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STATISTICAL ANALYSIS PLAN **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

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Abbreviations

AE	Adverse event
ATC	Anatomical-Therapeutic-Chemical
BIE	Blinded image evaluation
CE-ECHO	Contrast-enhanced echocardiography
CHMP	Committee for Medicinal Products for Human Use
EBD	Endocardial border delineation
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ICH	International Council for Harmonisation
IMP	Investigational medicinal product
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
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOC	System organ class
TEAE	Treatment-emergent adverse event
WHO-DD	World Health Organization Drug-Dictionary

1 INTRODUCTION

The statistical analysis plan (SAP) defines the methods and analyses that GE HealthCare plans to use to analyze the data from Protocol GE-191-008. This SAP is based on the final study protocol (Amendment A01 Version 2.0) dated 04 October, 2022. This SAP complies with guidance promulgated by the International Conference on Harmonization (ICH) and the US Food and Drug Administration (FDA). If the protocol is subsequently amended, this SAP may be amended as well. Should the SAP and the protocol be inconsistent with respect to the planned analyses, the language of the SAP prevails.

2 STUDY OBJECTIVES, DESIGN AND PROCEDURES

2.1 Objectives

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

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.

2.2 Study Design

This is 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 echo cardiogram will be enrolled into the study. Per FDA Advice/information request dated 2023, February 16th, a reduction of the sample size to 30 evaluable subjects was agreed.

OPTISON will be administered according to pediatric body weight (≥20 to ≤28 kg, >28 to ≤40 kg, and >40 kg). Each patient will receive administrations of 2 different dose levels. Up to 3 injections per dose level are allowed to be administrated if significant technical issue(s) have occurred e.g., patient related needs, scanner malfunction, or drug injection error, with the total injected dose not to exceed the value defined in Table 1. Injections should be at least 10 minutes apart between the 2 dose levels to allow for clearance of the previous dose.

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

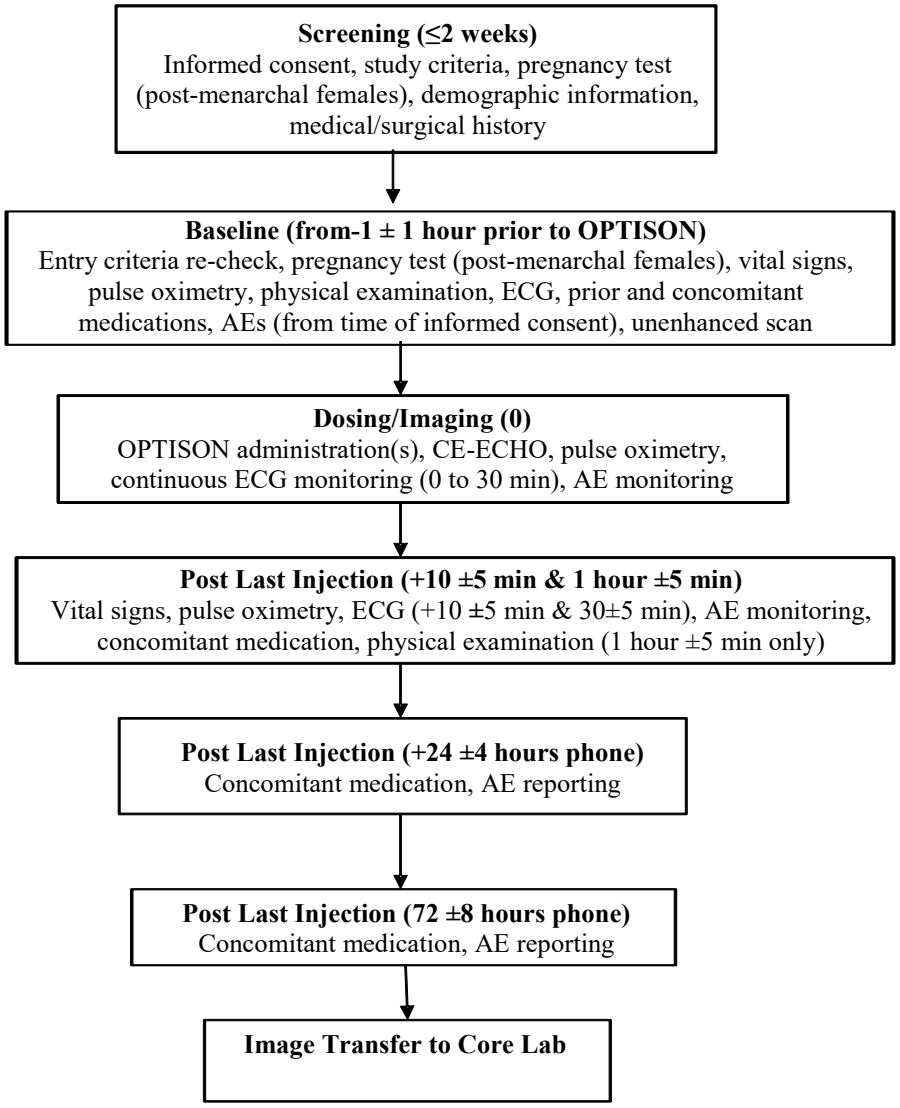
For echocardiographic imaging, standard apical 4-chamber and 2-chamber views will be obtained at baseline using tissue harmonic imaging, thereafter with contrast using a contrast-imaging mode. Additional views may be obtained at the clinician's discretion. All image views will be obtained prior to and 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. Echocardiographic Analyses as per independent review charter includes:

