Clinical Study Protocol **GE Healthcare**
GE-191-008

Title: A Phase 4, Open-Label, Non-randomized, Multicenter Study to Evaluate Safety and Efficacy of Intravenous Administration of OPTISON™ for Contrast-Enhanced Echocardiography in Pediatric Patients

REVISED TO INCORPORATE AMENDMENT A01

Sponsor

GE Healthcare Ltd. and its Affiliates (hereinafter referred to as the "sponsor")

Medical Director

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Confidentiality Statement

Investigator's Signature Page

I have read this protocol and all associated case report forms and agree to conduct this study in full accordance with the stipulations of the protocol described herein.

Signature

Date

Print Name

1 SYNOPSIS

Name of Sponsor/Company: GE Healthcare Ltd. and its Affiliates **Name of Finished Product:** OPTISON™ **Name of Active Ingredient:** Perflutren Protein – Type A Microspheres Injectable Suspension, USP	**Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:** **Volume:** **Reference:**	**(For National Authority Use Only)**

Title of Study:
A Phase 4, Open-Label, Non-randomized, Multicenter Study to Evaluate Safety and Efficacy of Intravenous Administration of OPTISON™ for Contrast-Enhanced Echocardiography in Pediatric Patients

Protocol Number: GE-191-008

Investigators and Study Centers: Up to 12 centers in North America and Europe.

Phase of Development: Phase 4

Objectives:

Primary:

- To determine a suitable dosage of OPTISON for contrast-enhanced echocardiography (CE-ECHO) in pediatric patients.

Secondary:

- To assess the safety profile of intravenous administrations of OPTISON in the pediatric population.
- To compare diagnostic confidence of left ventricular endocardial border delineation (LV EBD) and wall motion between non-contrast and OPTISON-enhanced echocardiography.
- To compare the diagnostic confidence in the evaluation of left ventricular ejection fraction (LVEF) between non-contrast and OPTISON-enhanced echocardiography.

Study Design:

This will be a Phase 4, open-label, non-randomized, multi-center, multi-dose study in North America and Europe. The study is designed to evaluate overall efficacy and safety of intravenous administration of OPTISON at various doses in pediatric patients undergoing transthoracic echocardiography.

Approximately 50 pediatric patients between ≥9 and <18 years of age weighing ≥20 kg who are clinically indicated for a transthoracic echocardiogram and are likely to have a limited transthoracic echocardiographic window based on body habitus and/or previous cardiac operation or have a previous suboptimal non-contrast echocardiogram will be enrolled into the study. OPTISON will be administered according to pediatric body weight (≥20 to ≤28 kg, >28 to ≤40 kg, and >40 kg). Each subject will receive administrations of 2 different dose levels. Injections should be at least 10 minutes apart between the 2 dose levels to allow for clearance of the previous dose.

For echocardiographic imaging, standard apical 4-chamber and 2-chamber views will be obtained at baseline using tissue harmonic imaging, thereafter with contrast using a contrast-imaging mode. Additional views may be obtained at the clinician's discretion. All image views will be obtained prior to and after contrast administration until 10 minutes post injection. All images will be recorded on disk and sent to image core lab for preparation of blinded image evaluation (BIE).

Efficacy assessment will be based on the results of the BIE. Clinical safety data will be collected throughout the study, beginning at baseline throughout 72 hours post OPTISON administration.

Primary Endpoint:

- Visualization of the 12 segments of the left ventricle wall in standard apical 4-chamber and 2-chamber views measured by the qualitative EBD visualization scale.

Secondary Endpoints:

- Overall safety profile in terms of occurrence of adverse events (AEs) and changes in vital signs, arterial oxygen saturation (SaO_2), physical examinations and 12-lead electrocardiograms (ECGs) following administration of different OPTISON doses in pediatric patients.

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- Left ventricular opacification (LVO) assessed by visual peak contrast intensity and peak left ventricular (LV) contrast filling, and duration of contrast enhancement following intravenous administration of OPTISON at various doses.
- Comparison of diagnostic confidence of LV EBD and wall motion between non-contrast and OPTISON-enhanced echocardiography at various doses.
- Comparison of diagnostic confidence in the evaluation of LVEF between non-contrast and OPTISON-enhanced echocardiography.

Selection of Subjects:

Inclusion Criteria:

(1) The subject is between ≥9 and <18 years of age and weighs ≥20 kg.
(2) The subject is clinically indicated to undergo a transthoracic echocardiogram.
(3) The subject has a suboptimal non-contrast echocardiogram defined as ≥2 contiguous segments in any given view that cannot be visualized.
(4) The subject is able to comply with study procedures.
(5) A parent or legal guardian of the subject has signed and dated an informed consent form, and age-appropriate pediatric assent has been obtained where appropriate.
(6) Post-menarchal female subjects must have a negative urine pregnancy test at screening and at pre-dose on the day of OPTISON administration.
(7) Post-menarchal female subjects must be practicing abstinence, or be using an effective form of birth control (e.g., intrauterine device, oral contraceptives, contraceptive implants or injections, diaphragm with spermicide, cervical cap, or consort use of condom) for at least 30 days before being enrolled in the study.

Exclusion Criteria

(1) The subject was previously enrolled in this study.
(2) The subject has received an investigational medicinal product within 30 days before or is scheduled to receive one from time of entry into this study until completion of the follow-up period proposed for this study.
(3) The subject has a known or suspected hypersensitivity to any of the components of OPTISON, blood, blood products, or albumin.
(4) The subject has pulmonary hypertension or unstable cardiopulmonary conditions.
(5) The subject has severe liver disease based on medical history.
(6) The subject had a recent (<6 months) neurological event.
(7) The subject presents any clinically active, serious, life-threatening disease, with a life expectancy of less than 1 month or where study participation may compromise the management of the subject or other reason that in the judgment of the investigator makes the subject unsuitable for participation in the study.
(8) The subject is a pregnant or lactating female, or is a female of childbearing potential not using an acceptable form of birth control (negative urine pregnancy test also required).

Number of Subjects/Centers Planned: Approximately 50 subjects at up to 12 centers in North America and Europe.

Treatment of Subjects:

Investigational Medicinal Product:

OPTISON (Perflutren Protein – Type A Microspheres Injectable Suspension [USP]) is a sterile non-pyrogenic suspension of perflutren-containing microspheres (5.0 to 8.0 x 10^8/mL with a mean diameter range of 3.0 to 4.5 µm) of heat-treated human serum albumin with perflutren for contrast enhancement during the indicated ultrasound imaging procedures. The approximate amount of perflutren gas in each milliliter of

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OPTISON is 0.19 mg corresponding to 7 µL. The vial contains a clear liquid lower layer and a white upper layer that, after resuspension by gentle mixing, provides a homogeneous, opaque, milky-white suspension for intravenous injection.

In adults, the recommended dose of OPTISON ranges from 0.5 to 3.0 mL injected into a peripheral vein with the maximum total dose not exceeding 8.7 mL in any one patient. However, there is no established dosage for children. For this study, the dose information is based on 2 publications using OPTISON in pediatric echocardiography. Each patient will receive 2 different doses of OPTISON administration for their CE-ECHO.

OPTISON will be administered according to pediatric body weight of ≥20 to ≤28 kg, >28 to ≤40 kg and >40 kg. According to the low volume of OPTISON required for these children, the volume of OPTISON will be diluted within the same volume of saline to facilitate the injection. In children weighing ≥20 to ≤28 kg, 0.1 or 0.2 mL of OPTISON per injection will be given in ascending order. The cumulative dose will not exceed 1.0 mL. If children weigh >28 to ≤40 kg, 0.2 or 0.3 mL of OPTISON per injection will be administered in ascending order with total dose not to exceed 1.5 mL. For children whose weight is >40 kg, 0.2 or 0.4 mL of OPTISON per injection will be administered in ascending order with total dose not to exceed 1.8 mL. The contrast will be administered as a slow bolus at a steady rate of approximately 0.05 mL per second for contrast-enhanced echocardiographic imaging followed by a 5.0 mL slow injection of saline.

Duration of Treatment:

Subject involvement in this study will be up to 3 weeks from time of screening to the completion of safety evaluation at 72 hours after OPTISON administration.

Efficacy and Safety Evaluation

Efficacy Evaluation:

Images will be evaluated by 3 independent blinded readers unaffiliated with enrolling centers.

Evaluation of EBD on 4- and 2-chamber views with non-contrast and OPTISON-enhanced echocardiography. A qualitative score will be assigned to each LV segment:

0 = no visualization of the LV endocardial border
1 = poor visualization
2 = fair visualization
3 = good/optimal visualization

The number of left ventricle segments visualized on 4- and 2-chamber views with non-contrast and OPTISON-enhanced echocardiography will be derived from the EBD score (segments rated as "fair visualization" or "good/optimal visualization").

Evaluation of LVO:

- Peak contrast intensity in the LV chamber is determined using categorical scales: none, low, medium, high, and blooming for different dose levels.
- Peak LV contrast filling will be categorized as:
  0 = none (0% filling)
  1 = faint (around 33% filling)
  2 = intermediate (around 67% filling)
  3 = full (100% filling)
- Contrast enhancement duration is determined from the time the contrast appears in the LV to the time the contrast almost dissipates from the left chamber.

Evaluation of image quality: a visual evaluation of the overall image quality will be categorized as one of the following:

- Excellent/good: homogeneous contrast enhancement with neither blooming nor shadowing artifacts.

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- Adequate: sufficient contrast enhancement (i.e., enough to visualize EBD) but less than ideal enhancement and/or short enhancement duration and/or with minimal blooming or shadowing artifacts.
- Poor: no or insufficient contrast enhancement/contrast duration or too much contrast enhancement leading to significant blooming and shadowing artifacts to assess EBD.

If any images are deemed as poor quality, an explanation is to be given as to the reason(s).

The diagnostic usefulness will be evaluated as follows:

- Non-diagnostic: the image quality is not sufficient to confidently visualize the endocardial border.
- Diagnostic: sufficient or excellent image quality for EBD assessments.

If any images are deemed non-diagnostic, an explanation is to be given as to the reason(s).

Diagnostic confidence for the assessment of LV EBD and wall motion will be scored for non-contrast and OPTISON-enhanced echocardiographic acquisitions separately using the following scale:

0 = no confidence
1 = low confidence
2 = moderate confidence
3 = high confidence

And the change in diagnostic confidence will be derived by comparing non-contrast and OPTISON-enhanced diagnostic confidence scores as follows (contrast-enhanced minus unenhanced):

≤-1 = confidence is lower with contrast-enhanced images
0 = confidence is the same between unenhanced and contrast-enhanced images
1 = confidence is slightly improved with contrast-enhanced images
2 = confidence is moderately improved with contrast-enhanced images
3 = confidence is greatly improved with contrast-enhanced images

Diagnostic confidence for the evaluation of LVEF (LVEF will be calculated using the formula: (end diastole volume – end systole volume)/end diastole volume) will be scored for non-contrast and OPTISON-enhanced echocardiographic acquisitions separately using the following scale:

0 = no confidence
1 = low confidence
2 = moderate confidence
3 = high confidence

And the change in diagnostic confidence will be derived by comparing non-contrast and OPTISON-enhanced diagnostic confidence scores as follows (contrast-enhanced minus unenhanced):

≤-1 = confidence is lower with contrast-enhanced images
0 = confidence is the same between unenhanced and contrast-enhanced images
1 = confidence is slightly improved with contrast-enhanced images
2 = confidence is moderately improved with contrast-enhanced images
3 = confidence is greatly improved with contrast-enhanced images

Further details of the independent assessment are given in the study's Independent Review Charter.

