxicology and possible risks and side effects of the investigational product (e.g., as described in the Reference Safety Information).
3) I shall provide information to all staff members involved in the study about their obligations as described in this document.
4) I shall submit the protocol, Informed Consent Form/Subject Information Sheet and other required documentation to the IRB/EC for review and approval.
5) I shall make no changes to the protocol without formal amendment (prepared in agreement with the Sponsor), except when necessary to protect the safety, the rights or welfare of subjects. In this last case I will inform the Sponsor of the change.
6) I shall require Informed Consent from each subject prior to enrollment into the study. The Informed Consent shall be documented by use of a written consent form approved by the IRB/EC.

7) I shall use the investigational product only in compliance with the study protocol and I shall be responsible for the security and accountability of clinical study supplies.

8) I shall notify the Sponsor immediately or no later than 24 hours by telephone and/or by fax of serious adverse events and submit written reports of serious adverse events, as outlined in the protocol, to Sponsor.

9) I shall submit a written report of adverse events to the IRB, as required by applicable regulatory requirements.

10) I shall complete the Sponsor's Case Report Form (CRF) in a timely and legible manner.

11) I shall maintain accurate source records (hospital or other institutional records), which will support the data entered into Case Report Forms and I shall maintain these as specified in the protocol.

12) I shall retain essential documents (Investigator's files) including study codes for at least 5 years (or longer if required by law or agreements with the Sponsor) after completion or discontinuation of the trial and, in any case, no documentation will be destroyed without prior written agreement with the Sponsor.

13) I shall allow monitoring visits by Sponsor's representatives a predetermined frequency.

14) I shall allow the authorized Sponsor representative and any competent and regulatory authorities to inspect the facilities and pertinent records at reasonable times and in a manner which ensure subject confidentiality.

15) I shall maintain confidentiality about all information concerning the investigational product, such as patent applications, formulas, manufacturing process, basic scientific data and formulation information supplied by the Sponsor and not previously published and I shall not disclose this information to a third party without the written consent of the Sponsor.

16) I shall permit the information developed in the clinical study to be used by the Sponsor in connection with the development of the investigational product and may be disclosed to the IRB and regulatory authorities.

Following completion of the study, the data may be considered for reporting at a scientific meeting and/or for publication in a scientific journal. A copy of the manuscript or abstract will be provided to the Sponsor for review before submission to a scientific journal for publication and/or a scientific meeting selection committee for oral or poster presentation. Subgroup or individual Investigator publications must not interfere or compromise publication of the multi-center results of this clinical study.

_____ _____

Investigator Date

_____

Investigator (Printed Name)

Appendix E: Administrative Structure

North America

Clinical Research Personnel	
[redacted]	[redacted]
CROs / Central Labs	
Study Monitoring	***Central Lab - TBD***
	PK Lab- TBD
TBD	***Central ECG - TBD***
	Central Imaging Lab - TBD