1. Evaluation of Left ventricular (LV) Endocardial Border Delineation (EBD)
2. Evaluation of diagnostic confidence for the assessment of LV EBD and wall motion
3. Evaluation of Left Ventricular Opacification (LVO)
4. Evaluation of Image Quality and Diagnostic Usefulness
5. Evaluation of Left Ventricular Function
6. Evaluation of diagnostic confidence for the assessment of LVEF
7. Evaluation of Duration of Contrast Enhancement.

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 pediatric population.

Figure 1 Study Diagram



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

2.3 Sample Size

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 3 displays the power calculation for the hypothesis testing with different sample sizes.

Table 3 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 was approximately 50 subjects, which would 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 was 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. Per FDA Advice/information request dated 2023, February 16th, a reduction of the sample size to 30 evaluable subjects was agreed.

2.4 Interim Analysis

There is no interim analysis planned for this study.

2.5 Randomization and Blinding

This is an open-label study. No blinding will be conducted for the subject allocation or application of the investigational medicinal product (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.

3 STUDY ENDPOINTS

3.1 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.

3.2 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 (SaO2), 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.

4 ANALYSIS POPULATIONS

4.1 Definition for Analysis Populations

Enrolled subjects are subjects who signed the informed consent.

4.1.1 Safety Population

The Safety Population will include all subjects enrolled in the study who receive ≥1 administration of OPTISON.

4.1.2 Full Analysis Set (FAS) Population

The FAS population will include 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.

4.1.3 Per-Protocol (PP) Population

The PP population will include all subjects in the FAS population who do not have important protocol deviation impacting the study primary endpoint.

5 ANALYSIS CONVENTIONS

Post-text tables and listings will be prepared in accordance with the current International Council for Harmonisation (ICH) Guidelines. The information and explanatory notes to be provided in the "footer" or bottom of each table and listing will include the following information:

1. Date and time of output generation
2. SAS® program name, including the path that generates the output
3. Any other output specific details that require further elaboration

In general, tables will be formatted with a column displaying findings recorded on the electronic case report form (eCRF) for all subjects combined. Row entries in tables are

made only if data exists for at least one subject (*i.e.*, a row with all zeros will not appear). The only exception to this rule applies to tables that list the termination status of subjects (*e.g.*, reasons for not completing the study). In this case, zeros will appear for study termination reasons that no subject satisfied. The summary tables clearly indicate the number of subjects to which the data apply and unknown or not performed are distinguished from missing data.

Supportive individual Subject Data Listings, as a minimum, will be sorted and presented by weight group, subject number, visit date, and dose level if applicable. Listings will also include days relative to the first dose of study drug, if applicable.

Specific algorithms are discussed for imputing missing or partially missing dates, if deemed appropriate, under specific data topics. Imputed or derived data are flagged in the individual subject data listings. Imputed data are not incorporated into any raw or primary datasets. These data are retained in derived (or analysis) datasets.