Safety Evaluation:

Safety parameters will include:

- Treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs)
- Vital signs: heart rate, respiratory rate and blood pressure
- Arterial oxygen saturation (SaO_2) by pulse oximetry
- Physical examination

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- Continuous ECG monitoring
- 12-lead ECGs

Statistical Methods and Planned Analysis:

All subjects who receive any study drug will be included in the safety population for safety analyses. Efficacy analyses will be performed on the intent-to-treat (ITT) and per-protocol populations. The ITT population includes all subjects enrolled in the study who have available non-contrast harmonic images and OPTISON-enhanced echocardiographic images, irrespective of quality of their CE-ECHO. The per-protocol population includes all subjects whose non-contrast harmonic images are available and whose OPTISON echocardiography images have adequate quality (sufficient contrast enhancement to visualize EBD). Demographic variables and patient characteristics will be summarized descriptively by treatment assignment and overall, independent of investigational medicinal product assignment. Demographic variables will include age, weight, height, gender, body mass index and race/ethnicity.

Efficacy: Since this is a dose-finding study, no hypotheses are being tested. Continuous variables will be summarized using means, standard deviations, medians, maxima, minima and sample size. Categorical data will be summarized using counts and percentages.

The suitable dosage of OPTISON for CE-ECHO in pediatric patients will be primarily determined by the improvement in the number of segments adequately visualized over non-contrast images, supplemented by LVO peak contrast intensity assessed as medium or better, peak LV contrast filling to around 67% or better, and duration of contrast enhancement ≥1.5 minutes. Image quality and diagnostic confidence, as well as overall safety of OPTISON in the pediatric population will also be taken into consideration for the dose selection.

Safety: Summary and descriptive statistics will be employed for safety analysis. TEAEs as reported on the case report forms (CRFs) will be mapped to System Organ Class (SOC) and preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA; current version or the immediately preceding version). The numbers and percentages of patients reporting each event will be summarized as follow:

- Overall number (frequency) of TEAEs (from dosing through 72±8 hours after the administration of the contrast agent).
- TEAEs by maximum severity, relationship to study drug and seriousness.
- TEAEs causing discontinuation from the study.

For vital signs, observed values and changes from baseline will be summarized with descriptive statistics, and a shift table analysis will be performed.

For ECGs, the overall ECG interpretation will be summarized. The observed values and changes from baseline in ECG intervals will be summarized with descriptive statistics, and a shift analysis will be performed for each ECG parameter. In addition, a summary will be made of clinically notable observed values and changes from baseline in QTc intervals.

Concomitant medications will be summarized using the World Health Organization (WHO) Drug Dictionary Anatomical-Therapeutic-Chemical (ATC) classification.

Physical examination results will be listed but not summarized.

Sample Size

The maximum sample size for this clinical investigation will be approximately 50 patients, which will provide adequate data to determine OPTISON efficacy in terms of LV EBD, LVO characteristics, wall motion assessment, diagnostic confidence for the evaluation of LVEF, and safety profile following intravenous administration in pediatric patients. The data will support dosing information in children.

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2.1 Overall Table of Contents

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ATC	Anatomical-Therapeutic-Chemical
BIE	Blinded image evaluation
CE-ECHO	Contrast-enhanced echocardiography
CRF	Case report form
CRO	Contract research organization
EBD	Endocardial border delineation
ECG	Electrocardiogram
GCP	Good Clinical Practice
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
IRB	Institutional/Independent Review Board
ITT	Intent-to-treat
LV	Left ventricular
LV EBD	Left ventricular endocardial border delineation
LVEF	Left ventricular ejection fraction
LVO	Left ventricular opacification
MedDRA	Medical Dictionary for Regulatory Activities
PMC	Post-marketing commitment
SAE	Serious adverse event
SOC	System organ class
SOPs	Standard operating procedures
TEAE	Treatment-emergent adverse event

4 BACKGROUND INFORMATION

Transthoracic echocardiography is an ideal tool for cardiac assessment, as it is noninvasive, portable, and efficacious in providing detailed anatomic, hemodynamic, and physiologic information. Unfortunately, up to 30% of adult studies are deemed technically difficult due to poor image quality, making these studies very challenging to interpret. Patients with large body habitus, chest wall deformities, and severe chronic lung disease are at higher risk of having suboptimal image quality ([Mulvagh et al. 2000], [Geleijnse et al. 1997], [Weissman et al. 2000]). Intravenous contrast-enhanced echocardiography (CE-ECHO) has been demonstrated to be a useful tool in optimizing endocardial border delineation (EBD) in adult patients ([Candido et al. 2003], [Clark and Dittrich. 2000], [Kornblith et al. 2000]) and in 3 studies only in pediatric patients ([Zilberman et al. 2003], [McMahon et al. 2005], [Kutty et al. 2016]).

Echocardiography plays a role in the diagnosis and assessment of congenital and acquired heart disease in infants and children. Despite the relatively high quality of echocardiographic imaging in children, echocardiographic images are not always diagnostic in some patients as a result of body habitus, previous cardiac operation, or both. However, there is limited experience with contrast agents in children to date ([Zilberman et al. 2003], [McMahon et al. 2005], [Kutty et al. 2016]).

OPTISON™ (Perflutren Protein-Type A Microspheres Injectable Suspension, USP) is a sterile non-pyrogenic suspension of microspheres of human serum albumin approved for use in adult patients with suboptimal echocardiograms to opacify the left ventricle and to improve the delineation of the left ventricular (LV) endocardial borders.

At the time of original new drug application (NDA) approval, a post-marketing commitment (PMC) was agreed to study the efficacy and safety of OPTISON in pediatric patients:

- To determine suitable dosage and administration instructions and any technical differences in instrumentation or settings that may affect OPTISON performance and safety in pediatric patients compared to adult patients.

The current study is therefore to fulfill the PMC requirement on dosage and safety assessment of OPTISON for the pediatric patients.

5 STUDY OBJECTIVES AND PURPOSE

The primary and secondary objectives of the study are as follows:

Primary:

- To determine a suitable dosage of OPTISON for CE-ECHO in pediatric patients.

Secondary:

- To assess the safety profile of intravenous administrations of OPTISON in the pediatric population.
- To compare diagnostic confidence of left ventricular endocardial border delineation (LV EBD) and wall motion between non-contrast and OPTISON-enhanced echocardiography.
- To compare the diagnostic confidence in the evaluation of left ventricular ejection fraction (LVEF) between non-contrast and OPTISON-enhanced echocardiography.

6 STUDY DESIGN

6.1 Overall Study Design and Plan

This will be a Phase 4, open-label, non-randomized, multi-center, multi-dose study in North America and Europe. The study is designed to evaluate efficacy and safety of intravenous administration of OPTISON at various doses in pediatric patients undergoing transthoracic echocardiography.

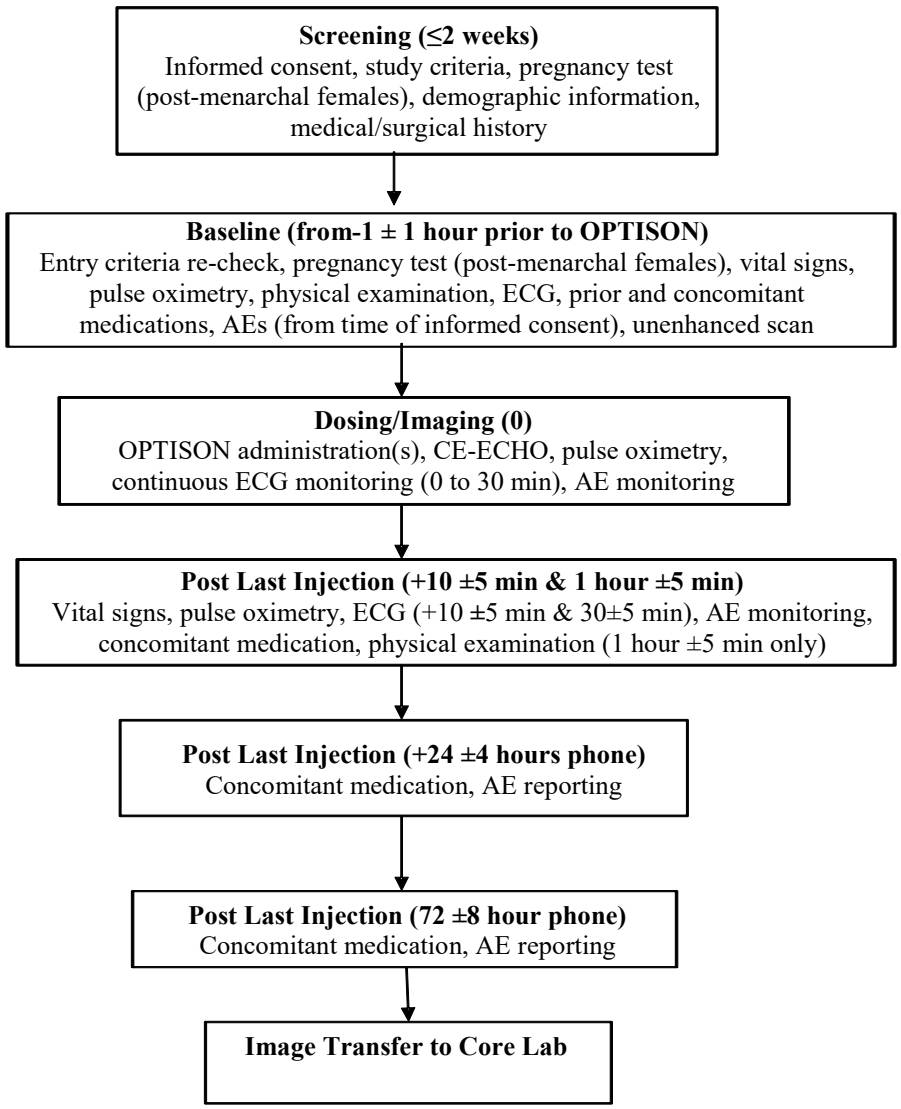
Approximately 50 pediatric patients between ≥9 and <18 years of age weighing ≥20 kg who are clinically indicated for a transthoracic echocardiogram and are likely to have a limited transthoracic echocardiographic window based on body habitus and/or previous cardiac operation or have a previous suboptimal non-contrast echocardiogram will be enrolled into the study.

