CONFIDENTIAL

A Prospective Multicenter Phase III Clinical Evaluation of the Safety and Efficacy of Lumason™/SonoVue® in Subjects Undergoing Pharmacologic Stress Echocardiography with Dobutamine for the Diagnosis of Coronary Artery Disease

Lumason™

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Abbreviations and Definition of Terms

ACC	American College of Cardiology
AE	Adverse Event
AHA	American Heart Association
ALT	Alanine aminotransferase
ASE	American Society of Echocardiography
AST	Aspartate aminotransferase
BMI	Body Mass Index
ºC	Degrees Centigrade
CA	Competent Authority
CAD	Coronary Artery Disease
CD	Compact Disc
CE	Contrast Enhanced
CE-DSE	Contrast Enhanced-Dobutamine Stress Echocardiography
CEUS	Contrast-Enhanced Ultrasound
CFR	Code of Federal Regulations
CI	Confidence Interval
CK-MB	Creatine Kinase-Myocardial B
CMRI	Cardiac Magnetic Resonance Imaging
CRF	Case Report Form
CRM	Clinical Research Manager
CT	Computed Tomography
CTR	Clinical Trial Report
DSE	Dobutamine Stress Echocardiography
DVD	Digital Versatile Disc
EAE	European Association of Echocardiography
EB	Endocardial Border
EBD	Endocardial Border Delineation
EC	Ethics Committee
ECG	Electrocardiogram
EudraCT	Database of European Clinical Trials
EU	European Union
ºF	Degrees Fahrenheit
FN	False Negative
FP	False Positive
FDA	Food and Drug Administration
GGT	Gamma-Glutamyl Transpeptidase
h	Hour
IB	Investigator's Brochure
ICH	International Conference on Harmonization

IND	Investigational New Drug
Investigational product	Compound under investigation
IP/IMP	Investigational Product/Investigational Medicinal Product
IRB	Institutional Review Board
ITD	Intention to Diagnose
kg	Killogram
LAD	Left Anterior Descending
LCx	Left Circumflex
LM	Left Main
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
LVO	Left Ventricle Opacification
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
MI	Mechanical Index
min	Minute
mL	Milliliter
mmHg	Millimeter of Mercury
MOD	Magnetic Optical Disc
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
mSv	Millisievert
NIMP	Non-Investigational Medicinal Product
PI	Package Insert
RBC	Red Blood cell Count (total erythrocyte count)
RCA	Right Coronary Artery
REB	Research Ethics Board
ROW	Rest of the World – countries outside of Americas, the European Union, and Japan.
S	Second
SAE	Serious Adverse Event
SAER	Serious Adverse Event Report
SAP	Statistical Analysis Plan
SF6	Sulfur Hexafluoride
SPC/SmPC	Summary of Product Characteristics
SPECT	Single Photon Emission Computed Tomography
SUSAR	Suspected Unexpected Serious Adverse Reaction
TN	True Negative
TP	True Positive
UCA	Ultrasound Contrast Agent
UE	Unenhanced
UE-DSE	Unenhanced-Dobutamine Stress Echocardiography
UEUS	Unenhanced Ultrasound

US	Ultrasound
USA	United States of America
USP	United States Pharmacopeia
WBC	White Blood Cell Count (total leukocyte count)
WHO	World Health Organization
βHCG	Beta Human Chorionic Gonadotropin
2D	2 Dimensional

1 Title of Study

A *Prospective Multicenter Phase III Clinical Evaluation of the Safety and Efficacy of Lumason™/SonoVue® in Subjects Undergoing Pharmacologic Stress Echocardiography with Dobutamine for the Diagnosis of Coronary Artery Disease*

2 Protocol Number

This study is being conducted under protocol number: BR1-142.
IND number: 46,958
EudraCT number: 2015-001962-25.

3 Introduction

Stress echocardiography is an established imaging modality for the detection of coronary artery disease (CAD). [1,2,3,4,5,6,7] The primary indication for conventional stress echocardiography is to indirectly detect angiographically significant (≥50% diameter) stenoses by inducing ischemia or to assess the patient's risk for a cardiac event.[5,8,9,10] Increasingly stress echo techniques have been shown to offer valuable prognostic information in the risk evaluation of stable chronic CAD.[5,10,11] Significant studies (> 100 patients) of dobutamine stress echocardiography (DSE) show a range for sensitivity of between 61–95%, while that for specificity ranged from 51–95%[11] for the diagnosis of CAD.[1] The detection of CAD with stress echocardiography is based on the observation of contractile dysfunction in any myocardial segment at rest or with stress; therefore, complete visualization of all left ventricular (LV) endocardial borders (EB) is necessary to document or exclude abnormalities of regional myocardial wall thickening confidently.[12,13] Interpretation of wall thickening is qualitative, highly dependent on the skill and experience of the reviewing physician, and is affected considerably by quality of endocardial border delineation (EBD).[12,13] Numerous patient factors may produce suboptimal images with poor EBD. Given, in addition, the challenges imposed by excessive cardiac motion due to hyperventilation and tachycardia at peak stress, non-diagnostic or poor-quality images may occur in up to 30% of patients.[12,13] The advent of digital side-by-side analysis, standardized reporting criteria, and generalized use of tissue harmonic imaging has reduced, but not overcome, this problem.[12]

Use of ultrasound contrast agents (UCAs) has shown to improve EBD during stress echocardiography and is recommended for use in patients with poor unenhanced image quality by both the American Society of Echocardiography (ASE) and the European Association of Echocardiography (EAE).[12,13] However, while Lumason™ (sulfur hexafluoride lipid-type A microspheres) with an approved trade name in the USA/SonoVue® (sulfur hexafluoride microbubbles) with an approved trade name for everywhere else in the world referred to hereafter as LUMASON, is approved for use in echocardiography both at rest and stress in the

European Union (EU) and Canada, no UCA is currently approved in the United States of America (USA) for use in stress echocardiography to improve LV EBD in patients with suboptimal unenhanced imaging.

Although generalized use of tissue harmonic imaging has improved image quality, interpretation of stress echocardiograms may still be limited in patients with poor acoustic windows.[12,14] Recent studies indicate that contrast-enhanced 2 dimensional (2D) echocardiography with harmonic imaging had excellent correlation with radionuclide, magnetic resonance (MR) and computed tomographic (CT) measurements of LV volumes and left ventricular ejection fraction (LVEF) with improved inter-observer agreement and physician interpretation confidence compared to unenhanced harmonic imaging in adult population.[15,16] Increasing accuracy of LVEF measurements have been associated with contrast-enhanced harmonic imaging over unenhanced harmonic imaging and fundamental imaging, respectively.[17,18]

Non-diagnostic or poor-quality images may occur in up to 30% of patients during stress because of challenges imposed by excessive cardiac motion due to hyperventilation and tachycardia at peak stress. There are documented benefits through published data regarding the value of the use of contrast agents in stress echocardiography with respect to improvement in image quality and EBD, which further translates into enhanced interpretation of regional and global function with decreased inter- and intra-observer variability.[4,12] Previous investigations have demonstrated that improving the quality of stress echocardiography images, using contrast agents enables superior visualization of EBD at rest and peak stress across a range of unenhanced baseline image quality (i.e., greatest improvement is seen in patients with the poorest baseline images)with completeness of wall-segment visualization, at both rest and peak stress.[6,19]The ASE[1] and American College of Cardiology (ACC)[20] recommendations for use of contrast enhancement in stress testing have been supported by peer reviewed publications where the importance of adequate visualization of segments for accurate diagnosis have been emphasized.[1,4,6,19 ,20]

Due to the increased routine use of DSE as a non-invasive cardiac imaging tool in adults with suspected or known CAD for the detection and/or evaluation of CAD and an increasing number of suboptimal, non-diagnostic quality images mostly with respect to the challenges imposed by excessive cardiac motion due to hyperventilation and tachycardia at peak stress, we aim to study the safety and efficacy of LUMASON-enhanced stress echocardiography with dobutamine in subjects with suboptimal LV EBD at unenhanced echocardiography. This study is being conducted as part of the Phase III program in support of a supplemental New Drug Application (sNDA) to be filed with the Food and Drug Administration (FDA).

4 Study Objectives

Primary Objectives:

- To assess the efficacy of LUMASON-enhanced dobutamine stress echocardiography (DSE) in subjects with suspected or known CAD having suboptimal left ventricular (LV) endocardial border delineation (EBD) at unenhanced echocardiography in terms of:

- Sensitivity and specificity for the detection or exclusion of CAD in unenhanced versus LUMASON-enhanced DSE using coronary angiography or clinical follow-up as the truth standard;
- Critical shift from suboptimal (≥2 adjacent segments inadequate on any apical view) at unenhanced dobutamine stress echocardiography (UE-DSE) to adequate images (reduction of suboptimal adjacent segments) for LV EBD at contrast-enhanced dobutamine stress echocardiography (CE-DSE).

Secondary Objectives:

- Change from peak stress non-contrast ultrasound (UE-DSE) versus peak stress contrast-enhanced ultrasound (CE-DSE) in total LV EBD score.
- To obtain safety data in subjects undergoing DSE with LUMASON.

5 Investigational Plan

5.1 Overall Study Design Description

This is a Phase III, prospective, multicenter, within-patient comparison of UE-DSE and CE-DSE that will be conducted at 10-15 sites in subjects with a suboptimal LV EBD on non-contrast 2D transthoracic echocardiography with harmonic imaging at rest and that are scheduled for coronary angiography. Suboptimal LV EBD is defined as published in guidelines, having ≥2 adjacent segments on any apical view that are suboptimal[1,12] during the rest non-contrast ultrasound examination.

Imaging conditions will be representative of those used in routine clinical practice and will include LV EBD, and LV wall motion using harmonic imaging modality with commercially available equipment.

Subjects who are enrolled in the study will undergo a rest non-contrast echocardiogram (UEUS) with the standard apical 4-, 2-, and 3-chamber views obtained with harmonic imaging. A 2 mL dose of LUMASON will be administered as an intravenous bolus injection during rest contrast enhanced echocardiography (CEUS). A recording of 2D transthoracic harmonic echocardiography images will be performed for both UEUS and CEUS rest imaging. At least 30 minutes after the completion of the rest unenhanced echocardiography (UEUS) and contrast enhanced-echocardiography (CEUS) the dobutamine infusion will begin. Once peak stress is achieved (with or without the addition of atropine), subjects will undergo unenhanced dobutamine stress echocardiography (UE-DSE). This will be followed by the contrast-enhanced dobutamine stress echocardiography (CE-DSE) with the administration of 2 mL of LUMASON. A recording of 2D transthoracic images will be performed for both UE-DSE and CE-DSE imaging. The on-site Investigators will provide an overall diagnosis of CAD for each subject from the unenhanced (UEUS and UE-DSE) and contrast-enhanced (CEUS and CE-DSE) echocardiographic images.

The truth standard in this study will be coronary angiography. If coronary angiography is not performed or is indeterminate subjects will be followed for up to 6 months to collect cardiac events.

An off-site assessment of the echocardiographic images will be performed by 3 blinded readers unaffiliated with any of the investigational sites, blinded to the subject's clinical information. The efficacy analysis will be based on the blinded reader evaluations of LV EBD, wall motion abnormalities and diagnosis of CAD.