This section details general conventions to be used for the statistical analyses. The following conventions will be applied to all data presentations and analyses.

- Summary statistics for categorical variables will consist of the number and percentage of responses in each level. The number and percentage of responses will be presented in the form XX (XX.X%).

- Summary statistics for continuous variables will consist of the sample size (n), mean, standard deviation (SD), median (1st and 3rd quartile), minimum, and maximum values.

- All mean and median values, as well as Q1 and Q3, will be formatted to one more decimal place than the measured value. Standard deviation values will be formatted to two more decimal places than the measured value. Minimum and maximum values will be presented with the same number of decimal places as the measured value.

- All p-values, if applicable, will be rounded to 4 decimal places. All p-values that round to 0.0000 will be presented as '<0.0001' and p-values that round to 1.0000 will be presented as '>0.9999'. Two-sided P-values < 0.05 will be considered to be statistically significant unless otherwise specified.

- All summary tables will include the analysis population sample size (i.e., number of subjects).

- Study Day 1 is defined as the date at which the subject receives the first dose of study medication. All study days are determined relative to Day 1.
- Study days prior to Day 1 will be calculated as:
    - Study Day = Assessment Date – First Injection Date
- Study days after Day 1 will be calculated as:
    - Study Day = Assessment Date – First Injection Date + 1.
- Baseline values will be defined as the last non-missing value recorded prior to first dose of study drug.
- Change from baseline will be calculated as follows: Change = Post-baseline value - baseline value.
- All pre- and post- investigational product assessments including unscheduled or repeat assessments will be included in the data listings. For unscheduled or repeat pre-dose assessments, the last assessment taken for a time point will be used in the data summaries (summary tables, figures, and statistical analysis); for all post-dose time points, the initial assessment for any given time point will be used in the safety data summaries (summary tables, figures, and statistical analysis, except the summary tables of clinically notable values and shift tables where all values will be included). For efficacy summaries, the site records in the eCRF which assessment was considered for the image interpretation. The corresponding assessment will be used in the efficacy summaries.
- Date variables will be formatted as DDMMMYYYY for presentation.
- Tables, figures, and listings will be presented in landscape orientation.
- SAS® Version 9.4 or higher will be the statistical software package used for all data analyses.

Definition of Analysis Windows

There are no visit windows for this study. For the statistical analyses, data will be analyzed by the nominal visit that was collected on the electronic case report form (eCRF).

Unscheduled visits will not be used in the by-visit analysis, but will be used for the following where appropriate: 1) derivations of baseline/last on-treatment measurements; 2) derivations of the maximum/minimum on-treatment values and maximum/minimum changes from baseline values for safety analyses; 3) data listings.

Definition of Missing Data Imputation

Missing values will not be substituted by estimated values but treated as missing in the statistical evaluation. Data for all enrolled participants will be presented in the data listings. A participant who is enrolled but who did not receive any amount of study drug will be included in those data listings for which they have data but will be excluded from all data summaries.

Missing or partial dates for AE or for medications may be imputed for determination of treatment emergent AE (TEAE) or concomitant medication, as follows.

- If the AE start date is completely missing, then the AE will be considered treatment emergent unless it can be determined that the AE end date/time occurred prior to IMP injection. If this is the case, the AE will not be considered treatment emergent.

- If the AE start date/time is partially missing and the partial date is not sufficient to determine if the event occurred after the IMP injection, then the AE will be considered treatment emergent unless it can be determined that the AE end date/time occurred prior to the injection.

6 STATISTICAL ANALYSES

6.1 Demographics, Baseline Characteristics, Medical History, and Prior/Concomitant Medications

6.1.1 Demographic and Baseline Characteristics

Age (years between birth and time informed consent form was signed), sex, race, ethnicity, and baseline height, weight, weight group (≥20 to ≤28, >28 to ≤40, >40 kg; as well as ≤40, >40 kg), and body mass index (BMI) will be summarized overall, independent of IMP assignment, based on Safety, FAS, and PP populations.

Categorical data (sex, weight group, race, and ethnicity) will be summarized using frequencies and percentages. The number of subjects with missing information will also be summarized.

Continuous data (age, baseline height, weight and BMI) will be summarized with the number of non-missing observations by mean, standard deviation, median, Q1, Q3, minimum and maximum values.