Each patient will undergo an echocardiographic procedure first without contrast administration then following intravenous bolus injection of OPTISON at various doses. The dose of OPTISON for each subject will be determined according to pediatric body weight group and 2 different dose levels will be tested in each subject. The details on OPTISON administration are presented in Section 8.1 of this protocol.

For echocardiographic imaging, standard apical 4-chamber and 2-chamber views will be obtained at baseline using tissue harmonic imaging, thereafter with contrast using a contrast-imaging mode. Additional views may be obtained at the clinician's discretion. All image views will be obtained prior to and after contrast administration until 10 minutes post injection. All images will be recorded on disk and sent to sponsor's image core lab for preparation of blinded image evaluation (BIE).

Efficacy of echocardiographic images will be evaluated by independent blinded readers based on characteristics of left ventricular opacification (LVO), diagnostic confidence of LV EBD and wall motion, and diagnostic confidence of LVEF. Clinical safety data will be collected throughout the study, beginning from dosing through 72 hours post OPTISON administration to determine the safety profile of intravenous administration of OPTISON at various doses in the pediatric population.

Figure 1 Study Diagram



6.2 Study Rationale

The study is to determine a suitable dosage and administration instructions for CE-ECHO with OPTISON in pediatric patients.

OPTISON is a registered product. It has been approved since 1997 for LVO and EBD in adult patients with non-diagnostic echocardiogram. Currently, several injections of 0.5 mL may be injected, up to a cumulative maximum of 8.7 mL. The current study will help to determine an appropriate dose for pediatric echocardiography.

6.2.1 Justification of Dose Selection and Efficacy Analysis

In the adult controlled clinical trials for the LVO and EBD indication, the duration of the useful imaging time for LV chamber opacification was 2.5 to 4.5 minutes for a dose of 0.5 to 3.0 mL [OPTISON PI].

McMahon et al conducted rest non-contrast and CE-ECHO in 20 children (9 to 18 years) with limited transthoracic echo windows [McMahon et al. 2005]. OPTISON was administered at a dose of 0.3 mL per injection for children <20 kg and 0.5 mL per injection for those ≥20 kg. The EBD was significantly improved in all patients with CE-ECHO and LVEF was measurable in all 20 patients using contrast echo versus only 11 with non-contrast echocardiography. Zilberman et al performed contrast stress echocardiography with OPTISON administration in 22 children (8 months to 19 years; mean age 9.3 ± 3.9 years) [Zilberman et al. 2003]. A dose of 0.1 mL was given for patients ≤25 kg, and 0.2 mL for patients >25 kg. These doses provided adequate opacification of the LV cavity for up to 60 seconds.

Similar to the two publications, OPTISON dose in this study will be administered based on body weight. According to the Centers for Disease Control and Prevention Pediatric Growth Charts [CDC Growth Charts], children >9 years of age usually weigh >28 kg and children >12 years of age usually weigh >40 kg for both boys and girls in the United States, but weight varies greatly for a specific age. Two dose levels will be tested in each weight cohort: 0.1 or 0.2 mL per injection to children weighing ≥20 to ≤28 kg, 0.2 or 0.3 mL per injection to those who weigh >28 to ≤40 kg, and 0.2 or 0.4 mL per injection to those who weigh >40 kg. It is expected that one or more of these dose levels will be able to provide adequate LVO and EBD in these groups of pediatric patients with a limited transthoracic echocardiographic window.

6.2.2 Justification of Safety Monitoring Plan

This study's safety monitoring plan is justifiable and adequate from a safety standpoint:

- The design of the safety plan permits a comparison of the safety response to OPTISON at baseline and post-investigational medicinal product (IMP) administration conditions in the same subject.
- Consideration of a 3-day safety monitoring follow-up permits the evaluation of late-appearing adverse effects that may emerge or progress after the administration of OPTISON.
- The measures used to assess safety are well-defined and reliable, and the proposed safety analyses are adequate to assess the effects of the administration of OPTISON.

6.3 Study Timeframe

The duration of each subject's participation is expected to be up to 3 weeks, including screening (2 weeks), procedure, and safety follow-up (24 hours and 72 hours post OPTISON administration).

The entire subject recruitment phase is expected to last up to 2 years.

6.4 Risks and Benefits to Subjects

Study participants may help future patients by contributing to a knowledge base for further evaluation of the use of OPTISON in pediatric patients with cardiac disease. For some patients participating in the study, the benefit might be detection or more precise evaluation of their cardiac disease.

OPTISON is well tolerated in adults. Adverse reactions to OPTISON are rare and usually of a non-serious nature. In general, the administration of human albumin has been associated with transient altered taste, nausea, flushing, rash, headache, vomiting, chills and fever. The shell of the OPTISON bubble is composed of albumin. Rare anaphylactic reactions have been associated with the administration of human albumin products. The reported adverse events (AEs) following the use of OPTISON in Phase 3 human clinical studies have been mild to moderate with subsequent full recovery.

For further information please refer to the currently approved OPTISON Package Insert [OPTISON PI].

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Procedures for Enrolment

Investigators are responsible for subject recruitment at their sites. A signed and dated (with time) informed consent form will be obtained from a parent or legal guardian of each study subject prior to study entry or any study-specific procedures. Age-appropriate pediatric assent will be obtained where appropriate. Subjects will be screened for entry through the use of clinical examination and historic assessment. A unique study-related subject number will be assigned at baseline before the unenhanced echocardiography procedure.

7.2 Inclusion Criteria

Subjects may be included in the study if they meet all of the following criteria:

(1) The subject is between ≥9 and <18 years of age and weighs ≥20 kg.

(2) The subject is clinically indicated to undergo a transthoracic echocardiogram.

(3) The subject has a suboptimal non-contrast echocardiogram defined as ≥2 contiguous segments in any given view that cannot be visualized.

(4) The subject is able to comply with study procedures.

(5) A parent or legal guardian of the subject has signed and dated an informed consent form, and age-appropriate pediatric assent has been obtained where appropriate.

(6) Post-menarchal female subjects must have a negative urine pregnancy test at screening and at pre-dose on the day of OPTISON administration.

(7) Post-menarchal female subjects must be practicing abstinence, or be using an effective form of birth control (e.g., intrauterine device, oral contraceptives, contraceptive implants or injections, diaphragm with spermicide, cervical cap, or consort use of condom) for at least 30 days before being enrolled in the study.

7.3 Exclusion Criteria

Subjects must be excluded from participating in this study if they meet any of the following criteria:

(1) The subject was previously enrolled in this study.

(2) The subject has received an IMP within 30 days before or is scheduled to receive one from time of entry into this study until completion of the follow-up period proposed for this study.

(3) The subject has a known or suspected hypersensitivity to any of the components of OPTISON, blood, blood products, or albumin.

(4) The subject has pulmonary hypertension or unstable cardiopulmonary conditions.

(5) The subject has severe liver disease based on medical history.

(6) The subject had a recent (<6 months) neurological event.

(7) The subject presents any clinically active, serious, life-threatening disease, with a life expectancy of less than 1 month or where study participation may compromise the management of the subject or other reason that in the judgment of the investigator makes the subject unsuitable for participation in the study.

(8) The subject is a pregnant or lactating female, or is a female of childbearing potential not using an acceptable form of birth control (negative urine pregnancy test also required).

7.4 Withdrawal and Termination Criteria

7.4.1 Subject Withdrawal

During the conduct of the study, the sponsor will review the safety data for trends and signals that would indicate the need for withdrawal of a subject. If a subject undergoes an intervention or has a significant change in disease status between the administration of OPTISON and the completion of study follow-up, the subject will be withdrawn from the study.

In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time. Investigator(s) also have the right to withdraw subjects from the study in the event of illness, AEs, or other reasons concerning the health or well-being of the subject, or in the case of lack of co-operation.

Should a subject decide to withdraw after administration of OPTISON, or should the investigator(s) decide to withdraw the subject, all efforts will be made to complete and report the observations up to the time of withdrawal as thoroughly as possible. A complete final evaluation at the time of the subject's withdrawal should be made and an explanation given of why the subject is withdrawing or being withdrawn from the study.

The reason for withdrawal must be noted in the Case Report Form (CRF). If the reason for withdrawal is a serious adverse event (SAE), monitoring will continue until the outcome is evident. The specific event or test result(s) must be recorded in the CRF.

If a subject is withdrawn before completion of CE-ECHO, the subject will be replaced. The replaced subject will be assigned a new subject number.

7.4.2 Study or Site Termination

GE Healthcare is committed to complete subject enrolment in approximately 2 years. However, if after one year, <10% of the 50 subjects have been enrolled at 12 sites, additional study modifications or study termination will be considered. The sponsor reserves the right to terminate the study at any time.

Should termination be necessary, both the sponsor and the investigators will ensure that adequate consideration is given to the protection of the subjects' interest. Termination of the study will be considered in the event of a significant safety finding occurring at any time during the conduct of the study.

Investigators have the responsibility to comply with International Council for Harmonisation (ICH) E6-Good Clinical Practice (GCP) guidance. The sponsor, the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), or the health authorities may terminate a center for the following (but not limited to) reasons:

(1) Failure of the investigator to comply with pertinent ICH E6-GCP guidelines and regulations.

(2) Occurrence of serious protocol violations.

(3) Submission of knowingly false information from the research facility to the sponsor, clinical monitor, or other party involved in the study.

(4) Failure of the investigator to enroll subjects into the study at an acceptable rate as agreed with the sponsor.

(5) Repeated failure to have imaging data transferred to the sponsor or the CRF completed and ready for review by the sponsor in the agreed time frame.

8 TREATMENT OF SUBJECTS

Each investigator is responsible for ensuring that deliveries of IMP and other study materials from the sponsor are correctly received, recorded, handled, and stored safely and properly in accordance with all applicable regulatory guidelines, and used in accordance with this protocol.

All IMP containers (opened, unopened, or empty) must be returned to the sponsor or destroyed on site after the study and overall drug accountability have been completed by the sponsor or representative. A list of IMP(s) and other materials that were returned, or destroyed, must be prepared and signed by the principal investigator or designee. If there are any discrepancies, an explanation for these should also be provided.

8.1 Investigational Medicinal Product

8.1.1 OPTISON

OPTISON (Perflutren Protein-Type A Microspheres Injectable Suspension, [USP]) is a sterile non-pyrogenic suspension of perflutren-containing microspheres (5.0 to 8.0 $\times 10^8$/mL with a mean diameter range of 3.0 to 4.5 μm) of heat-treated human serum albumin with perflutren for contrast enhancement during the indicated ultrasound imaging procedures. The approximate amount of perflutren gas in each milliliter of OPTISON is 0.19 mg corresponding to 7 μL. The vial contains a clear liquid lower layer and a white upper layer that, after resuspension by gentle mixing, provides a homogeneous, opaque, milky-white suspension for intravenous injection. OPTISON will be provided in 3-mL vials for single use. OPTISON should be stored refrigerated at 2°C to 8°C (36°F to 46°F). Investigators must follow the package insert instruction to prepare and administer OPTISON.