A fourth blinded reader will assess the coronary angiography images and provide a diagnosis of CAD. The fourth reader will be blinded to the specifics of the protocol including the patient population and the results of the ultrasound examination, but will receive clinical information such as the medical history on the subject.

Safety will be evaluated in this study by the collection of concomitant medications and the monitoring of adverse events, vital signs, electrocardiograms (discrete & continuous), laboratory tests and continuous pulse oximetry.

This study is planned to be conducted in the United States, Canada and Europe.

5.2 Discussion of Study Design

The use of UCAs has shown to improve EBD during stress echocardiography and is recommended for use in patients with poor unenhanced image quality by both the ASE and the EAE.[12,13,21] However, while LUMASON (under the trade name SonoVue) is approved for use in echocardiography both at rest and stress in the EU, no UCA is currently approved in the USA for use in stress echocardiography to improve LV EBD in patients with suboptimal unenhanced imaging.

The mechanism of action of LUMASON during stress echocardiography would be exactly the same as during an exam at rest. LUMASON would be used in subjects with suboptimal images to enhance the LV cavity and improve the detection of the EB. This Phase III study will, therefore, be conducted with a consistent design (dose, study population and endpoints of both LV EBD score and Sensitivity/Specificity for detection of CAD) based on the Phase III studies previously conducted to obtain approval for the use of LUMASON in echocardiography at rest[22,23,24] and a previous large, multicenter, dose-ranging study of LUMASON in stress echocardiography[25].

The study is designed to assess the safety and efficacy of LUMASON at improving the visualization of the LV EBD during pharmacologic stress echocardiography examinations and for detection or exclusion of CAD.

The study population will consist of adult subjects referred for pharmacologic stress echocardiography and with suboptimal image quality during unenhanced ultrasound imaging at rest who have known or suspected CAD. Subjects to be enrolled in the current study are representative of subjects who could benefit most from CEUS stress echocardiography.

Imaging conditions will be representative of those used in routine clinical practice and will include LV EBD using harmonic imaging modality. Each subject will undergo an unenhanced

echocardiogram (UEUS) followed by a LUMASON-enhanced echocardiogram (CEUS) both at rest. The same applies to the stress echocardiogram when echocardiographic images will be obtained immediately after dobutamine administration (UE-DSE) and then LUMASON will be administered and LUMASON-enhanced stress images (CE-DSE) will be captured. Thus, each subject will serve as its own control and is designed as an intra-patient controlled study.

The current guidelines of American Heart Association (AHA)[26] and ASE[12] while they suggest ultrasound contrast use in difficult to image patients with suboptimal image quality at their presenting rest echocardiography exams, their definition of a suboptimal study is when 2 contiguous segments are not visualized in any of the standard 3 apical views. The same criteria will be applied in adults during peak stress imaging, representative of those used in routine clinical practice and will include assessment of LV EBD using harmonic imaging modality.

Taking into consideration the clinical importance, both the diagnostic performance and the LV EBD visualization are defined as co-primary efficacy endpoints for the study.

- First co-primary efficacy endpoint is a comparison of diagnostic performance (sensitivity and specificity) for the detection or exclusion of CAD in unenhanced (UE-DSE) versus Lumason-enhanced DSE (CE-DSE) using coronary angiography or clinical events based on 6 month follow-up as the truth standard. Subjects will be considered to have positive CAD if they have at least one vessel with stenosis ≥ 50% based on the assessment of off-site reader for coronary angiography or have one of the clinical events (cardiac death, non-fatal myocardial infarction, revascularization and need for revascularization) within the 6 month follow-up.
- Second co-primary efficacy endpoint is critical shift from suboptimal (≥2 adjacent segments inadequate on any apical view) at unenhanced dobutamine stress echocardiography (UE-DSE) to adequate images (reduction of suboptimal adjacent segments) for LV EBD at contrast-enhanced dobutamine stress echocardiography (CE-DSE).

The secondary efficacy endpoint of LUMASON studies (change from baseline in total of LV EBD score) is the same endpoint for adult studies for LV EBD indication at rest echo examinations.

The muscle and cavity of the LV will be divided into 17 segments (17-segment model). The 17-segment model will be utilized as recommended by the ASE guidelines. [1,12,27] The standard views of a 2D echocardiogram as defined by the ASE are all used as part of a 2D echocardiogram at rest and stress.[26] The clinical utility of improvement in LV EBD resulting from opacification of the 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.[12]

Unevaluable image are defined as images with inadequate visualization of segments comprising all coronary artery territories due to 1 or more of the following artifacts: motion artifacts, lateral wall drop out, swirling or attenuation artifacts. From the published literature[6] and previous Bracco-sponsored study[25] 8% of the UE-DSE images are expected to be unevaluable.

Stress can be induced by either physical exercise or pharmacologic agents. Physical stress was the first reported and used form of stress echocardiography, but it has several disadvantages since during exercise it is not possible to perform close echocardiographic monitoring and recordings are not reliable because of hyperventilation. Pharmacologic stress overcomes these disadvantages.

The pharmacologic stress agent used in this study will be dobutamine. Dobutamine is a direct-acting inotropic agent whose primary activity results from stimulation of the beta-adrenoreceptors of the heart while producing comparatively mild chronotropic, hypertensive, arrhythmogenic, and vasodilative effects. Dobutamine acts primarily on beta-1 adrenergic receptors, with little effect on beta-2 or alpha receptors. In the USA, dobutamine is indicated when parenteral therapy is necessary for inotropic support in the short-term treatment of patients with cardiac decompensation due to depressed contractility resulting either from organic heart disease or from cardiac surgical procedures. In the EU and other countries, dobutamine is also indicated for use in stress echocardiography as an alternative to exercise in patients for whom routine exercise cannot be satisfactorily performed. Even if not approved for use in stress echocardiography in the USA, dobutamine is recommended as the pharmacologic stressor of choice for stress echocardiography by the ACC Foundation Appropriateness[20] Criteria Task Force, the ASE, and the AHA. [1,26] Dobutamine became the preferred stressor in USA as the AHA/ACC guidelines stated that "dobutamine stress echo has higher sensitivity than vasodilator stress echo for detection of CAD."[28,29] The recent 2007 recommendations on stress echocardiography of the ASE conclude that "although vasodilators may have advantages for assessment of myocardial perfusion, dobutamine is preferred when the test is based on assessment of regional wall motion." [1]

Based on the Phase III studies conducted to obtain approval for use of LUMASON in echocardiography at rest[22,23,24] and a previous large, multicenter, dose-ranging study of LUMASON in stress echocardiography[25] the proposed dose of LUMASON is 2 mL bolus injection during the rest examination, and then again as a single 2 mL bolus injection after administration of the stress agent, dobutamine, and the UEUS stress examination is completed. There will be at least a 30 minute period between rest and stress LUMASON injections.

The off-site assessments of echocardiographic images will be performed by experienced cardiologists, unaffiliated with the enrolment centers and blinded to the subject's clinical information. Blinded off-site assessments are used to evaluate diagnostic agents in order to eliminate the potential bias associated with on-site assessments due to the investigator's knowledge of the subject's medical history, clinical findings from other modalities, or other clinical information. Thus, the off-site assessments allow for a blinded evaluation of efficacy which is not possible for the on-site assessments.

All subjects will have a truth standard of either coronary angiography or, if coronary angiography is indeterminate or not performed, they will be followed for up to 6 months to collect cardiac events. Although we will study the sensitivity and specificity in detecting anatomic stenoses ≥50% in severity, not all the subjects will undergo coronary angiography. Therefore, subjects who do not undergo coronary angiography in the study will be followed for cardiac events (cardiac death, non-fatal myocardial infarction, revascularization and need for

revascularization) at 6 months. The proposed cardiac events as hard end points in the study are widely used, well recognized, robust, measurements of a non-invasive test to predict cardiac events.[5,7,10]

The coronary angiography images will be reviewed by a single, off-site reader who is an experienced cardiologist to provide a centralized reading for the confirmation of the diagnosis of CAD. He/she will be blinded to the results of the stress echo examination. Medical historical information will be provided to the reader. This will allow the gold standard to achieve the maximum accuracy and reliability. It will also serve to reduce the variability of readings performed by many different unblinded readers on-site across all study sites.

5.2.1 Risk-Benefit Considerations

Despite substantial technical improvements in imaging modalities that have an impact on image quality on the equipment side, approximately 30% of patients continue to undergo stress echocardiographic examinations that are non-diagnostic or inconclusive due to poor image quality, with subsequent poor evaluation of cardiac function, leading to the need for more invasive techniques. [1,12 ,30] These patients would benefit from a contrast agent that can opacify the LV and improve the delineation of EBs.

Given the fact that the subjects to be enrolled in the present clinical trial will be subjects already having suboptimal echocardiographic images and have no evidence of intracardiac shunts, inclusion in the study is unlikely to add any risk for the subjects. Due to the fact that subjects who are contraindicated for dobutamine will already be excluded from this study, dobutamine used as the stressor in this study will not add any additional risk to the subjects.

Even though the subjects have been scheduled for coronary angiography most likely have already undergone other diagnostic imaging modalities such as DSE or SPECT, the contrast enhanced DSE will still add incremental value with better EBD which will translate into better recognition of wall motion abnormalities. Furthermore, CEUS stress echocardiography will prevent the risk of those subjects being exposed to radiation through other imaging modalities in the down streaming of appropriate assessment of cardiac function and structures.

LUMASON efficacy and safety is based on data from 75 clinical trials in 6307 subjects and post-marketing surveillance over 12 years of market use in an estimated 2.7 million patients. Hypersensitivity to any of the components of LUMASON, as for other contrast agents, cannot be excluded, and there is potential for unpredictable, serious hypersensitivity reactions, including anaphylactoid reactions. Further, such reactions may lead to life-threatening conditions if they occur in critically ill patients. However, serious hypersensitivity reactions after LUMASON administration are rare (estimated occurrence of approx. 1 in 10,000 exposures) and suboptimal EBD and inaccurate assessment of LV function (global and regional wall motion assessment) and/or cardiac anatomy are frequent in a patient population with suspected CAD.[12,13,31] Echocardiography is an imaging modality that represents a fundamental investigation used in the initial and ongoing management of patients with suspected or known CAD because it is noninvasive, provides easy access, anytime and any day, can be performed at the bedside of the patient, and can be repeated as often as needed for monitoring. The balance of the benefit of LUMASON in comparison with its risks is also positively affected by the availability of acute

care settings and physicians that can promptly and effectively recognize and manage serious adverse events.

Although optional noninvasive modalities (SPECT, CMRI and CT angiography) may provide diagnostic accuracy, valuable and detailed data, all of them pose greater risk than transthoracic echocardiograms.[32] [33] Probably the most critical drawback is the time delay in obtaining the data needed for urgent risk assessment and treatment, but safety concerns including exposure to radioactive material and risky patient transportation to the nuclear/MRI department are also present.[21] Furthermore, if an imaging agent is used, these imaging modalities carry the same risk of rare, serious hypersensitivity reactions and the same risk of serious, life-threatening complications.