In adults, the recommended dose of OPTISON ranges from 0.5 to 3.0 mL injected into a peripheral vein with the maximum total dose not exceeding 8.7 mL in any one patient. However, there is no established dosage for children. For this study, the dose information is based on 2 publications using OPTISON in pediatric echocardiography (see Section 6.2.1). Each patient will receive 2 different doses of OPTISON administration for CE-ECHO; the actual doses of OPTISON will be based on pediatric body weight of ≥20 to ≤28 kg, >28 to ≤40 kg, and >40 kg and up to 3 injections per dose level are allowed for acquiring different imaging views (Table 1). According to the low volume of OPTISON required for these children, the volume of OPTISON will be diluted within the same volume of saline to facilitate the injection.

Table 1 OPTISON Dosing Table

Body weight (kg)	Dose/injection (mL) Dose level 1	Dose/injection (mL) Dose level 2	Total dose (mL) not to exceed (3 injections/dose level)
≥20 to ≤28	0.1	0.2	1.0
>28 to ≤40	0.2	0.3	1.5
>40	0.2	0.4	1.8

The contrast will be administered as a slow bolus at a steady rate of approximately 0.05 mL per second for contrast-enhanced echocardiographic imaging followed by a 5.0 mL slow injection of saline. Injections should be at least 10 minutes apart to allow for clearance of the previous dose.

8.1.2 Comparator

Unenhanced grey-scale echocardiography will be the comparator.

8.1.3 Registration of Investigational Medicinal Product Complaints

In the event of an IMP complaint (e.g., breakage, leakage, particulate matter, discoloration), the investigator or recipient of the IMP is requested to report the problem on the IMP shipping documentation (e.g., 'Delivery Note for Product', Drug Shipping and Receiving Form, or equivalent form). This should be promptly forwarded to the person indicated on the shipping documentation. Once received, the Clinical Supplies Manager will register the complaint and determine if the complaint is minor or significant according to sponsor procedures. All complaints will be followed-up and the appropriate action will be implemented according to sponsor procedures.

8.2 Method of Numbering Subjects and Assigning Subjects to Treatment Groups

No treatment will be assigned to a subject before it is ascertained that the subject has met all of the inclusion criteria and none of the exclusion criterion and that written informed consent (and age-appropriate pediatric assent where appropriate) has been obtained. An allocation number will be assigned to each consented subject so that all of the subjects from a center will be given allocation numbers in successive order of inclusion. The subject's allocation number and vial number are separate and independent.

8.3 Blinding

This is an open-label study. No blinding will be conducted for the subject allocation or application of the IMP in this study.

For determination of efficacy and optimal dose level, 3 qualified independent blinded readers will conduct BIE of echocardiographic images. Non-contrast and OPTISON-enhanced images will be presented in random order to the 3 readers without dosing information.

8.4 Prior and Concomitant Therapy

Any prior, concomitant, or procedural medications or therapy given to or taken by the subject within 24 hours before and up to the end of the observation period (3 days post OPTISON

administration) will be recorded in the CRF along with the indication. Either the generic or the trade name may be recorded. The sponsor will encode all therapy and medication according to a current well-recognized dictionary of medical codes.

8.5 Contraception and Pregnancy Avoidance Procedure

Females of child-bearing potential are "fertile, following menarche and until becoming post-menopausal, unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy" [CTFG Guidance 2014].

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy or vasectomized with confirmation of success.

Females of child-bearing potential who are sexually active with a non-sterile male partner must be using an acceptable method of contraception (as defined below) from at least 30 days before being enrolled in the study.

Acceptable methods of contraception considered as highly effective are those with no higher than a 1% failure rate. Such methods include [CTFG Guidance 2014]:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation, provided there is no concern about interaction with the IMP:
  - oral
  - intravaginal
  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation, provided there is no concern about interaction with the IMP:
  - oral
  - injectable
  - implantable
- Intrauterine device
- Intrauterine hormone-releasing system, provided there is no concern about interaction with the IMP
- Bilateral tubal occlusion
- Vasectomized partner (provided that the vasectomized partner is the sole sexual partner of the female of child-bearing potential and that the vasectomized partner has received medical confirmation of the surgical success)
- Sexual abstinence (only if the subject refrains from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject).

Acceptable, but not highly effective, birth control methods that result in a failure rate of more than 1% per year include [CTFG Guidance 2014]:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide
- cap, diaphragm or sponge with spermicide

Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process. Females of child-bearing potential must have a negative result for a urine human chorionic gonadotropin pregnancy test at screening and at pre-dose (according to the Study Schedule of Events, Table 2).

8.6 Treatment Compliance

Subjects will receive OPTISON administration under direct supervision of study personnel. Each administration volume will be checked and the volume per administration will be recorded in each subject's CRF.

9 STUDY PROCEDURES

All efficacy and safety measurements obtained during the course of the study are summarized in the Study Schedule of Events (Table 2).

Table 2 Study Schedule of Events

Variables	Pre-procedure		Dosing/ Imaging (0)	Post-last OPTISON Administration				
	Screening (≤2 weeks)	Baseline (from -1 ± 1 h)		10 min (±5 min)	30 min (±5 min)	1 h (±5 min)	24 h (±4 h)	72 h (±8 h)
Informed Consent	X							
Entry Criteria	< ---------- >							
Demographic Information	X							
Medical/Surgical History	X							
Pregnancy test [a]	X	X						
Physical Examination		X				X		
Vital Signs		X		X		X		
SaO_2 by Pulse Oximetry		X	X	X		X		
12-Lead ECG Recording		X		X	X			
Prior and concomitant Medications [b]		< ---------- >						
Continuous ECG Monitoring [c]			< ---------- >					
OPTISON Administration			X					
Image Acquisition		X	X					
Phone Calls							X	X
AEs and SAEs Monitoring	X	<---------- >						

AE = Adverse event; ECG = Electrocardiogram; SAE = Serious adverse event; SaO_2 = Oxygen saturation

a Urine β-hCG pregnancy test at screening and at pre-dose; post-menarchal females only.

b Any prior or concomitant medication taken by the subject within 24 hours before OPTISON injection and up to the end of the 72-hour follow-up period.

c Continuous ECG monitoring is from time of first OPTISON injection until 30 minutes after the last OPTISON injection

9.1 Screening Period

The screening evaluations may occur within 2 weeks prior to baseline day 0. The subject's study participation must be documented in the institution's files including subject's identification, date of inclusion and the study number.

The screening evaluations include the following:

- Signed, dated, and timed informed consent must be obtained from parents or legal guardian of all subjects prior to their entering the study or any study specific procedure. Age-appropriate pediatric assent must be obtained where appropriate.
- Assessment of inclusion and exclusion criteria. All subjects must satisfy all the inclusion criteria and none of the exclusion criteria listed in Sections 7.2 and 7.3.
- Collection of demographic information including age and gender.
- Obtaining medical/surgical history.
- Urine β-hCG pregnancy test (post-menarchal females).
- Recording of AEs (from time of consent).

9.2 Baseline Period

The subjects will enter the baseline period on the day of the echocardiography. The baseline period will begin at 1 ± 1 hour prior to OPTISON administration and continue until time of first injection of OPTISON.

The following procedures must be completed before OPTISON administration:

- Re-assessment of inclusion and exclusion criteria.
- Urine β-hCG pregnancy test (post-menarchal females)
- Perform a physical examination.
- Vital signs (heart rate, blood pressure and respiratory rate) will be measured and recorded.
- Monitoring and recording pulse oximetry.
- 12-lead electrocardiograms (ECGs) will be performed with the subject in the supine position.
- Recording prior and concomitant medication taken within 24 hours prior to procedure.
- Record any AEs.
- Acquiring unenhanced echocardiography images.

9.3 Treatment Period

The treatment period includes OPTISON administration, imaging procedure and safety follow-up. The following procedures will be performed:

- OPTISON must be prepared and administered according to the package insert instruction (also see Section 8.1.1).

- The volume per OPTISON administration, number of bolus injections, and total volume administered will be recorded.
- CE-ECHO images will be acquired according to instruction in Section 10.2.2.
- Continuous ECG monitoring from time of first OPTISON injection until 30 minutes after the last OPTISON injection.
- Monitoring and recording pulse oximetry.
- Subjects will be monitored for the occurrence of AEs and SAEs.

9.4 Post-Treatment Period

After completion of contrast administration and imaging acquisition, the subjects will be monitored at the following time points for safety assessment:

- At 10 ± 5 minutes and 30 ± 5 minutes after the last OPTISON administration, 12-lead ECG will be recorded. Investigators may also record 12-lead ECG at additional time points if thought to be clinically relevant; however, only 12-lead ECG recordings at 10 ± 5 minutes and 30 ± 5 minutes will be considered as study data.
- At 10 ± 5 minutes and 1 hour ± 5 minutes after the last OPTISON administration, vital signs and pulse oximetry will be obtained.
- A physical examination will be performed at 1 hour ± 5 minutes. The occurrence of AEs and SAEs will be monitored and recorded.
- At 24 ± 4 hours and 72 ± 8 hours after the last OPTISON administration, the subject's parents or legal guardian will be contacted by telephone for assessing AEs/SAEs and recording concomitant medications.
- The subject's parent/guardian will be provided with an emergency phone number and instructed to contact study site personnel with any questions or concerns. The subject's parent/guardian may be called at any time if considered necessary by study personnel.

10 EFFICACY, SAFETY, AND OTHER VARIABLES

10.1 Study Endpoints

10.1.1 Primary Endpoint

- Visualization of the 12 segments of the left ventricle wall in standard apical 4-chamber and 2-chamber views measured by the qualitative EBD visualization scale.

10.1.2 Secondary Endpoints

The secondary endpoints will be the following:

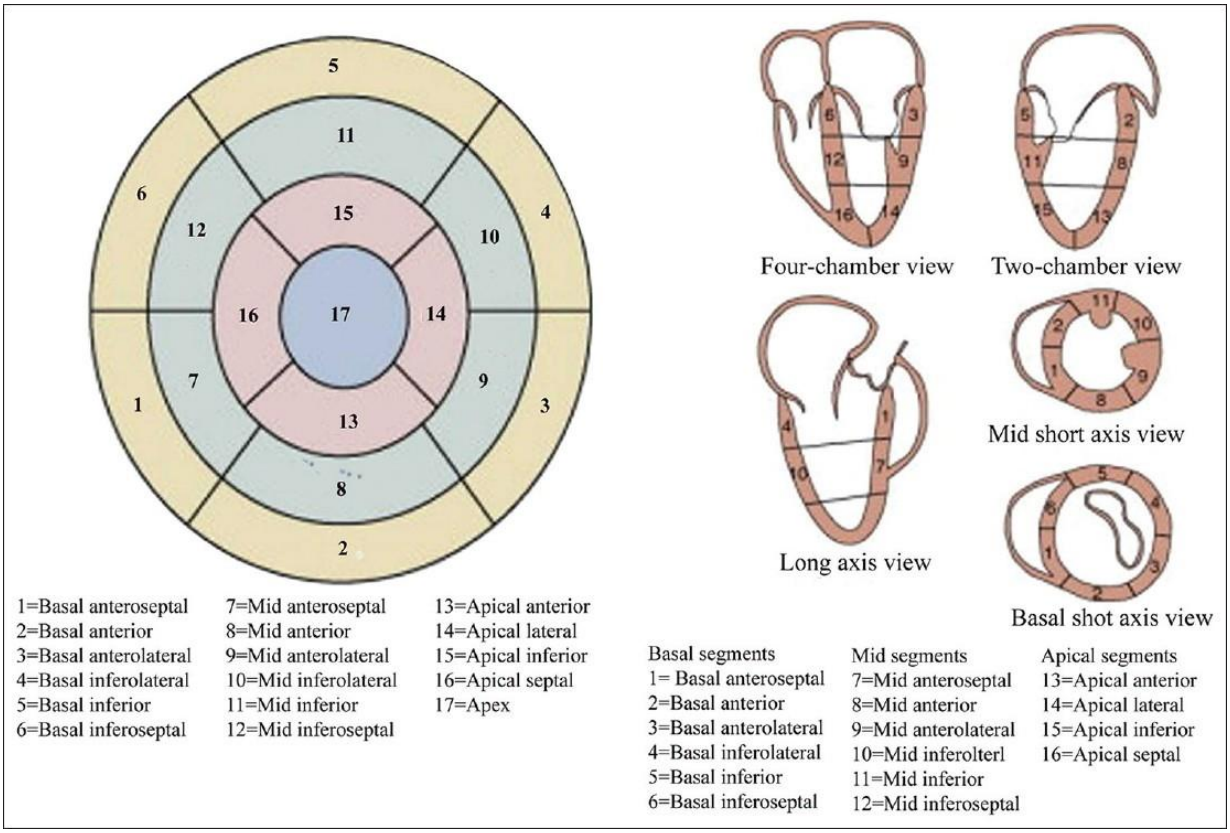
- Overall safety profile in terms of occurrence of AEs and changes in vital signs, arterial oxygen saturation (SaO_2), physical examinations and 12-lead ECGs following administration of different OPTISON doses in pediatric patients.
- LVO assessed by visual peak contrast intensity and peak LV contrast filling, and duration of contrast enhancement following intravenous administration of OPTISON at various doses.
- Comparison of diagnostic confidence of LV EBD and wall motion between non-contrast and OPTISON-enhanced echocardiography at various doses.
- Comparison of diagnostic confidence in the evaluation of LVEF between non-contrast and OPTISON-enhanced echocardiography.

10.2 Efficacy Assessment

Efficacy will be assessed by 3 independent blinded readers unaffiliated with enrolling centers and based on characteristics of LV EBD, LVO, diagnostic confidence for the assessment of LV EBD and wall motion, and for the evaluation of LVEF for both non-contrast and CE-ECHO.

10.2.1 Efficacy Variables and Method of Measurement

Figure 2 Segmentation schemes for segmental analysis of LV EBD (adapted from [Lang et al. 2015])



Basal segments	Mid segments	Apical segments
2 = Basal anterior	8 = Mid anterior	13 = Apical anterior
3 = Basal anterolateral	9 = Mid anterolateral	14 = Apical lateral
5 = Basal inferior	11 = Mid inferior	15 = Apical inferior
6 = Basal inferoseptal	12 = Mid inferoseptal	16 = Apical septal

Table 3 Efficacy Variables and Method of Measurement

Efficacy Variable	Method of Measurement
EBD	Evaluation of EBD on 4- and 2-chamber views with non-contrast and OPTISON-enhanced echocardiography. A qualitative score will be assigned to each ventricular segment: 0 = no visualization of the LV endocardial border 1 = poor visualization 2 = fair visualization 3 = good/optimal visualization The number of left ventricle segments visualized on 4- and 2-chamber views with non-contrast and OPTISON-enhanced echocardiography will be derived from the EBD score (segments rated as "fair visualization" or "good/optimal visualization").
LVO	• Peak contrast intensity in the LV chamber is determined by blinded readers using categorical scales: none, low, medium, high, and blooming for different dose levels. • Peak LV contrast filling will be categorized as: 0 = none (0% filling) 1 = faint (around 33% filling) 2 = intermediate (around 67% filling) 3 = full (100% filling) • Contrast enhancement duration is determined from the time the contrast appears in the LV to the time the contrast almost dissipates from the left chamber.
Image quality	Independent blinded readers assess CE-ECHO images for overall image quality as excellent/good, adequate, or poor. • Excellent/good: homogeneous contrast enhancement with neither blooming nor shadowing artifacts. • Adequate: sufficient contrast enhancement (i.e., enough to visualize EBD) but less than ideal enhancement and/or short enhancement duration and/or with minimal blooming or shadowing artifacts. • Poor: no or insufficient contrast enhancement/contrast duration or too much contrast enhancement leading to significant blooming and shadowing artifacts to assess EBD. If any images are deemed as poor quality, an explanation is to be given as to the reason(s). The diagnostic usefulness will be evaluated as follows: • Non-diagnostic: the image quality is not sufficient to confidently visualize the endocardial border. • Diagnostic: sufficient or excellent image quality for EBD assessments. If any images are deemed non-diagnostic, an explanation is to be given as to the reason(s)
Diagnostic confidence for the assessment of LV EBD and wall motion	Diagnostic confidence for the assessment of LV EBD and wall motion will be scored for non-contrast and OPTISON-enhanced echocardiographic acquisitions separately using the following scale: 0 = no confidence 1 = low confidence 2 = moderate confidence 3 = high confidence And the change in diagnostic confidence will be derived by comparing non-contrast and OPTISON-enhanced diagnostic confidence scores as follows (contrast-enhanced minus unenhanced): ≤-1 = confidence is lower with contrast-enhanced images 0 = confidence is the same between unenhanced and contrast-enhanced images 1 = confidence is slightly improved with contrast-enhanced images 2 = confidence is moderately improved with contrast-enhanced images 3 = confidence is greatly improved with contrast-enhanced images

Table 3 Efficacy Variables and Method of Measurement

Efficacy Variable	Method of Measurement
Diagnostic confidence for the evaluation of LVEF	Diagnostic confidence for the evaluation of LVEF (LVEF will be calculated using the formula: (end diastole volume – end systole volume)/end diastole volume) will be scored for non-contrast and OPTISON-enhanced echocardiographic acquisitions separately using the following scale: 0 = no confidence 1 = low confidence 2 = moderate confidence 3 = high confidence And the change in diagnostic confidence will be derived by comparing non-contrast and OPTISON-enhanced diagnostic confidence scores as follows (contrast-enhanced minus unenhanced): ≤-1 = confidence is lower with contrast-enhanced images 0 = confidence is the same between unenhanced and contrast-enhanced images 1 = confidence is slightly improved with contrast-enhanced images 2 = confidence is moderately improved with contrast-enhanced images 3 = confidence is greatly improved with contrast-enhanced images

The suitable dosage of OPTISON for CE-ECHO in pediatric patients will be primarily determined by the improvement in the number of segments adequately visualized over non-contrast images, supplemented by LVO peak contrast intensity assessed as medium or better, peak LV contrast filling to around 67% or better, and duration of contrast enhancement to ≥1.5 minutes. Image quality and diagnostic confidence, as well as overall safety of OPTISON in the pediatric population, will also be taken into consideration for the dose selection.

10.2.2 Image Acquisition

For each echocardiographic imaging, standard apical 4-chamber and 2-chamber views will be obtained at baseline using tissue harmonic imaging, thereafter with contrast using a contrast imaging mode. Additional views may be obtained either prior to or after contrast administration under clinician's discretion.

For details, please refer to the Imaging Manual provided as a separate document.

10.2.3 Comparator or Reference Imaging

Non-contrast harmonic images will be used as comparator in this study.

10.2.4 Image Interpretation

All echocardiographic images will be evaluated by 3 independent blinded readers for characteristics of LV EBD, LVO, diagnostic confidence for the assessment of LV EBD and wall motion, and for the evaluation of LVEF. The BIE process is described in detail in the Independent Review Charter.

10.3 Safety Assessments

The investigator(s) and the sponsor will review the safety data. The following safety data will be collected and evaluated (see Table 2):

- Vital signs: systolic/diastolic blood pressure, heart rate, respiratory rate, and continuous pulse oximetry.
- Continuous ECG monitoring.
- 12-lead ECG recording.
- Physical examination.
- Post-treatment events (treatment-emergent AEs [TEAEs] and SAEs).

Pre-specified normal limits for vital signs and ECG intervals are provided in Section 12.4.

10.3.1 Vital Signs

Vital signs will be measured at the various pre- and post-treatment time point ranges described in Table 2. Vital sign parameters include measurements of heart rate, respiratory rate, systolic and diastolic blood pressures, and pulse oximetry. Before vital signs are measured, subject should be resting for at least 5 minutes (if possible). The same position will be used each time vital signs are measured for a given subject and blood pressure will be measured from the arm contra-lateral to the site of OPTISON administration whenever possible.

Any finding that is classified by the investigator as a clinically significant (i.e., that resulted in a change in subject management) worsening compared to a previous finding will be considered an AE or TEAE as applicable and will be documented on the subject's CRF, and followed until the outcome is known.

10.3.2 Electrocardiograms

Continuous ECG monitoring will begin from time of first OPTISON injection and continue until 30 minutes after the last dose of OPTISON. 12-lead ECGs will be recorded at baseline prior to contrast administration and 10 minutes and 30 minutes after the last OPTISON administration.

All ECG recordings will be read at the investigational site. Any finding that is classified by the investigator as a clinically significant worsening compared to a previous finding will be considered an AE or TEAE as applicable and will be documented on the subject's CRF and followed until the outcome is known.

10.3.3 Prior and Concomitant Medication

Prior and concomitant medication will be recorded during the study procedures and at follow-up appointments throughout the study period.

10.3.4 Physical Examination

A licensed physician or qualified designee will conduct the physical examinations at the baseline visit and 1 hour after the last OPTISON administration. The same individual should conduct the physical examination at both required time points. The physical examination will include recording an assessment for the presence of abnormalities of the following: general appearance, respiratory and cardiovascular system. Neurological examination will include level of consciousness, motor function, and sensory function.

Any finding that is classified by the investigator as a clinically significant worsening compared to a previous finding will be considered an AE or TEAE as applicable and will be documented on the subject's CRF, and followed until the outcome is known.