Of note, at the patient level, the effective dose of a single nuclear cardiology stress imaging scan ranges from 10 to 27 mSv. The corresponding equivalent dose exposure is 500 chest X-rays [Cardiolite® (Tc-99m Sestamibi)], 1200 chest X-rays (Thallium) and 1300 chest X-rays (with dual imaging protocol). According to the latest estimates the risk of cancer for a middle aged patient ranges from 1 in 1000 (for Cardiolite) to 1 in 400 (for a dual isotope scan).[21] Therefore, contrast-enhanced stress echocardiography should be preferred due to wider availability and for its radiation free nature. Overall, the effectiveness and suitability of LUMASON as a contrast agent for use with echocardiography to obtain improve LV EBD has been demonstrated. Clinical studies have shown LUMASON to be safe and well tolerated with minimal risk to subjects. In conclusion, the benefit-to-risk ratio for LUMASON is high, indicating an advantage to subjects undergoing stress echocardiography.

With the marked increase in costs associated with cardiovascular care and interventions, it is vital that non-invasive imaging procedures be highly accurate in detecting whether a patient is at risk for coronary events, not just whether they have a significant coronary stenosis. It is also vital that these stress imaging procedures be equally accurate in identifying which patients are at risk for future cardiac events.

5.3 Study Duration

The procedure associated with the LUMASON administration will be completed within approximately 1 hour. Safety monitoring will begin at the time of signing Informed Consent and continue for 72 hours after the last LUMASON administration or until the subject undergoes cardiac intervention, whichever comes first. Subjects will be followed up to 6 months to collect truth standard data (coronary angiography or cardiac events) as described in Section 8.6.4.

5.4 Study Population

The study will be conducted in subjects who have suboptimal image quality on UEUS echocardiography at rest and are scheduled for coronary angiography. It is expected that 10-15 study centers will participate in the study. Approximately 175 subjects will be enrolled in order to obtain 45 subjects with positive CAD and 45 with negative CAD based on the truth standard. The prevalence of positive CAD subjects will be tracked based on the onsite diagnosis of CAD from the truth standard. If the overall yield of positive CAD subjects is lower than the assumed

30%,[34] then additional subjects will need to be enrolled in order to obtain a sufficient number of positive CAD subjects.

5.4.1 Inclusion Criteria

Enroll a subject in this study if the subject meets the following inclusion criteria:

- Provides written Informed Consent and is willing to comply with protocol requirements;
- Is at least 18 years of age;
- Has suspected or known CAD and is scheduled to undergo coronary angiography within 6 months after the LUMASON DSE.
- Has undergone a previous echocardiography prior to enrollment; resulting in suboptimal unenhanced images at rest, defined as ≥ 2 suboptimal adjacent segments in any apical view.

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:

- Is a pregnant or lactating female. Exclude the possibility of pregnancy:
  - by testing on site at the institution (serum or urine βHCG) within 24 hours prior to the start of LUMASON administration(s),
  - by surgical history (e.g., tubal ligation or hysterectomy),
  - post menopausal with a minimum 1 year without menses;
- Has any known hypersensitivity to 1 or more ingredients of LUMASON (sulfur hexafluoride or to any components of LUMASON);
- Has any known hypersensitivity to dobutamine;
- Has an ongoing or recent (within the last 30 days) acute myocardial infarction;
- Has known right-to-left, bidirectional or transient cardiac shunt (ruled out with agitated saline study performed before administration of LUMASON);
- Has electrolyte (especially potassium and magnesium) abnormalities;
- Has unstable pulmonary and/or systemic hemodynamic conditions e.g.:
  - decompensated or inadequately controlled congestive heart failure (NYHA Class IV);
  - hypovolemia;
  - uncontrolled hypertension, i.e. resting systolic blood pressure >200 mmHg or diastolic blood pressure >110 mmHg;
  - unstable angina;
  - acute coronary syndrome;
  - aortic dissection;
  - acute pericarditis,
  - myocarditis, or endocarditis;
  - stenosis of the main left coronary artery;

- hemodynamically significant outflow obstruction of the left ventricle, including hypertrophic obstructive cardiomyopathy;
- hemodynamically significant cardiac valvular defect;
- acute pulmonary embolism;

- Has uncontrolled cardiac arrhythmias;
- Has significant disturbance in conduction;
- Has hypertrophic subaortic stenosis;
- Has an acute illness (e.g., infections, hyperthyroidism, or severe anemia);
- Was previously entered into this study or received an investigational compound within 30 days before admission into this study;
- Has been treated with any other contrast agent either intravascularly or orally within 48 hours of the first LUMASON administration;
- Has any medical condition or other circumstances which would significantly decrease the chances of obtaining reliable data, achieving study objectives, or completing the study and/or postdose follow-up examinations;

In addition, due to the use of Atropine in subjects who have not reached targeted heart rate with peak dobutamine infusion, subjects with the following will be excluded:

- Glaucoma;
- Pyloric stenosis;
- Prostatic hypertrophy.

5.4.3 Discontinuation Criteria

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

- Withdraws consent;
- 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 subject follow-up in accordance with Section 9.1.5.

6 Investigational Product/Investigational Medicinal Product

The investigational product (IP)/investigational medicinal product (IMP) is LUMASON/SONOVUE.

In the USA and Canada, LUMASON will be supplied as a single 3-part kit with the following components:

- 1 clear glass vial labeled as LUMASON (sulfur hexafluoride lipid-type A microspheres) for injectable suspension,

- 1 prefilled syringe containing 5 mL Sodium Chloride 0.9% Injection, USP (Diluent) and its plunger rod
- 1 Mini-spike

In Europe, SONOVUE will be supplied in a kit which includes 2 individual plastic containers each with the following components:

- 1 vial containing 25 mg of lyophilized powder labeled as SONOVUE
- 1 pre-filled syringe containing 5 mL sodium chloride NaCl 0.9%w/v and its plunger rod
- 1 MiniSpike transfer system

6.1 Description and Labeling

LUMASON is formulated as a 25-mg sterile, non-pyrogenic lyophilized powder in a septum-sealed vial. The ingredients in each vial of LUMASON, after reconstitution, are listed in Table A

Table A: Description of Investigational Product/Investigational Medicinal 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 (SF6)	8 µL/ml*

*Contents in the microspheres/microbubbles

Each vial of LUMASON will bear a two-part label with the information detailed in Appendix A. Affix the second part of the two-part label from the vial to the vial label page.

6.2 Storage

Store the LUMASON kits at controlled room temperature at 25°C (77°F) excursions are permitted to 15-30 °C (59-86°F). No special storage conditions are required for SONOVUE containers. Do not freeze. All kits/containers must be stored in a secure area with limited access.

6.3 Blinding and Randomization

This is an open-label, nonrandomized study.

6.4 Handling and Preparation

Prior to administration, the lyophilized powder should be reconstituted under aseptic technique conditions by injecting the prefilled 5 mL sodium chloride syringe into the LUMASON/SONOVUE vial. After adding the sodium chloride solution, the vial should be shaken vigorously for 20 seconds, after which a homogeneous white milky liquid is obtained.

Detailed instructions for the reconstitution of both LUMASON and SONOVUE are presented in Appendix B.

Store the reconstituted IP/IMP at room temperature in the vial supplied. Although the suspension is useable for up to 3 hours in the USA and up to 6 hours in Canada/ Europe the more conservative time period of 3 hours will be used in this study. Upon standing for more than 15 minutes, buoyancy causes some of the larger microspheres/microbubbles to rise to the surface. Therefore, gently agitate the reconstituted vial in a top-to-bottom manner to resuspend the sulfur hexafluoride microspheres/microbubbles before administration.

A separate kit/container should be used for the rest (CEUS) and stress (CE-DSE) LUMASON administrations. In the case of incomplete use of vial contents, the product cannot be reused.

6.5 Administration

Once reconstituted, LUMASON will be administered as a single 2 mL bolus injection during the rest examination, and then again as a single 2 mL bolus injection after peak stress is achieved with the administration of the stress agent, dobutamine (with or without the addition of atropine), and the UE-DSE stress examination is completed. Each LUMASON injection will be performed intravenously through a peripheral line using a 20 gauge catheter followed immediately with 5 mL of saline in order to flush the intravenous line of any remaining contrast agent. There will be at least a 30 minute period between the rest and the stress LUMASON injections.

6.6 Accountability

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

No IP/IMP is to be used outside of this study. Record the use of the IP/IMP on the appropriate Drug Accountability Record. All containers of IP/IMP 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 IP/IMP to the Investigator must be returned to the Sponsor accompanied by a completed, signed copy of the appropriate IP/IMP inventory reconciliation form.

7 Non-Investigational Medicinal Products

7.1 Dobutamine

Dobutamine is a non-investigational medicinal product (NIMP) in this study due to its use as the pharmacologic stress agent during stress echocardiographic imaging. Refer to the dobutamine Package Insert (PI)/Summary of Product Characteristics (SPC) for details on the storage, handling, preparation and administration.

7.2 Atropine

Atropine is a non-investigational medicinal product (NIMP) in this study due to its use as an adjunct to dobutamine in achieving peak stress during DSE. Refer to the atropine PI/SPC for details on the storage, handling, preparation and administration.

8 Methodology

8.1 Study Schedule

See Appendix C for the timing of required observations and evaluations.

8.2 Written Informed Consent

Obtain written Informed Consent from the subject prior to the implementation of study procedures required by the protocol. The subject must sign the Informed Consent approved by the Institutional Review Board (IRB)/Ethics Committee (EC)/Research Ethics Board (REB) (including specific local requirements e.g., data protection where applicable). Give the subject a copy of the signed and dated written informed consent form including any other written information regarding the study. Document the Informed Consent process in detail in the source record at the site.

8.3 Subject Numbering

Assign a four 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.

8.4 Subject Evaluations

8.4.1 Demographics

Obtain demographic information on each subject (including; height, weight, age, sex and race) and record on the CRF.

8.4.2 Medical History

Obtain a general medical history with relevant findings along with a special focus on cardiac history after the subject has signed the Informed Consent and within 24 hours prior to the first LUMASON administration. Record the subject's medical history/cardiac history on the appropriate Medical History or Cardiac Medical History sections of the CRF.

8.4.3 Pregnancy Test

If the subject is female, and of child bearing potential, exclude the possibility of pregnancy:

- by testing (serum or urine βHCG) within 24 hours prior to the start of IP/IMP administration;
- by surgical history (e.g., tubal ligation or hysterectomy);
- post-menopausal with a minimum of 1 year without menses.

8.4.4 Concomitant Medications

Record all medications (prescription and over-the-counter) taken within 24 hours prior to the first LUMASON administration in the Concomitant Medication section of the CRF. Additionally, record newly prescribed pharmacological treatments in this section up through 24 hours after the last LUMASON administration. Any medication taken for treatment of an adverse event that occurred after the subject signed the informed consent form should be recorded.

Record administration time and dosage information for medications administered as part of the pharmacologic stress procedure in a separate section on the CRF. Do not record on the concomitant medication CRF page.

8.4.5 Safety Assessments

8.4.5.1 Adverse Events

Monitor subjects for any untoward medical occurrences from the time of signing of Informed Consent through 72 hours after the last LUMASON administration or until the subject undergoes cardiac intervention, whichever comes first. Adverse events will be collected at the 24 hour postdose visit with a follow up phone call/contact at 72 hours. Any untoward medical occurrences as a result of the administration of the NIMP(s) (dobutamine or atropine) will also be considered adverse events. However, the known response pattern resulting from the administration of NIMP(s) for the purpose used in this study will not be recorded as adverse events.

Record all untoward medical events in the Adverse Event section of the CRF as specified in Section 9. Only postdose untoward medical occurrences will be tabulated as adverse events.

All serious adverse events that occur during the study monitoring period are required to be collected regardless of the relationship to LUMASON or the NIMPs on the Serious Adverse Event Report (SAER) Form.

In addition, the investigator should report any serious adverse events that occur after the monitoring period that he/she believes may be related to the LUMASON or the NIMPs on the SAER Form.

8.4.5.2 Physical Examination

Perform a physical examination within 24 hours prior to the first LUMASON administration and record any significant changes at 24 hours after the last LUMASON administration on the Adverse Event CRF. The subject's baseline physical examination will be recorded on the Medical/Physical exam section of the CRF.

8.4.5.3 Vital Signs

Collect blood pressure and heart rate at the time points listed below. Obtain all the vital signs in a position that is consistent for all time points for each subject.

Rest echocardiography:	within 1 hour prior to the Rest LUMASON (CEUS) administration 30 minutes after the Rest LUMASON (CEUS) administration
Stress echocardiography:	immediately prior to the Stress LUMASON (CE-DSE) administration 30 minutes after the Stress LUMASON (CE-DSE) administration 1 hour after the Stress LUMASON (CE-DSE) administration* 24 hours after the Stress LUMASON (CE-DSE) administration*

**If stress echocardiography (CE-DSE) is not performed, obtain vitals at 1 and 24 hours after the rest LUMASON administration.*

8.4.5.4 Laboratory Evaluations

Collect blood samples within 24 hours prior to the first LUMASON administration and 24 hours after the last LUMASON administration. A central laboratory will perform evaluations for the analytes listed in Table B:

Table B: Laboratory Analytes

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

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

8.4.5.5 Electrocardiograms

A 12-Lead electrocardiogram (ECG) will be performed in all subjects at the following time points:

Rest echocardiography: within 1 hour prior to the Rest LUMASON (CEUS) administration
30 minutes after the Rest LUMASON (CEUS) administration

Stress echocardiography: immediately prior to the Stress LUMASON (CE-DSE) administration
30 minutes after the Stress LUMASON (CE-DSE) administration
1 hour after the Stress LUMASON (CE-DSE) administration*
24 hours after the Stress LUMASON (CE-DSE) administration*

**If stress echocardiography* (CE-DSE) *is not performed, obtain ECGs at 1 and 24 hours after the rest LUMASON* (CEUS) *administration.*

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

Attach a photocopy of the ECG reports to the appropriate page of the CRF. A dual snap electrode will be supplied to the site by the central ECG laboratory in order to allow the acquisition of both discrete (static) 12-lead ECGs and continuous ECG monitoring described in section 8.4.5.6.

8.4.5.6 Continuous Electrocardiograms

Continuous ECG monitoring will be performed starting 10 minutes prior to the first administration of LUMASON (CEUS) and will continue through 30 minutes after the last administration of LUMASON.

Continuous ECG examinations will be evaluated on site by the Investigator.

8.4.5.7 Pulse Oximetry

Pulse oximetry will be monitored starting at 10 minutes prior to the first administration of LUMASON (CEUS) and continuing through 30 minutes after the last LUMASON administration (CE-DSE).

Pulse oximetry will be evaluated by the on-site Investigator.

8.5 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. Focus will be set at the far field below the mitral valve.

Every effort must be made to keep the optimal transducer position for standard views. At each site, image acquisition should be performed by the same sonographer throughout each subject's study. See Figure 1 for the order of imaging procedures.

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

Prior to rest administration of LUMASON (CEUS), further evaluation for intracardiac shunt will be demonstrated with agitated saline study to assess the interatrial septum, unless an intracardiac shunt has been previously ruled out. If no evidence of intracardiac shunt is confirmed by the cardiologist, the subject will further undergo a CEUS echocardiography examination.

8.5.1 Rest Echocardiography

At rest, UEUS will be acquired using harmonic imaging with the standard apical 4-, 2-, and 3-chamber views. Following the rest UEUS echocardiogram, a rest CEUS echocardiogram will be performed with administration of a single 2 mL bolus injection of LUMASON. A recording of 2D transthoracic harmonic echocardiography imaging will be performed from 30 seconds prior to injection of LUMASON and continue until all images are acquired. For the rest CEUS images,

the apical 4-chamber view will be acquired first followed by the apical 2- and 3-chamber views.

8.5.2 Pharmacologic Stress with Dobutamine

For stress, dobutamine will be administered intravenously by infusion pump at least 30 minutes after the rest LUMASON administration starting at a rate of 5 µg/kg/min. At 3 minute intervals, the infusion rate can be increased to 10, 20, 30, and 40 µg/kg/min to reach one of the following peak stress endpoints:

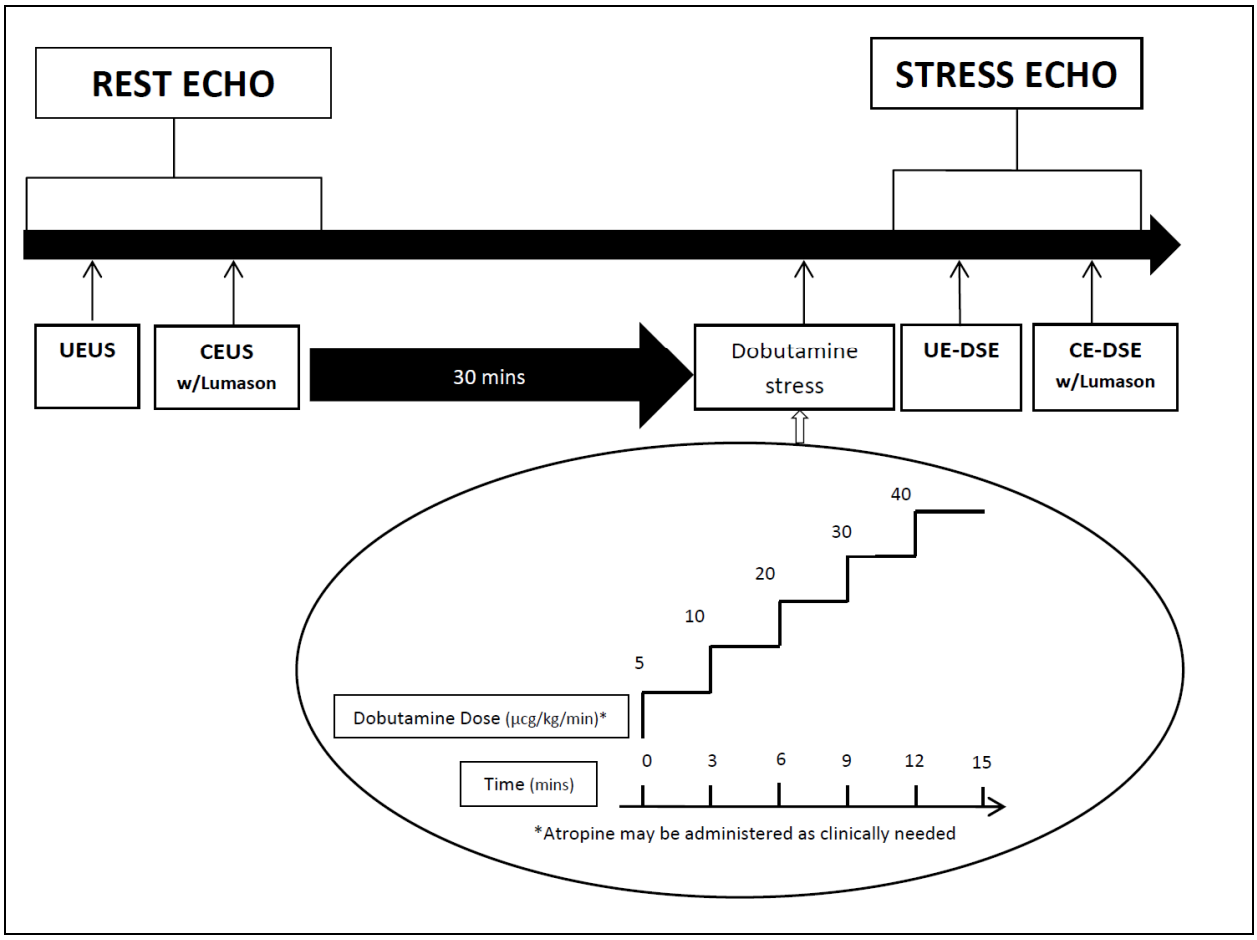
- Target heart rate: 0.85 x (220 - age);
- Development of new or worsening regional wall motion abnormalities;
- Peak dose;
- Significant arrhythmias;
- Severe hypertension (systolic blood pressure >200 mmHg or diastolic blood pressure >110 mmHg);
- Hemodynamically significant hypotension;
- Intolerable symptoms.

If a peak stress endpoint is reached, the test can be stopped before reaching the maximal dose of dobutamine. If no peak stress endpoint is reached after the maximum dose of dobutamine, 0.25 mg to 0.5 mg of atropine can be administered intravenously at peak infusion to increase heart rate. If peak stress is still not reached, atropine, in divided doses of 0.25 to 0.5 mg up to a total of 2.0 mg, should be used as needed to achieve target heart rate.

8.5.3 Stress Echocardiography

Immediately after the administration of dobutamine, with or without the addition of atropine, a stress UE-DSE echocardiogram will be acquired at peak stress using harmonic imaging with the standard apical 4-, 2-, and 3-chamber views. Following the stress UE-DSE echocardiogram, a stress CE-DSE echocardiogram will be performed with administration of a single 2 mL bolus injection of LUMASON. A recording of 2D transthoracic harmonic echocardiography imaging will be performed from 30 seconds prior to injection of LUMASON and continue until all images are acquired. For both US-DSE and CE-DSE images, the apical 4-chamber view will be acquired first followed by the apical 2- and 3-chamber views.

Figure 1: Imaging Procedures



8.6 Efficacy Evaluations

8.6.1 On-Site

The on-site Investigators will provide an overall diagnosis of CAD (Section 8.6.3.3) for each subject based on wall motion abnormalities from the results of the unenhanced (UEUS & UE-DSE) and contrast-enhanced (CEUS and CE-DSE) echocardiographic images.

8.6.2 Off-Site

The off-site efficacy evaluations of the echocardiographic images will be performed by 3 independent, experienced readers, unaffiliated with the investigational sites and blinded to any clinical information for subjects enrolled in this study. These off-site blinded readers will score EBD (Section 8.6.3.1), LV wall motion (Section 8.6.3.2) and overall diagnostic conclusion (Section 8.6.3.3).