10.3.5 Adverse Events

The AE profile of OPTISON has not yet been established in pediatric patients. Study personnel must remain vigilant for the occurrence of AEs, particularly those that may be life threatening. Personnel who are trained in the management of acute anaphylaxis and other emergencies and who have access to appropriate clinical supplies must be immediately available for at least 30 minutes to observe for possible anaphylactoid reactions after dosing. Treatment of SAEs should be primarily supportive of vital functions.

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject which does not necessarily have to have a causal relationship with the study treatment. AEs will be asked for from time of informed consent through 72 ±8 hours after the last administration of contrast agent.

A TEAE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of OPTISON, whether or not considered related to that product. Only symptoms/signs that begin or worsen in severity and/or frequency after OPTISON administration/use will be recorded as TEAEs in the CRF.

The subjects will be closely observed and questioned for any kind of AE during the study procedures and at follow-up appointments throughout the study period with non-leading questioning (e.g., "How do you feel?"). The subjects will be instructed to immediately report any symptoms and signs to the study staff (i.e., between formal observations).

See section 10.3.8 for documentation of SAEs in the CRF.

10.3.6 Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

a) Results in death.

b) Is life threatening.

- The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c) Requires in-patient hospitalization or prolongation of existing hospitalization.

- In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d) Results in persistent or significant disability or incapacity.

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e) Is a congenital anomaly or birth defect.

f) Is another important medical event.

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Subjects (or, when appropriate, a caregiver, surrogate, or the subject's legally authorized representative) will report AEs to the investigator and/or qualified designee.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the IMP or study procedures, or that cause the subject to discontinue the study.

10.3.7 Other Significant Adverse Events

Any events that lead to an intervention (including premature discontinuation of OPTISON, dose reduction or significant additional concomitant therapy), other than those reported as SAEs, will be reported and evaluated as other significant AEs.

10.3.8 Adverse Event and Serious Adverse Event Reporting

All AEs should be recorded using acceptable diagnoses, if possible. If an AE has already been reported it is not necessary to report each individual sign and symptom of that AE as a separate AE. For example, if myocardial infarction is reported as an AE, there is no need to report elevated creatine phosphokinase and abnormal ECG, or other related signs, symptoms, or laboratory values as separate AEs. However, if both occurred in isolation and myocardial infarction was not diagnosed, then each event would be reported as an AE.

The intensity of all AEs will be graded as mild, moderate, or severe using the following definitions:

Mild:	Tolerable.
Moderate:	Interferes with normal activity.
Severe:	Incapacitating (causes inability to perform usual activity or work).

The investigator will be instructed to closely monitor each subject who experiences an AE (whether ascribed to the IMP or not) until the outcome of the AE has been determined.

Both the investigator and GE Healthcare will perform a causality assessment on any AE, to assess whether or not there is a reasonable possibility (evidence to suggest) that the IMP caused the event.

SAEs will be recorded in the CRF and on the SAE reporting form if they occurred as follows:

- After a subject first signed informed consent and throughout the subject's follow-up period*, whether or not considered related to the OPTISON, and
- After the subject's follow-up period, and for which a causal relationship to the OPTISON is a reasonable possibility (evidence to suggest) that the OPTISON caused the event. Causality evaluation for any AE shall be documented.

(*Follow-up period is defined as the protocol-stipulated period of 72±8 hours after administration of the IMP or, for subjects prematurely withdrawn from a study, the duration of a subject's participation.)

All serious and non-serious AEs must be followed for a final outcome until the end of the follow-up period. An outcome of "unknown" is not considered to be an acceptable final outcome. An outcome of "not yet resolved" is an acceptable final outcome for non-serious AEs at the end of a subject's participation in a study, and for SAEs at database lock.

The sponsor will report all SAEs to local health authorities, IECs/IRBs and investigators as required by local regulations and sponsor standard operating procedures (SOPs).

In this study, pediatric patients with known or suspected heart disease may be enrolled. Therefore, it is anticipated that echocardiography (with or without OPTISON) may detect abnormalities that clearly must have been pre-existing, and that some of these may result in new/prolonged hospitalization, surgery, etc. In such cases, the newly detected pre-existing abnormality need not be reported as an AE or SAE.

Study centers are instructed to report all SAEs to the sponsor within 24 hours.

Notification of SAEs (using the standard SAE form provided by the sponsor) must be sent by email to the attention of "Covance DDSS" at SAEIntake@Covance.com immediately (within 24 hours) of becoming aware of an SAE. Covance will forward the SAE form to GE Healthcare within 24 hours to GPV.DrugSafety@GE.com

For any protocol or safety-related questions please contact the Medical Director:

[redacted]
GE Healthcare Life Sciences
Pollards Wood, Nightingales Lane
Chalfont St Giles, Bucks HP8 4SP
United Kingdom
T: [redacted]

10.3.9 Urgent Safety Measures

In accordance with the principles of GCP as laid out in ICH E6, the investigator(s) has/have primary responsibility for assuring subject safety throughout the performance of study procedures. An urgent safety measure is defined as any measure which an investigator may need to implement which is a deviation from, or a change in, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IEC/IRB approval/favorable opinion.

The investigator may take appropriate urgent safety measures to protect the subjects of a clinical trial against any immediate hazards to their health or safety. However, the investigator must inform the sponsor within 24 hours of having taken such measures.

The sponsor in turn shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the licensing authority and the relevant IEC/IRB of the measures taken and the circumstances giving rise to those measures.

All urgent safety measures must be reported to the Medical Director using the contact numbers listed in Section 10.3.8 within 24 hours of having to take such a measure(s). Such reports can be initiated by telephone but must be officially documented by the investigator (by email or fax) and must include details of what measures were taken and the circumstances giving rise to those measures.

10.3.10 Pregnancy Reporting

This process is aimed at ensuring the appropriate monitoring of the potential risk related to IMP exposure of pregnant women and/or fetuses regarding congenital abnormalities or birth defects in their offspring. It also ensures compliance with applicable international and local regulations.

The requirements are applicable to all subjects following exposure to IMP.

Female trial subjects: The trial subject must be advised by the investigator to inform him/her immediately if she suspects she may be pregnant and believes that conception occurred within 30 days after administration of OPTISON.

When a female trial subject reports a pregnancy (post-IMP administration) to the investigator, a pregnancy test should be arranged for the trial subject by the investigator within 7 days of the pregnancy being reported.

Notification of pregnancy (using the standard pregnancy form provided by the sponsor) must be sent by email to the attention of "Covance DDSS" at SAEIntake@Covance.com immediately (within 24 hours) of obtaining a positive pregnancy test. Covance will forward the pregnancy form to GE Healthcare within 24 hours to GPV.DrugSafety@GE.com. The investigator should include an estimated date of conception when communicating with the sponsor/CRO.

A reported pregnancy will be followed to determine the outcome. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

10.4 Other Variables

Other study relevant information, such as demographic data, medical and surgical history, and prior and concomitant medication will be collected at screening and during the study period as appropriate in the CRFs.

10.5 Appropriateness of Measurements

All assessments and measurements are appropriate and generally regarded as standard medical practice.

11 DATA HANDLING AND QUALITY ASSURANCE

11.1 Completing and Signing Case Report Forms

For electronic CRFs, data will be entered by trained site personnel with reasons given for any missing data. Any errors should be corrected within the electronic system. The audit trail will record all changes made, the date and time of the correction, and the person correcting the error. The appropriate electronic signature will be provided.

Any data recorded directly in the CRF, for which no other written or electronic record will be maintained in the subject's medical record, will be considered source data and should be signed by the investigator(s) (e.g., results of physical examinations, vital signs, or the IMP administration procedure).

11.2 Clinical Data Management

The sponsor or Contract Research Organization (CRO) will be responsible for the processing and quality control of the data. Data management will be carried out by the sponsor or CRO. The handling of data, including data quality control, will comply with all applicable regulatory guidelines.

11.3 Data Quality Assurance

All subject data relating to the study will be recorded on electronic CRFs unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The sponsor or designee is responsible for the data management of this study including quality checking of the data.

The sponsor assumes accountability for actions delegated to other individuals (e.g., CROs).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

11.4 Record Retention

All study documentation at the investigator site and sponsor site will be archived in accordance with ICH E6-GCP and the sponsor's quality standards and SOPs.

All study documentation at the Investigator site and sponsor site will be retained for a minimum of 15 years following completion or discontinuation of the study, unless notified otherwise by the sponsor or a longer period is required by local legislation. The Investigator must request written agreement from the sponsor before destruction of archived study documentation. No records may be transferred to another location or party without written notification to the sponsor.

12 STATISTICAL METHODS AND PLANNED ANALYSIS

12.1 General Statistical Considerations

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed using SAS® software. All summary tables and data listings will be separated by dose levels. The last pre-administration observation will be used as the baseline value for calculating post-administration changes from baseline. All data obtained on the CRF and entered into the database will be provided in separate data listings showing individual subject values. The planning and reporting of statistical analysis will be carried out as described in the sponsor's SOPs governing clinical studies.

The relevant variables will be presented using the appropriate descriptive statistics, categorical variables will be summarized by counts and percentages and continuous variables by means, standard deviations, medians, maxima, minima and sample size. No specific clinical data will be imputed beyond the point of discontinuation for subjects who withdraw prematurely from the study. Demographic variables and other baseline patient characteristics will be summarized descriptively by dose level and overall, independent of IMP assignment. Demographic variables will include age, weight, height, gender, body mass index and race/ethnicity.

12.2 Subject Characteristics

A table will be provided with the following information:

- Number of subjects enrolled.
- Number of subjects included in the intent-to-treat (ITT) analysis.
- Number of subjects included in the per-protocol analysis.
- Number of subjects included in the safety analysis.
- Number of subjects withdrawn from the study and the reason for withdrawal.

Demographic information (age, height, weight, and body mass index) will be summarized using descriptive statistics. Gender and race/ethnicity will be summarized by counts and percentages.

Medical/surgical history data will be summarized by counts and percentages.

12.2.1 Determination of Safety and Efficacy Population

The study analysis populations are defined as follows:

Safety population

All subjects enrolled in the study who receive administration of OPTISON.

ITT population

All subjects enrolled in the study who have available non-contrast harmonic images and OPTISON-enhanced echocardiographic images, irrespective of the quality of CE-ECHO.

Per-protocol population

All subjects whose non-contrast harmonic images are available and whose OPTISON echocardiography images have adequate quality (sufficient contrast enhancement to visualize EBD).

12.3 Efficacy Analysis

Since this is a dose-finding study, no hypotheses are being tested. All efficacy results will be summarized for the ITT and per-protocol populations.

12.3.1 Primary Efficacy Analysis

Visualization of the 12 segments of the left ventricle wall in standard apical 4-chamber and 2-chamber views will be measured by the qualitative EBD visualization scale. Results will be summarized for non-contrast and OPTISON dose levels. Additionally, the mean number of segments visualized will be derived from the EBD score and summarized (segments rated as "fair visualization" or "good/optimal visualization" will be considered visualized).