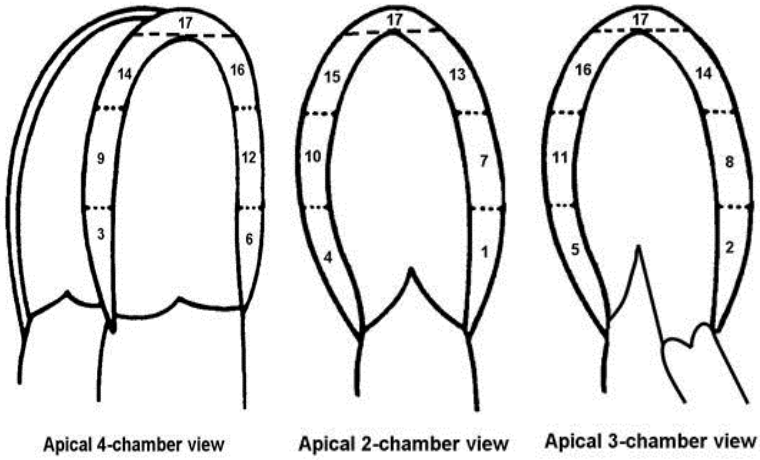
8.6.3 Efficacy Measurements

If the subject does not complete the echocardiography examinations (rest, stress or any views are missing) the subject will not be included in the efficacy evaluation for on- or off-site.

8.6.3.1 Endocardial Border Delineation

The assessment of EBD will be performed by dividing the heart into 17 segments using the standard apical 4-, 2-, and 3-chamber views (see Figure 2) according to the ASE[1,12,27] guidelines:

Figure 2: 17-Segment Model



LV EBD will be graded for each segment of the 17-segment model shown in Figure 2 by each off-site reader using the following 3-point scale:

0 = Inadequate (endocardial border not visible)

1 = Sufficient (endocardial border barely visible)

2 = Good (endocardial border clearly visible)

For LV EBD, separate assessments will be collected for the unenhanced (UE-DSE) and contrast-enhanced (CE-DSE) images at peak stress.

8.6.3.2 LV Wall Motion

LV wall motion will be graded by each off-site reader using the following 3-point scale at rest and peak stress for unenhanced (UEUS and UE-DSE) and contrast-enhanced (CEUS and CE-DSE) images:

1 = Normal

2 = Abnormal (Mild-Moderate, Severe Hypokinesis, Akinetic and/or Dyskinetic)

3 = Unevaluable

8.6.3.3 Overall Diagnostic Conclusion

The overall diagnostic conclusion will be determined by the on-site Investigator and the off-site reader using the scoring system in Table C:

1 = Normal

2 = Abnormal (Mild-Moderate, Severe Hypokinesis, Akinetic and/or Dyskinetic)

3 = Unevaluable (Inadequate visualization of segments comprising all coronary artery territories due to motion artifacts, lateral wall drop out, swirling or attenuation artifacts)

Table C: Overall Diagnostic Conclusion Based on LV Wall Motion

Rest	Stress	Overall Diagnostic Conclusion
1 Normal	1 Normal	Normal
1 Normal	2 Abnormal	Abnormal
2 Abnormal	2 Abnormal (no change or worsening)	Abnormal
3 Unevaluable	3 Unevaluable	Unevaluable*

**A score of 3 – unevaluable at either rest or stress will result in an overall diagnostic conclusion of unevaluable.*

Separate assessments will be collected for unenhanced (UEUS and UE-DSE) and contrast-enhanced (CEUS and CE-DSE).

8.6.4 Truth Standard

8.6.4.1 Coronary Angiography

The results of coronary angiography will be collected on-site for any subject who has the procedure within 6 months following the LUMASON administration(s). All coronary angiography images should be archived and stored on dedicated digital media according to the standard practice at the institution. Investigational sites are required to maintain the original images as a record of the study. A copy of the images shall be archived on portable digital media (e.g., digital versatile disc [DVD], magnetic optical disc [MOD], compact disc [CD], etc.) that will be forwarded to the central imaging laboratory according to the instructions in the Imaging Manual. If a subject does not undergo coronary angiography or coronary angiography is indeterminate, 6 month follow-up data will be collected to determine if the subject has CAD based on clinical information (see Section 8.6.4.2).

A fourth blinded reader will assess the coronary angiography images and provide a diagnosis of CAD. The off-site reader will be blinded to the specifics of the protocol including the patient population and the results of the echocardiographic examination, but will receive clinical information such as the medical history.

For subjects with coronary angiography as the truth standard, a subject will be diagnosed by the blinded off-site coronary angiography reader as Positive if percentage diameter stenosis of any of the vessels (RCA, LAD, LCx and LM) is ≥50%; otherwise, a subject will be considered as Negative.

8.6.4.2 6 Month Follow Up to Collect Cardiac Events

A 6 month follow up will be required for those subjects who have not had a coronary angiography or had coronary angiography with indeterminate results within the 6 months following the LUMASON administration(s).

Subjects requiring 6 month follow up will be monitored by a review of their hospital and clinic records as well as a phone call follow-up at 6 months to collect cardiac events, as defined by the incidence of: a) cardiac death; b) non-fatal myocardial infarction as defined by a greater than two fold serial elevation in serum creatine kinase-myocardial B (CK-MB) fraction levels; c) coronary revascularization; and d) documentation of the need for coronary revascularization. If a coronary angiography is performed during the 6 month follow-up, this will be collected and accepted as the truth standard for the subject. Each site will have study personnel who will be responsible for collecting phone/clinical record or coronary angiography images during the follow up period.

8.6.5 Off-Site Assessment Methodology

Digital recordings of the echocardiographic images will be blinded, randomized and assessed off-site at a core laboratory. The off-site assessment of the digital recordings will be performed by 3 independent, experienced readers, unaffiliated with the investigational sites and blinded to any clinical information for subjects enrolled in this study.

In addition, the coronary angiography images will be evaluated by a fourth blinded reader who will provide a diagnosis of CAD. This off-site reader will be blinded to the specifics of the protocol including the patient population and the results of the ultrasound examination, but will receive clinical information such as the medical history.

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.

Changes to the off-site image assessment will either be reviewed and approved via a protocol amendment or described in the separate off-site image assessment document.

9 Reporting Safety Information

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

AE collection begins when a subject signs the Informed Consent and continues through the follow-up period defined in the section 8.4.5.1 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/IMP and/or NIMP(s).

Any untoward medical occurrence that occurs from the time of signed Informed Consent to the time immediately prior to IP/IMP administration will be tabulated in the Clinical Trial Report as a "predose event."

9.1 Adverse Events

9.1.1 Definitions

An adverse event (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 in subject 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),

Dobutamine: latest version of PI/SPC approved in the country where the site is located which administered the NIMP to the study subject,

Atropine: latest version of PI/SPC approved in the country where the site is located which administered the NIMP to the study subject.

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. For Europe, the new version will be provided to all investigators, ECs and CAs. 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.

9.1.2 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 D) 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 IP/IMP 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/IMP and/or NIMP(s), 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 Serious Adverse Event Report or any other way chosen by the Investigator. Do not use the Case Report Form.

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

Table D: Serious Adverse Event Reporting – Sponsor Contact Personnel

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

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

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

9.1.3 Breaking the Study Blind

This is an open-label, nonrandomized study.

9.1.4 Data Collection

The Investigator will collect adverse events through non-leading questions and examination of the subjects/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 9.1.1).
- **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 predose 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 predose in a laboratory test is reported as an adverse event, record the stop date as the date of collection of the first postdose sample that shows a return to the predose 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/IMP/NIMP:** Make every effort to determine the cause of each adverse event. Classify the correlation between the IP/IMP/NIMP 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/IMP and/or NIMP;

AND

b) The event follows a known response pattern to the IP/IMP and/or NIMP 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 predose event or is definitely due to causes separate from the administration of the IP/IMP, i.e.,

- documented pre-existing condition
- technical and manual procedural problems
- concomitant medication and/or NIMP
- 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 IP/IMP 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, obtain 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.

9.1.5 Subject Follow-Up

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

9.2 Special Situations

The Investigator must report all special situations **within 24 hours** by telephone or by fax using the Special Situation 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/IMP and/or NIMP, medication error with IP/IMP and/or NIMP (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 notification of lactation or pregnancy of a subject or partner must be reported by the Investigator to the Sponsor within 24 hours using the appropriate

(i.e., Lactation or 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 communicated in a timely fashion by the Sponsor to the applicable regulatory authorities, IRBs/ECs/REBs and also to concerned investigators and subjects.

9.3 Laboratory Evaluations

9.3.1 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 predose to postdose 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**.

9.3.2 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 predose level or clinically stabilizes, or until the Investigator or physician of record determines that further follow-up is unnecessary.

9.3.3 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.

9.4 Electrocardiograms

If a worsening from predose to postdose is observed on an ECG, repeat the ECG at the discretion of the Investigator, in addition to obtaining an ECG at the postdose time points required by the protocol. The Investigator will assess any worsening from predose to postdose 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.

9.5 Continuous ECGs

If a worsening is observed during the continuous ECG monitoring, the Investigator will assess the worsening 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 CRF.

9.6 Physical Examinations

If a worsening from predose to postdose is observed in a physical examination, repeat the physical examination at the discretion of the Investigator, in addition to obtaining a physical examination at the postdose time points required by the protocol. Document any change from predose to postdose in the Physical Examination section of the Case Report Form. The Investigator will evaluate any worsening at the postdose 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.

9.7 Vital Signs

If a worsening from predose to postdose is observed in vital signs, repeat the vital sign measurement at the discretion of the Investigator, in addition to obtaining vital sign measurements at the postdose time points 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.

9.8 Pulse Oximetry

If a worsening is observed during the continuous pulse oximetry monitoring, the Investigator will assess the worsening 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 CRF.

10 Statistical Methods

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. Unless otherwise specified, the statistical tests will be 2-sided at 0.05 level of significance.

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.

10.1 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, weight, BMI, and other relevant study entry criteria.

10.2 Analysis Population

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

Efficacy Analysis Population – all subjects who receive LUMASON, and have data available at peak stress for both UE-DSE and CE-DSE and a definitive diagnosis (Positive, Negative) for CAD from truth standard (coronary angiography or 6 months follow-up data) will be included in the modified Intent-to-Diagnose (ITD) population.

10.3 Concomitant Medications

Concomitant medications will be coded according to therapeutic area using the WHO drug reference list. Concomitant medications will be presented in data listings and summarized by frequency counts according to anatomical and therapeutic area for all subjects dosed.

10.4 Extent of Exposure

Descriptive statistics will be presented to summarize the volume (mL) of IP/IMP administered. Dose administration for IP/IMP will be listed for each injection by subject. The peak dose of dobutamine and total dose of atropine will be summarized and listed for each subject.

10.5 Safety Analysis

The safety data will be summarized for all subjects dosed. Summary tables, including clinically significant changes wherever applicable, will be presented for the following safety endpoints:

- Adverse Events
- Clinical Laboratory Evaluations
- Electrocardiograms
- Vital Signs
- Special Situations

For vital signs and ECGs, the baseline for time points at rest will be the last measurement prior to the IP/IMP administration at rest; while the baseline for time points at stress will be the measurement which is collected after administration of the pharmacologic stress agent, dobutamine, and prior to LUMASON administration at stress (CE-DSE). 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 IP/IMP. Adverse event will be summarized separately for the NIMP(s). Only those events which occur from the start of IP/IMP administration through the follow-up period defined in the protocol will be tabulated in the Clinical Trial Report as "adverse events" see Section 9.1.1.

10.6 Efficacy Analysis

The echocardiographic images will be assessed by 3 off-site blinded readers independent of the investigational sites; accordingly, the efficacy analysis will be performed separately for each off-site reader. An additional, fourth, blinded reader will assess the coronary angiography images and provide a diagnosis of CAD.

10.6.1 Primary Efficacy Endpoint – Sensitivity and Specificity for Detection and Exclusion of CAD

10.6.1.1 Truth Standard Diagnosis

For subjects with coronary angiography as the truth standard, a subject will be diagnosed by the blinded off-site coronary angiography reader as Positive if percentage diameter stenosis of any of the vessels (RCA, LAD, LCx and LM) is ≥50%; otherwise, a subject will be considered as Negative.

For the remaining subjects who didn't have coronary angiography as the truth standard, a subject will be Positive if the subject had cardiac death, documented myocardial infarction, documented

revascularization or need for revascularization during follow up; otherwise, the subject will be considered as Negative.

10.6.1.2 Evaluation of CAD

The overall diagnostic echocardiographic conclusion will be classified by the off-site readers as Normal, Abnormal or Unevaluable for unenhanced (UEUS and UE-DSE) and LUMASON-enhanced (CEUS and CE-DSE) echocardiography respectively.

The diagnostic performance of echocardiography (UE-DSE and CE-DSE) will be derived based on the diagnosis from stress echocardiography and diagnosis from truth standard (see Table E).

Table E: Cross Tabulation of Diagnosis: Truth Standard vs. Stress Echocardiography

Truth Standard (TS)	Stress Echocardiography		
	Normal	**Unevaluable***	**Abnormal**
Negative	*TN*	*FP*	*FP*
Positive	*FN*	*FN*	*TP*
TN = true negative; TP = true positive; FN = false negative; FP = false positive			

**Unevaluable is defined as: inadequate visualization of segments comprising all coronary artery territories due to motion artifacts, lateral wall drop out, swirling or attenuation artifacts.*

The null hypothesis is that there is no difference in sensitivity/specificity between CE-DSE and UE-DSE at peak stress; the alternative hypothesis is that CE-DSE is superior to UE-DSE in terms of higher sensitivity/specificity:

H_0: $Diag_{CE} - Diag_{UE} = 0$,

H_a: $Diag_{CE} - Diag_{UE} > 0$;

where $Diag_{CE}$ and $Diag_{UE}$ denote sensitivity/specificity for CE-DSE and UE-DSE at peak stress respectively.

The sensitivity and the specificity along with their 95% confidence intervals (CIs) will be calculated, and the difference in sensitivity/specificity will be tested using McNemar's Chi-square 2-sided test.

In addition, inter-reader agreement for the detection/exclusion of CAD for CE-DSE and UE-DSE will be evaluated by kappa statistic.

10.6.2 Co-Primary Efficacy Endpoint – Critical Shift of Subjects with Suboptimal Images to Adequate Images

The co-primary endpoint: The proportion of subjects with suboptimal images (≥2 adjacent segments with inadequate LV EBD in any of the 3 apical views) at UE-DSE converted to adequate images (reduction of suboptimal adjacent segments to obtain adequate images) at CE-DSE will be estimated along with the 95% CIs.

Additionally, the following supportive analyses will be performed:

- proportion of subjects with optimal images at UE-DSE to suboptimal (≥2 adjacent segments with inadequate LV EBD in any of the 3 apical views) at CE-DSE in terms of LV EBD visualization will be estimated along with the 95% CIs.
- The percentage of patients with at least one segment with inadequate border delineation will also be summarized for each of the views for both UE-DSE and CE-DSE.
- Inter-reader agreement among 3 off-site readers on the critical shift of subjects with suboptimal images at UE-DSE to adequate images at CE-DSE will be evaluated by kappa statistic.

10.6.3 Secondary Efficacy Endpoints

For the secondary efficacy endpoint of change in total LV EBD scores at peak stress from UE-DSE to CE-DSE, the total LV EBD score is calculated as the sum of the individual scores (0, 1, or 2) assigned to each of the 17 ventricle segments with a range from 0 to 34.

For each blinded reader assessment, paired t-test will be used to compare total LV EBD score between the CE-DSE and UE-DSE. Summary statistics will be provided for the total LV EBD score from UE-DSE and CE-DSE. The change in total LV EBD score from UE-DSE to CE-DSE will be summarized along with the 95% CIs and tested by paired t-test. The cross tabulated (for UE-DSE vs. CE-DSE) distribution of the individual scores (0, 1, or 2) assigned to each of the 17 ventricle segments will be presented.

Inter-reader agreement among 3 off-site readers on the EBD score for each segment from UE-DSE and CE-DSE will be evaluated by kappa statistic.

10.6.4 Success Criteria

The study will be considered a success if:

- for the primary efficacy endpoint of sensitivity/specificity, CE-DSE is superior to UE-DSE for at least 2 out of the 3 blinded readers and for the same 2 readers a lower limit of 2-sided 95% CI for sensitivity/specificity is ≥ 50% (a rate considered greater than chance);
- for co-primary efficacy endpoint of proportion of subjects with critical shift of suboptimal images at UE-DSE to adequate images at CE-DSE, for at least 2 out of the 3 blinded readers lower limit of 2-sided 95% CI is above 35%.

10.7 Sample Size

Software nQuery Advisor 7.0 is used for sample size determination in this study.

As suggested by FDA during the type C meeting on 13Aug2014, and as also reinforced and documented in the official meeting minutes issued by FDA on 24Sep2014, this study needs to demonstrate 1) a significant improvement in diagnostic performance endpoints, i.e., sensitivity/specificity from unenhanced stress imaging (UE-DSE) to contrast-enhanced stress

imaging (CE-DSE) in term of detection and exclusion of CAD, and 2) lower the CI of CE-DSE sensitivity and specificity greater than 50% (a rate greater than chance).

For significant improvement in diagnostic performance endpoints, the null hypothesis is that there is no difference in sensitivity/specificity between CE-DSE and UE-DSE at peak stress; the alternative hypothesis is that CE-DSE is superior to UE-DSE in terms of higher sensitivity/specificity:

H_0: $Diag_{CE} - Diag_{UE} = 0$,

H_a: $Diag_{CE} - Diag_{UE} > 0$;

where $Diag_{CE}$ and $Diag_{UE}$ denote sensitivity/specificity for CE-DSE and UE-DSE at peak stress respectively.

With 2-sided significance level (alpha) of 5%, and the probability of a Type II error (β) of 0.20, assuming expected difference between CE-DSE and UE-DSE is 25%,[1] and the proportion of discordant pairs is 40%, 45 subjects will be needed for sensitivity/specificity respectively.

For lower CI of CE-DSE sensitivity/specificity greater than 50%, assuming sensitivity/specificity of CE-DSE = 65%, about 45 CAD positive subjects are needed to have 95% confidence that the lower limit of sensitivity >50%, and 45 CAD negative subjects are needed to have 95% confidence that lower limit of specificity>50%.

Assuming approximately 30% of subjects enrolled will be CAD positive,[13] in order to obtain 45 CAD positive subjects, 150 subjects in total need to be enrolled. With a total of 150 subjects enrolled, this will also provide more than enough CAD negative subjects according to the above calculations. The prevalence of positive subjects will be tracked based on on-site CAD diagnosis from the truth standard and if less than 30% of subjects are CAD positive, additional subjects will need to be enrolled to obtain sufficient CAD positive subjects.

For the co-primary endpoint of critical shift from suboptimal at UE-DSE images to adequate images at CE-DSE, sample size assumptions are based on the results from the previous pivotal phase III Bracco-sponsored study ([redacted].[22] This study had 44.8%-46.0% of the subjects converted from suboptimal at UEUS to adequate at LUMASON-enhanced echocardiography (CEUS) at rest. When the sample size is 150, a 2-sided 95% CI for a single proportion using the large sample normal approximation will extend 8% from the observed proportion for an expected proportion of 45% subjects with critical shift from suboptimal at UE-DSE images to adequate images at CE-DSE. This can be translated as lower limit of 95% CI >35% for the observed proportion of subjects with critical shift.

Considering approximately 15% of subjects enrolled are anticipated to dropout, approximately 175 subjects will need to be enrolled.

10.8 Data Handling

All data collected will be entered into the database and displayed in the data listings and/or tables. Details of the data handling procedures will be specified in the SAP.

10.9 Interim Analyses

No interim analysis is planned.

11 Ethics and Good Clinical Practice

11.1 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.

11.2 Informed Consent and Subject Information

All subjects must sign and personally date an approved Informed Consent Form after receiving detailed written and verbal information about the reason, the nature and the possible risks associated with the administration of the IP/IMP and NIMP(s). 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 the EU Directive 2001/20/EC, 2005/28/EC, and related guidance, and applicable laws and regulations of Health Canada.

The subject must be made aware and agree that personal information may be scrutinized during inspection or audit by competent authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available in accordance to the Guidelines for Data Processing within the Framework of Clinical Drug Trials 24 July 2008.

11.3 Institutional Review Board/Ethics Committee/Research Ethics Board 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/REB, as required in chapter 3 of the ICH E6 Guideline, and as applicable, Title 21 CFR 56.107 through 56.115 and EU Directive 2001/20/EC, 2005/28/EC, and related guidance, and applicable laws and regulations of Health Canada. Written IRB/EC/REB approval must be obtained by Sponsor prior to shipment of IP/IMP or subject enrollment.

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

11.4 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 to 54.6 and the EU Directive 2001/20/EC, 2005/28/EC and related guidance.

11.5 Safety Monitoring Boards

There will be no safety monitoring board for this study.

12 Administrative Considerations

12.1 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 EU Directive 2001/20/EC and 2005/28/EC related guidance, and applicable laws and regulations of Health Canada. 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.

12.2 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/Competent Authorities including but not limited to FDA, Health Canada or any European Economic Area State in which the trial is being conducted and to the relevant ECs in accordance with EU Directive 2011/C 172/01 about Communication from the Commission — Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use (‘CT-3’).

12.3 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/EC/Competent Authority (CA)/REB 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).

12.4 Curriculum Vitae

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

12.5 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 F. A complete and controlled list of the Investigators, study sites, and IRBs/ECs/REBs involved in this study can be found in the Trial Master File maintained by the Sponsor.

12.6 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, and meet the definition of a serious breach the Clinical Research Manager (CRM) takes appropriate action according to the requirements and timelines stated the Code of Federal Regulations (CFR), European Guidance and local regulations as applicable.

12.7 Monitoring Procedures

12.7.1 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 IP/IMP 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, subjects' 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.

12.7.2 Case Report Form

The Sponsor will provide a Case Report Form for each subject. The Investigator must record all data on the Case Report Form and archive a copy of the completed Case Report Form 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.

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

12.7.3 Inspection and Auditing

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

12.8 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 and by the applicable local regulations. For non-European sites, this time period must be at least 2 years after the last approval of the marketing application of the IP/IMP 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 IP/IMP. For EU sites, according to EU Directive 2005/28/EU this time period must be at least 5 years after study completion. This period may be extended if required by local law.