12.3.2 Secondary Efficacy Analysis

The following secondary endpoints will be summarized:

- The LV peak contrast intensity, determined as none, low, medium, high or blooming, will be summarized as a categorical variable by OPTISON dose level.
- The peak LV contrast filling, determined as none, faint, intermediate or full, will be summarized as a categorical variable by OPTISON dose level.
- The contrast enhancement duration in the LV chamber determined from the time contrast appears in the LV to the time it almost dissipates from the chamber as determined by investigators, will be summarized with descriptive statistics by OPTISON dose level.
- Image quality, categorized as excellent/good, adequate or poor will be summarized as a categorical variable for OPTISON dose levels.
- Diagnostic usefulness, evaluated as non-diagnostic or diagnostic, will be summarized as a categorical variable for non-contrast and OPTISON dose levels.

- The diagnostic confidence of the assessment of LV EBD and wall motion, assessed using a 5-point scale in comparison to non-contrast echocardiographic images (confidence was lower with contrast-enhanced images, same between unenhanced and contrast-enhanced images, slightly improved with contrast-enhanced images, moderately improved, greatly improved), will be summarized as a categorical variable by OPTISON dose level.

- The diagnostic confidence of the evaluation of LVEF, assessed using a 5-point scale in comparison to non-contrast echocardiographic images (confidence was lower with contrast-enhanced images, same between unenhanced and contrast-enhanced images, slightly improved with contrast-enhanced images, moderately improved, greatly improved), will be summarized as a categorical variable by OPTISON dose level.

- LV function assessed by LVEFs, calculated using the formula: (end diastole volume – end systole volume)/end diastole volume for each ventricle, will be summarized with descriptive statistics by OPTISON dose levels and compared with non-contrast images.

A composite assessment of dose suitability will be made at the subject level and results will be tabulated by OPTISON dose level. A dose will be considered suitable of it meets the following criteria:

(1) There is an increase in the number of segments adequately visualized over non-contrast images

(2) LVO peak contrast intensity assessed as medium or better

(3) Peak LV contrast filling to around 67% or better

(4) Duration of contrast enhancement ≥1.5 minutes

12.3.3 Handling of Uninterpretable Images

In the ITT analysis of the primary endpoint, the un-interpretable images will be categorized as none for contrast intensity and 0 visualization of EBD. In the secondary analyses, the un-interpretable images will be considered as 0 enhancement duration, poor for image quality, and non-diagnostic for diagnostic usefulness. Additionally, the number of un-interpretable images will be summarized by non-contrast and dose level. Uninterpretable images will be excluded from the per-protocol population analysis.

12.3.4 Reader Differences

Summaries of the image readings by reader will be presented.

12.4 Safety Analysis

The safety of the intravenous administration of OPTISON in the pediatric population will be measured by the incidence of AEs, vital signs, SaO_2 by pulse oximetry, physical examination and ECG results.

12.4.1 Vital Signs

Vital signs will be measured at the various pre- and post-treatment time point ranges described in Table 2. Vital sign parameters include measurements of systolic and diastolic blood pressure, heart rate, respiratory rate, and pulse oximetry. Normal ranges for the observed values of heart rate, respiratory rate, systolic blood pressure and weight by age group are defined in Table 5.

The presentation of vital signs focuses on the descriptive summaries at baseline, at 10 minutes, and at 1 hour post-OPTISON administration, and the change from baseline to the 10-minute and 1-hour post-OPTISON assessments. All subjects in the safety population who have a baseline and a post-baseline assessment of vital signs will be included in the presentation of the results.

In addition to the change from baseline results, the shifts from baseline to the post baseline visit will be summarized in tabular form for heart rate and systolic blood pressure. Subjects will be classified at baseline and the post-baseline visit in one of the following categories based on a comparison of the vital sign result to the reference range for that vital sign parameter: below normal, normal, or above normal. A shift table will be constructed in which each subject is cross-classified based upon the reference range categories. The number and percentage of subjects in each shift table cell will be displayed.

12.4.2 Electrocardiograms

A 12-lead ECG will be measured at the various pre- and post-treatment time point ranges described in Table 2. The ECGs will be characterized as normal or abnormal, and, if abnormal, whether the abnormality is clinically significant. These overall readings will be summarized. Descriptive statistics will be used to describe the observed values and change from baseline for ECG intervals (heart rate, PR, RR, QRS, QT, QTcB [Bazett], QTcF [Fridericia]). Summaries will be presented by dose level, at each post-baseline time point. QTcB and QTcF will be defined as follows,

$$QTcB = QT/\sqrt{RR}$$

$$QTcF = QT/\sqrt[3]{RR}$$

Normal ranges for the observed values and change from baseline by age group are defined in Table 6 and Table 7.

In addition to the observed values and change from baseline results, the shifts from baseline to the post-baseline visit will be summarized in tabular form for each ECG parameter. Subjects will be classified at baseline and the post-baseline visit in one of the following categories based on a comparison of the ECG result to the reference range for that ECG parameter: below normal, normal, or above normal. A shift table will be constructed in which each subject is cross-classified based upon the reference range categories. The number and percentage of subjects in each shift table cell will be displayed. In addition, observed values and changes in QTc consistent with the [ICH E14] guidance will be specified in the statistical analysis plan, and a categorical analysis of study QTc values based on these pre-specified values will be performed. The results will be summarized in a table.

12.4.3 Prior and Concomitant Medications

Prior and concomitant medications will be recorded and coded using the Anatomical-Therapeutic-Chemical (ATC) classification text from the World Health Organization (WHO) Drug Dictionary. Medications will be presented by ATC Class 1 and preferred term.

12.4.4 Physical Examination

Physical examination findings at baseline and after OPTISON administration will be summarized.

12.4.5 Adverse Events

An AE will be considered as *treatment-emergent* if it starts or worsens after OPTISON administration. Any AE with an onset on the day of IMP administration for which the time of onset is missing will be assumed to be treatment-emergent.

All summaries of AEs will be based on TEAEs and will be prepared by dose level and overall. AEs will be mapped to System Organ Class (SOC) and preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA; current version or the immediately preceding version). The number and percentage of subjects experiencing AEs will be summarized by SOC and preferred term. Summaries by maximum severity, and summaries and tabular listings by relationship to study treatment will also be provided. SAEs and AEs leading to discontinuation of study treatment will be presented by SOC and preferred term.

The sponsor will discuss other significant AEs, defined as laboratory abnormalities that qualify as AEs (other than those meeting the definition for serious) and any events that led to an intervention (including premature discontinuation from the study, dose reduction, or significant additional concomitant therapy), in addition to those reported as SAEs.

12.5 Sample Size Determination

Although this is a dose-finding study and no hypotheses are being tested, it was thought useful to estimate sample size based on one of the study endpoints as a guide. Since there is no historical literature about LV EBD results for OPTISON, the power estimation is based on the endpoint of contrast enhancement duration.

A paired t-test was used for this power analysis with a one-sided significance level of 0.025. The null hypothesis is that the mean value of (contrast enhancement duration for the high dose – contrast enhancement duration for the low dose within the same patient ≤0 minutes), and the alternative hypothesis is that the mean value of (contrast enhancement duration for the high dose – contrast enhancement duration for the low dose within the same patient) >0 minutes. Based on the results from [Cohen et al. 1998], we assume that the estimated mean value of (contrast enhancement duration for the high dose – contrast enhancement duration for the low dose within the same patient) equals 0.7 minutes, with a standard deviation of 1.5 minutes. Table 4 displays the power calculation for the hypothesis testing with different sample sizes.

Table 4 Power Calculation

Total sample size	Power
30	70%
40	82%
50	90%
60	94%
70	97%
80	98%

Therefore, the sample size for this clinical investigation will be approximately 50 subjects, which will provide at least 90% power to detect an efficacy difference of 0.7 minutes between the high and low doses, with the greater duration for the high dose. In addition, a sample size of 50 is considered to be adequate to address the other efficacy endpoints (LV EBD, LVO characteristics, wall motion assessment, and diagnostic confidence for the evaluation of LVEF) and OPTISON's safety profile following intravenous administration in pediatric subjects. The data will support dosing information in children.

12.6 Significance Level

Since no hypotheses are being tested in this study, this section is not applicable.

12.7 Procedures for Missing, Unused and Spurious Data

Missing values will not be substituted by estimated values, but treated as missing in the statistical evaluation. All data from all subjects dosed in the study will be included in all listings, plots, summary tables, and statistical analyses when appropriate.

12.8 Rules for Excluding Subjects from Analysis

All dosed subjects will be included in the analyses unless otherwise specified. The sponsor will make any decisions regarding whether any subjects or any individual values belonging to a subject will be excluded from the evaluations when the protocol violation is considered to have a negative impact on the scientific aspects and interpretation of the study results. If the subject has received any IMP, all available safety data will be used. The reason(s) for any exclusion will be described in the report.

12.9 Procedures for Reporting Deviations from Original Statistical Plan

Any deviations from the statistical analysis outlined in this protocol will be described, and reasons for the deviations listed, in the final Clinical Study Report.

13 SPECIAL REQUIREMENTS AND PROCEDURES

13.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH GCP Guidelines
- Applicable laws and regulations

Before starting this study, the protocol (authorized by the sponsor), Informed Consent Form, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IEC/IRB for evaluation and reviewed and approved by the IEC/IRB. The protocol will also be submitted to regulatory bodies/local health authorities in accordance with local regulations as required. The study will not start before the IEC/IRB gives written approval or a favorable opinion in accordance with ICH E6-GCP and all applicable regulatory bodies/local health authorities give approval or a favorable opinion as required.

No changes from the final approved (authorized) protocol will be initiated without the IEC's/IRB's prior written approval or favorable opinion of a written amendment, except when necessary to eliminate immediate hazards to the subjects or when the change involves only logistics or administration. The sponsor will authorize and the principal investigator(s) will sign the protocol amendment prior to submission to the IEC/IRB. Protocol amendments should be submitted to the IEC/IRB without delay. Any significant deviation from the protocol when no approved amendment exists will be regarded as a protocol violation, and will be addressed as such during the reporting of the study.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IEC/IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IEC/IRB
- Notifying the IEC/IRB of SAEs or other significant safety findings as required by IEC/IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IEC/IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

13.2 Investigator's Responsibilities

13.2.1 Overall Responsibilities

The investigator(s) is/are responsible for conducting the study in full accordance with the Protocol and the Declaration of Helsinki, the *Good Clinical Practice: Consolidated Guideline*, approved by the ICH, and any applicable national and local laws and regulations. Information regarding any investigational centers participating in this study that cannot comply with these standards will be documented.