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 IP/IMP.

12.9 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 notification of the end of study and sent to the involved ECs and CAs. It will be reviewed and approved by the Investigator when required by local authorities.

12.10 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/EC/CA/REB 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.

12.11 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.

12.12 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/EC/REB before the study can be continued.

12.13 Definition of the End of the Study

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

12.14 Premature Termination of the Study

If Sponsor, the Investigator, or IRB/EC/CA/REB 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.

12.15 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's 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/EC/CA/REB as long as they are required to keep it confidential.

12.16 Insurance Policy

Whenever applicable, Sponsor will provide verification of insurance coverage for damages emerging from the study and involving test subjects treated with the IP/IMP. 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.

13 Confidentiality

All information provided to the Investigator dealing with the IP/IMP 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.

14 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 (Signature) Date

_____

Investigator (Printed Name)

_____ _____

For Bracco (Signature) Date

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Appendix A: Investigational Product/Investigational Medicinal Product Labeling

Investigational Product/Investigational Medicinal Product Labeling

North America

The following 2-part label will be applied to the kit:

LUMASON Kit Label

Lumason™ IND 46,958 Protocol No. BR1-142 Contents: 1 Lumason™ vial of 25 mg lyophilized powder 1 pre-filled syringe of 5 mL Sodium Chloride injection USP, 0.9% Sodium Chloride (DILUENT) 1 mini-spike Store at 25 °C (77 °F) excursions permitted to 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	Lumason™ IND 46,958 Protocol No. BR1-142 Contents: 1 Lumason™ vial of 25 mg lyophilized powder 1 pre-filled syringe of 5 mL Sodium Chloride injection USP, 0.9% Sodium Chloride (DILUENT) 1 mini-spike Store at 25 °C (77 °F) excursions permitted to 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

The following 2-part label will be positioned on each vial of LUMASON:

LUMASON 2-Part Vial Label

Lumason™ 25mg Protocol: BR1-142 Store at 25 °C (77 °F) excursions permitted to 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._____ Batch No.: Exp. Date: Bracco Diagnostics Inc., Monroe Twp, NJ 08831	Lumason™ 25mg Protocol: BR1-142 Store at 25 °C (77 °F) excursions permitted to 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._____ Batch No.: Exp. Date: Bracco Diagnostics Inc., Monroe Twp, NJ 08831

The above labels are "master labels." The actual labels used for each country are filed in the Trial Master File of the study.

Europe

The following label will be applied to the kit which includes 2 clear plastic containers:

SonoVue Kit Label

SonoVue® (BR1) - 8 µl/mL
Dispersion for intravenous injection after reconstitution *Code number. Lxxx/xx*
Study No. BR1-142
EudraCT No.
Centre No. **Subject No**.
Content: 2 vials of 25 mg lyophilized powder (SonoVue®)
2 pre-filled syringes with 5 mL NaCl 0.9% w/v
Batch No.:xxxxx/xxxxx Expiry date: mm/yyyy – mm/yyyy
Direction for use: Administer within 3 hours after reconstitution.
See paragraphs 6.4-6.5 and Appendix B of the study protocol.
No special storage conditions are required. Do not freeze.
FOR CLINICAL TRIAL USE ONLY
Return to IMP Manufacturer all the material, including empty packaging and unused or partially used product
Sponsor: Bracco Imaging S.p.A. (+39-02-2177.1) Via Folli, 50 - I -20134 Milano
IMP Manufacturer: Bracco Imaging S.p.A. (+39-0125-56170.1) Via Ribes, 5 - I -10010 Colleretto Giacosa

The following 2-part label will be positioned on each vial of SonoVue:

SonoVue Vial Label

SonoVue®(BR1) - 8 µl/mL *Code No. Lxxx/xx* 25 mg lyophilized powder to be reconstituted with 5 mL NaCl 0.9% - For intravenous injection after reconstitution **Study No. BR1-142** Centre No. **Subject No.** Batch No: Exp. Date: mm/yyyy No special storage conditions are required. **FOR CLINICAL TRIAL USE ONLY** Sponsor: Bracco Imaging S.p.A - I - 20134 Milano	SonoVue®(BR1) - 8 µl/mL *Code No. Lxxx/xx* 25 mg lyophilized powder to be reconstituted with 5 mL NaCl 0.9% - For intravenous injection after reconstitution **Study No. BR1-142** Centre No. **Subject No.** Batch No: Exp. Date: mm/yyyy No special storage conditions are required. **FOR CLINICAL TRIAL USE ONLY** Sponsor: Bracco Imaging S.p.A - I - 20134 Milano

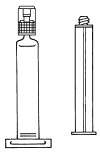
The above labels are "master labels." The actual labels used for each country are filed in the Trial Master File of the study.

Appendix B: LUMASON/SONOVUE Reconstitution

LUMASON Reconstitution

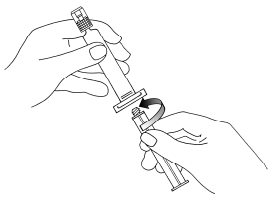
LUMASON is supplied within a kit containing the following:

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

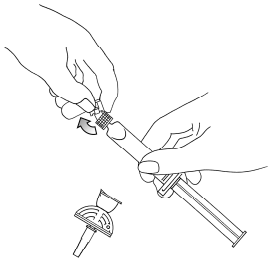
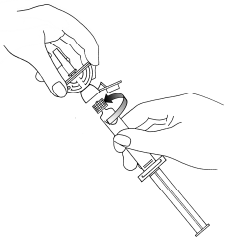
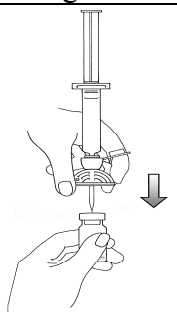
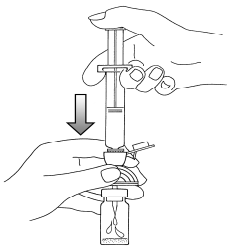
		
Glass vial	Prefilled syringe and its plunger rod	Mini-Spike

Reconstitution steps:

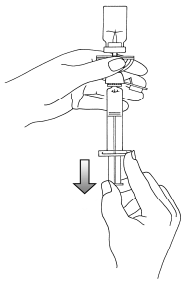
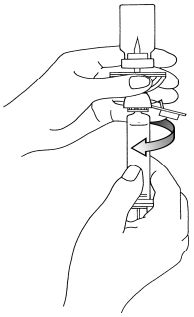
- Prior to LUMASON reconstitution, inspect the kit and its components for signs of damage. Do not use the kit if the protective caps on the vial and prefilled syringe are not intact or if the kit shows other signs of damage.
- Perform all LUMASON reconstitution steps under aseptic conditions. The LUMASON 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.

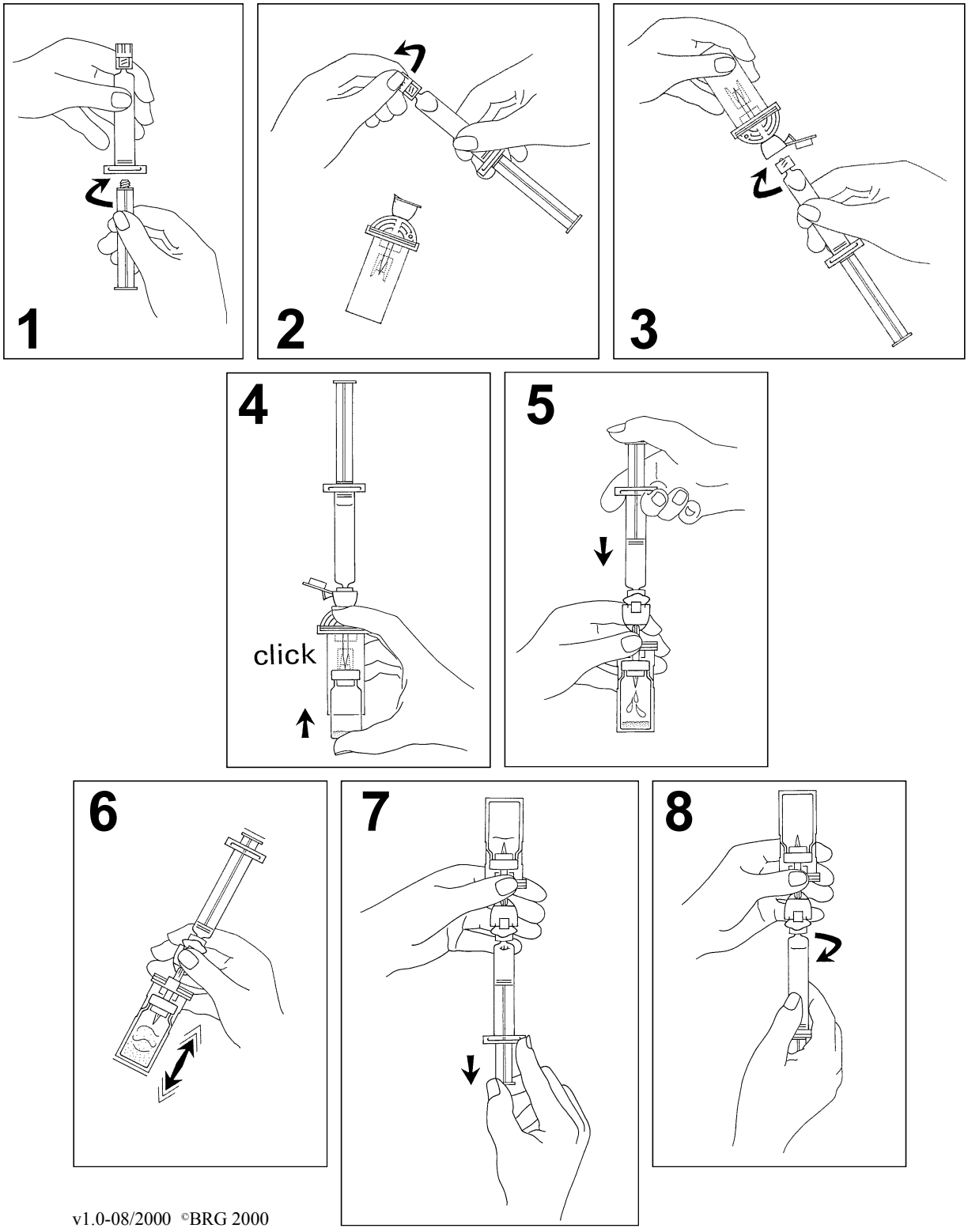
LUMASON Reconstitution (continued)

2. Open the Mini-Spike 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.

LUMASON Reconstitution (continued)

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.

SONOVUE Reconstitution



v1.0-08/2000 ©BRG 2000

1. Connect the plunger rod by screwing it clockwise into the syringe.
2. Open the MiniSpike transfer system blister and remove syringe tip cap.
3. Open the transfer system cap and connect the syringe to the transfer system by screwing it in clockwise.
4. Remove the protective disk from the vial. Slide the vial into the transparent sleeve of the transfer system and press firmly to lock the vial in place.
5. Empty the contents of the syringe into the vial by pushing on the plunger rod.
6. Shake vigorously for 20 seconds to mix all the contents in the vial to obtain a white milky homogeneous liquid.
7. Invert the system and carefully withdraw SONOVUE into the syringe.
8. Unscrew the syringe from the transfer system.