13.2.2 Parental Permission/Subject Informed Assent

Written and oral information about the study in an understandable language will be given to the subject and subject's parent/legal guardian. Each subject's willingness to participate in the study will be documented in a signed and dated informed assent form and parental informed permission form before any procedures or assessments are performed and after the aims, methods, anticipated benefits, potential hazards, and insurance arrangements in force are explained. Age-appropriate pediatric assent will be obtained where required.

It will also be explained to the subject and the subject's parent/ legal guardian that they are free to refuse to participate in the study and free to withdraw from the study at any time without prejudice to future treatment.

The informed consent/assent process will be documented in the subject's medical record and the investigator will sign, date and time the informed consent form after the subject's parent/legal guardian have signed, dated and recorded the time.

The investigator(s) will keep the original consent forms and copies will be given to the subjects or the subject's parent/legal guardian.

13.2.3 Direct Access to Source Data/Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data reported on the CRF or entered in the electronic CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The monitor(s), auditor(s), authorized personnel of the sponsor, health authority inspector(s) or their agents, and authorized members of IECs/IRBs will be given direct access to source data and documentation (e.g., medical charts/records, laboratory results, printouts, videotapes, etc.) for source data verification, provided that subject confidentiality is maintained in accordance with local requirements.

13.2.4 Confidentiality Regarding Study Subjects

The investigator(s) must assure that the privacy of the subjects, including their personal identity and all other personal medical information, will be maintained at all times. In CRFs and other documents or image material (including materials from all examinations, e.g., ultrasound examinations) submitted to the sponsor, subjects will not be identified by their names, but by an identification code (e.g., initials and study subject number).

Personal medical information may be scrutinized for the purpose of verifying data recorded in the CRF. This may be done by the monitor(s), properly authorized persons on behalf of the sponsor, the quality assurance unit, or regulatory authorities. Personal medical information will always be treated as confidential.

13.2.5 Data Protection

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject and subject's parent/legal guardian must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject and subject's parent/legal guardian who will be required to give consent for their data to be used as described in the informed consent.

The subject and subject's parent/legal guardian must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IEC/IRB members, and by inspectors from regulatory authorities.

13.3 Protocol Deviations

Any deviation from the protocol when no approved amendment exists must be documented as a protocol deviation and reported according to local requirements. If appropriate, corrective and preventative action must be implemented to avoid repetition. Protocol deviations and any potential impact on the study results will be discussed during the reporting of the study.

Waivers or protocol exceptions will not be granted prospectively by the sponsor under any circumstances.

13.4 Study Monitoring

Study monitoring will be performed in accordance with ICH E6-GCP, the sponsor SOPs, the protocol, and applicable local regulations.

13.5 Audit and Inspection

According to ICH E6-GCP, the sponsor or regulatory authorities may audit the investigational site. The sponsor's Quality Assurance Unit, independent of the Clinical Research and Development Department, is responsible for auditing the study.

The investigator(s) must accept that regulatory authorities may conduct an inspection to verify compliance of the study with GCP.

13.6 Financial Disclosure

According to 21 CFR, Part 54, the sponsor is required to completely and accurately disclose or certify information concerning the financial interests of a clinical investigator (or investigating institution) who is not a full-time or part-time employee to the FDA. Therefore, the investigator(s) (or investigating institution) must either provide the sponsor with sufficient, accurate financial certification that none of the following financial arrangements (further defined in 21CFR Part 54.2) exist with the sponsor or fully disclose the nature of the arrangement. This financial disclosure also applies to any financial arrangements that exist between the sponsor and the investigator's spouse(s) or dependent children:

- Compensation for participation in the study is affected by the outcome of the study.
- Significant equity (greater than $50,000) interest in the sponsor's company.
- Proprietary interest in the tested product.
- Significant payments of other sorts, exceeding a monetary value of $25,000.

13.7 Insurance

This study is covered under the sponsor's Liability Insurance Policy (under General Electric Insurance Company and/or a company designated by the study sponsor). A Certificate of Insurance and/or an information leaflet containing essential information about the insurance coverage can be provided upon request.

13.8 Publication Policy

The investigator and/or Institution shall have the right to publish the results of their work conducted under this protocol, subject to providing the sponsor with the opportunity to review the contents of any proposed abstract or publication concerning the work, including any results of the study, in advance of publication and if necessary to delay publication for a limited time not to exceed 60 days in order to protect the confidentiality or proprietary nature of any information contained therein. The sponsor will make every reasonable effort to consider and release each proposed publication within 30 days, or proposed abstract within 15 days, of submission.

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15 APPENDICES

15.1 Information on Investigational and Registered Products

The currently approved OPTISON package insert or Summary of Product Characteristics will be provided to all investigators as a separate document. The package insert/Summary of Product Characteristics provides information on the efficacy and safety of the OPTISON, and is used for assessing expectedness of Serious Adverse Drug Reactions, in order to determine regulatory reportability. An unexpected Adverse Drug Reaction is a reaction, for which the nature, seriousness, severity or outcome is not consistent with the applicable product information presented in the package insert/Summary of Product Characteristics.

15.2 Equipment Parameters

Please refer to the Imaging Manual.

15.3 Pediatric Normal Ranges

Table 5 Pediatric Vital Signs Normal Ranges

Age Group	Respiratory Rate	Heart Rate	Systolic Blood Pressure	Weight in kilos	Weight in pounds
Newborn	30 – 50	120 – 160	50 – 70	2 – 3	4.5 – 7
Infant (1-12 months)	20 – 30	80 – 140	70 – 100	4 – 10	9 – 22
Infant (1-12 months)	20 – 30	80 – 140	70 – 100	4 – 10	9 – 22
Preschooler (3-5 yrs.)	20 – 30	80 – 120	80 – 110	14 – 18	31 – 40
School Age (6-12 yrs.)	20 – 30	70 – 110	80 – 120	20 – 42	41 – 92

REF: Source: http://www.rnceus.com/psvt/psvtvs.html

Table 6 Pediatric ECG Normal Ranges

ECG Variable	Mean (sd)	1st %ile	5th %ile	Median	95th %ile	99th %ile
Heart Rate (bpm)	83.0 (12.1)	59.0	65.0	82.0	104	114.0
RR Interval (ms)	738.5 (107.8)	526.3	576.9	731.7	923.1	1040.0
PR Interval (ms)	132.8 (17.3)	100.0	108.0	130.0	162.0	180.0
QRS Interval (ms)	80.0 (9.0)	64.0	70.0	80.0	96.0	102.0
QT Interval (ms)	354.7 (24.3)	300.0	318.0	352.0	396.0	416.0
QTc Bazett (ms)	414.0 (19.6)	367.0	383.0	414.0	443.0	460.0
QTc Data-derived (ms)	399 (17.3)	357.4	372.0	398.7	424.1	439.6
QTc Fridericia (ms)	393.2 (17.1)	353.3	366.3	392.9	419.0	433.0

Note: Pre-pubertal group (N = 1537) defined as females whose age was ≤ 8 years (n = 224) and males whose age was ≤ 9 years (n = 1313)-Abbreviations: RR, Duration of ventricular cardiac cycle (an indication of ventricular rate); PR, Time from the onset of atrial depolarization (P wave) to onset of ventricular depolarization (QRS complex); QTcb, Bazett's method for QT interval correction; QTcd, Data-derived corrected method for QT interval correction; QTcf, Fridericia's method for QT interval correction.

Table 7 Mean and Percentiles of ECG Cardiac Measures Among Pubertal Children

ECG Variable	Mean (sd)	1st %ile	5th %ile	Median	95th %ile	99th %ile
Heart Rate (bpm)	77.4 (11.8)	54.0	60.0	76.0	98.0	109.0
RR Interval	792.9 (121.1)	555.6	612.2	780.0	1000.0	1132.0
PR Interval (ms)	136.8 (18.5)	100.0	112.0	136.0	170.0	109.0
QRS Interval (ms)	83.0 (9.1)	68.0	70.0	80.0	96.0	106.0
QT Interval (ms)	368.8 (27.0.)	310.0	330.0	368.0	412.0	440.0
QTc Bazett (ms)	415.6 (21.3)	362.0	381.0	416.0	448.0	468.0
QTc Data-derived (ms)	403.9 (19.1)	357.0	373.3	404.0	433.1	450.1
QTc Fridericia (ms)	399.4 (19.0)	354.5	369.4	399.6	429.0	443.9

Note: Pubertal group (N = 3656) defined as females whose age was > 8 and ≤ 13 years (n = 881) and males whose age was > 9 and ≤ 14 years (n = 2775).
Abbreviations: RR, Duration of ventricular cardiac cycle (an indication of ventricular rate); PR, Time from the onset of atrial depolarization (P wave) to onset of ventricular depolarization (QRS complex); QTcb, Bazett's method for QT interval correction; QTcd, Data-derived corrected method for QT interval correction; QTf, Fridericia's method for QT interval correction.

Source: Child and Adolescent Psychiatry and Mental Health 2007;1:11

16 CLINICAL PROTOCOL AMENDMENT SUMMARIES

16.1 Amendment A01

16.1.1 Reasons for Amendment

- The requirement for a minimum of 10 subjects to be enrolled in each body weight group is removed.
- The requirement to record the vial code in the CRF is removed.

16.1.2 Description of Changes

Where appropriate, the changes documented below are also made in the synopsis.

Section 6.1, Overall Study Design and Plan, Third Paragraph

Previously read:

Each patient will undergo an echocardiographic procedure first without contrast administration then following intravenous bolus injection of OPTISON at various doses. The dose of OPTISON for each subject will be determined according to pediatric body weight group and 2 different dose levels will be tested in each subject. A minimum of 10 subjects will be enrolled in each body weight group. The details on OPTISON administration are presented in Section 8.1 of this protocol.

Now reads:

Each patient will undergo an echocardiographic procedure first without contrast administration then following intravenous bolus injection of OPTISON at various doses. The dose of OPTISON for each subject will be determined according to pediatric body weight group and 2 different dose levels will be tested in each subject. The details on OPTISON administration are presented in Section 8.1 of this protocol.

Section 8.6, Treatment Compliance

Previously read:

Subjects will receive OPTISON administration under direct supervision of study personnel. Each administration volume will be checked and the vial code and volume per administration will be recorded in each subject's CRF.

Now reads:

Subjects will receive OPTISON administration under direct supervision of study personnel. Each administration volume will be checked and the volume per administration will be recorded in each subject's CRF.

SIGNATURE PAGE

Date / Name	Justification / Role
Signed By: [redacted] Date of signature: 04-Oct-2022 08:33:15 GMT+0000	Justification: Approved Role: [redacted]
Signed By: [redacted] Date of signature: 04-Oct-2022 08:53:37 GMT+0000	Justification: Approved Role: [redacted]
Signed By: [redacted] Date of signature: 04-Oct-2022 12:59:26 GMT+0000	Justification: Approved Role: [redacted]

This is a representation of an electronic record that was signed electronically in accordance with GxP guidelines and controls. The information above is a valid manifestation of the electronic signature(s) and is legally binding