Do not use if the liquid obtained is clear and/or if solid parts of the lyophilisate are seen in the suspension.

Appendix C: Study Schedule

	Rest						Stress					Follow-Up		
	UEUS				IP/IMP	CEUS		UE-DSE	IP/IMP	CE-DSE				
Event	**Within -24 hr**	**-1 hr**	**-10 min**	**Immed prior**	**0 min**	**30 min**	**STRESSOR**	**Immed prior**	**0 min**	**30 min**	**1 hr**	**24 hr**	**72 hr**	**6 mos**
Written Informed Consent [a]	×													
Pregnancy Test (if necessary)	×													
Adverse Events Monitoring [b]	×	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	×	
Concomitant Medications [c]	×	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	⇒	×		
Medical History [d]	×													
Physical Examination	×											×		
Vital Signs [e,f]		×				×		×		×	×	×		
Laboratory Evaluations [g]	×											×		
Electrocardiogram - discrete [f]		×				×		×		×	×	×		
Electrocardiogram – continuous			×	⇒	⇒	⇒	⇒	⇒	⇒	×				
Pulse oximetry			×	⇒	⇒	⇒	⇒	⇒	⇒	×				
IP/IMP Administration					×				×					
Imaging Procedure				×	×			×	×					
Dobutamine Administration [h]							×							
Truth Standard												×	⇒	×
Follow-up Contact for AE													×	

[a] Obtain prior to implementation of any study procedure.

[b] Start monitoring from the time of signing Informed Consent up to 72 h after the last LUMASON administration or until cardiac intervention, whichever comes first. Collect adverse events for LUMASON and NIMPs (dobutamine, atropine).

[c] Record all medications (prescription and over-the-counter) taken within 24 hours prior to first LUMASON administration up through 24 hours after the last LUMASON administration.

[d] Includes Demographics, Cardiac Medical History and General Medical History.

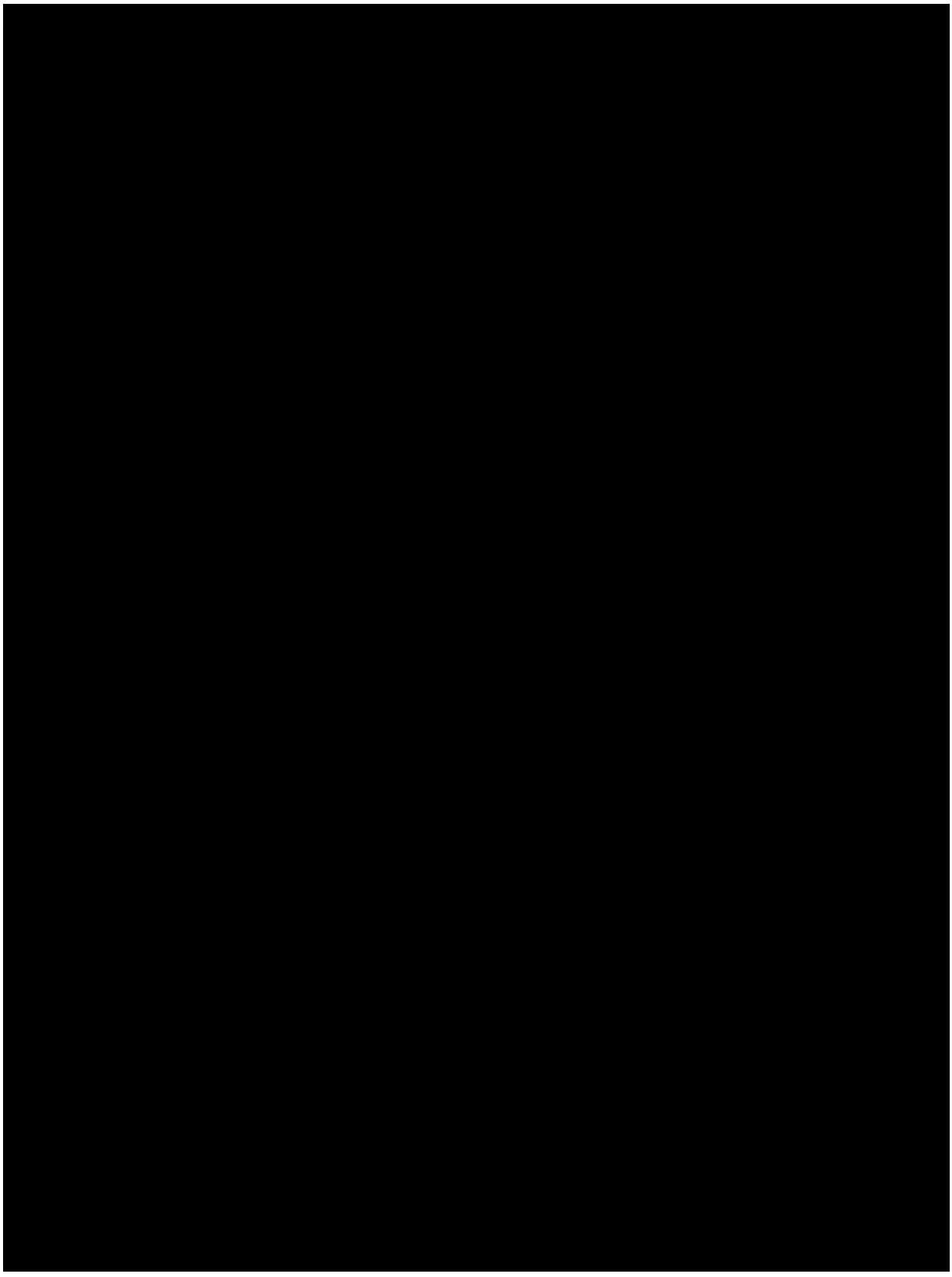
[e] Includes systolic and diastolic blood pressure, heart rate.

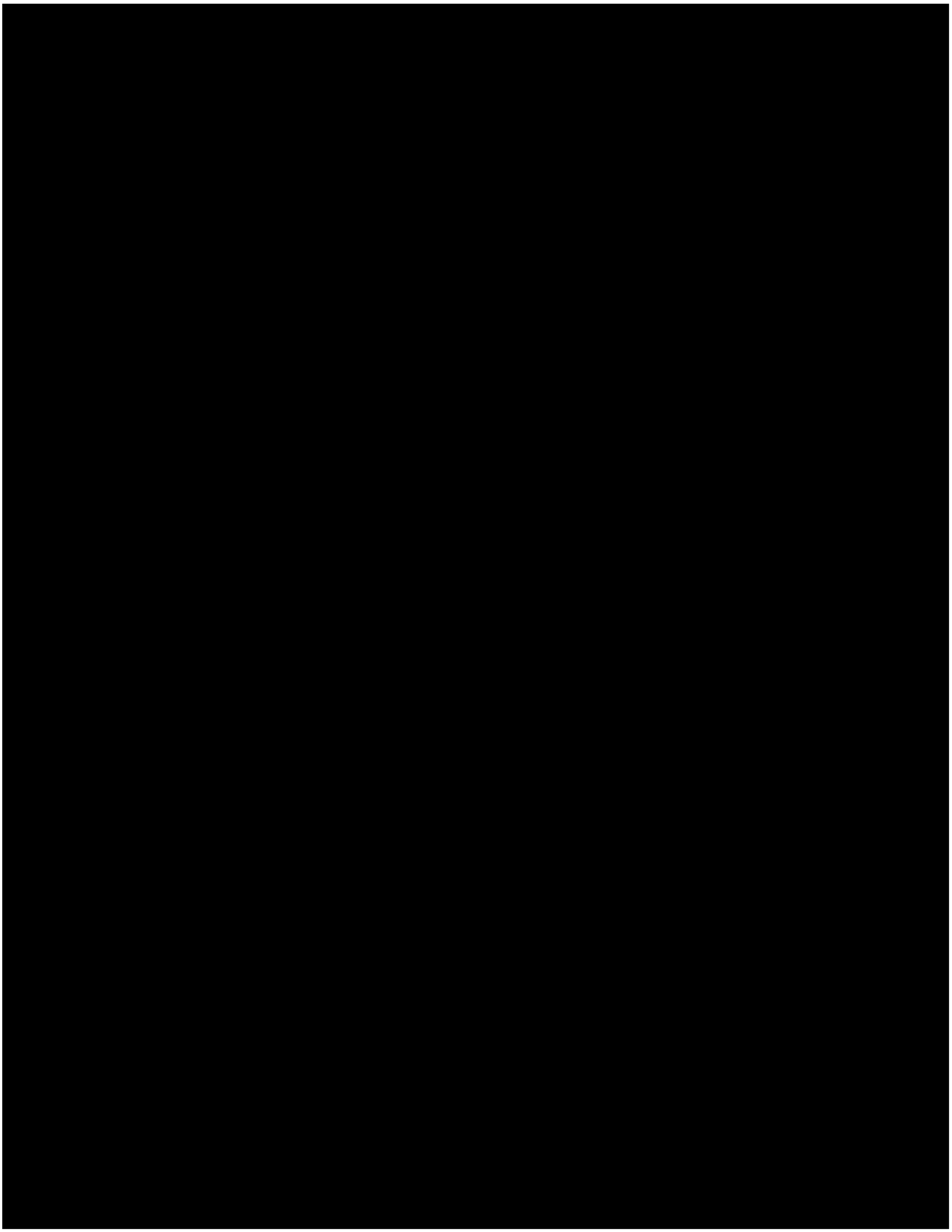
[f] If stress echocardiography (CE-DSE) is not performed, obtain at 1 and 24 hours after the rest LUMASON administration.

[g] Collect blood samples according to the instruction manual from the central laboratory.

[h] Atropine may be administered with dobutamine if needed to achieve peak stress.

Appendix D: Serious Adverse Event Report





Appendix E: Investigator Statement

Investigational Product	**Lumason™/SonoVue®**
Protocol No.	**BR1-142**
Protocol Title:	**A Prospective Multicenter Phase III Clinical Evaluation of the Safety and Efficacy of Lumason/SonoVue in Subjects Undergoing Pharmacologic Stress Echocardiography with Dobutamine for the Diagnosis of Coronary Artery Disease**
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, the European Directive 2001/20/EC, 2005/28/EC and related guidance, 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. I shall report serious breaches according to applicable regulations.
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. I shall also require a Data Protection Form.

INVESTIGATOR STATEMENT

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) (for sites outside the European Economic Area, add the following) I shall submit a written report of adverse events to the IRB/EC, 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/EC and competent 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 F: Administrative Structure

Administrative Structure

North America

Clinical Research Personnel	
[redacted]	[redacted]
CROs	
Study Monitoring	***Central Laboratory***
[redacted]	[redacted]
Central ECG Laboratory	***Central Imaging Laboratory***
TBD	TBD

Europe

Clinical Research Personnel	
[redacted]	
CROs	
Study Monitoring	***Central Laboratory***
TBD	[redacted]
Central ECG Laboratory	***Central Imaging Laboratory***
TBD	TBD