6.1.2 Medical History

Medical/surgical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0. It will be summarized and presented using system organ class (SOC) and preferred term (PT). The SOC will be presented in alphabetical order and the PT will be presented in alphabetical order within each SOC. The number and percentage of subjects with a particular condition/diagnosis will be summarized overall and by weight group (≤40, >40 kg) based on the Safety population. Subjects reporting more than one condition/diagnosis within a body system will be counted only once for that body system.

6.1.3 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 Drug Dictionary (WHO-DD) version, March 1, 2021. They will be summarized overall and by weight group (≤40, >40 kg) based on the Safety population. A prior medication is defined as any medication taken prior to the first dose of study drug. A concomitant medication is defined as any medication that started prior to the first dose of study drug and continued to be taken after the first dose of study drug or any medication that started after the first dose of study drug, but not after the last dose of study drug. Medications with unknown or incomplete end date will be considered concomitant unless there is clear evidence that the medication was stopped prior to the first dose of study drug. The number and percentage of subjects who have taken medications will be summarized by ATC Class 3 and generic drug name, separately for prior and concomitant medications.

6.2 Subject Disposition

The number of subjects for each of the following categories will be summarized, overall and by weight group (≤40, >40 kg):

- Enrolled subjects
- Subjects who received at least one dose of study drug (i.e., the safety population).
- Subjects included in the FAS population
- Subject included in the PP population
- Subjects who completed the study
- Subjects who withdrawn from the study and the reason for withdrawal

All protocol deviations will be listed. Deviations will be classified as Important or Non-important. Important deviations excluding from the PP population will be identified.

6.3 Study Drug Exposure and Compliance

The total treatment exposure (OPTISON injection in mL as well as number of administrations) received at two dose levels will be summarized by descriptive statistics overall and by weight group (≤40, >40 kg) based on Safety population. A summary of each subject's dosing information will be presented in a listing. The injection used for image interpretation will be identified in this listing. Reasons why OPTISON dose was repeated will also be listed.

6.4 Efficacy Analysis

6.4.1 General Considerations

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

Assessments to be used for image interpretation are identified by the site and recorded in the eCRF. These identified assessments will be used for efficacy analyses.

Efficacy summaries will be presented for each reader.

6.4.2 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:

0 = no visualization of the LV endocardial border

1 = poor visualization

2 = fair visualization

3 = good/optimal visualization

Results for each segment will be summarized for non-contrast and OPTISON dose levels overall and by weight group (≤40, >40 kg). A category "Visualized", corresponding to scores 2 or 3, will also be presented. Additionally, the number of segments visualized (segments rated as "fair visualization" or "good/optimal visualization") will be derived from the EBD score and summarized for non-contrast and OPTISON dose levels, overall and by weight group. 95% CI will be presented. A Paired t-test will be used to compare the number of segments visualized between non-contrast and each OPTISON dose level.

The number and percentage of subjects with an increase in the number of segments adequately visualized over non-contrast images will also be tabulated overall and by weight group for each OPTISON dose levels.

The Total LV EBD score, calculated as the sum of the individual scores assigned to each of the segment (range 0 to 36), will be tabulated overall and by weight group for non-contrast and each OPTISON dose level. The change from non-contrast to each OPTISON dose level will also be presented. 95% CI will be presented. A Paired t-test will be used to compare Total LV EBD scores between non-contrast and each OPTISON dose level.

A suboptimal echocardiogram is defined as ≥ 2 contiguous segments in any given view that cannot be visualized. The number and percentage of subjects with suboptimal echocardiogram will be presented for non-contrast and each OPTISON dose level, overall and by weight group.

6.4.3 Secondary Efficacy Analyses

The following secondary endpoints will be summarized, overall and by weight group (≤40, >40 kg):

- The LV peak contrast intensity, determined as None, Low, Medium, High or Blooming, will be summarized as a categorical variable for each OPTISON dose level.

- The peak LV contrast filling, categorized as None (0% filling), Faint (around 33% filling), Intermediate (around 67% filling) or Full (100% filling), will be summarized as a categorical variable for each 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 for each OPTISON dose level.

- Image quality, categorized as Excellent/good, Adequate or Poor will be summarized as a categorical variable for each OPTISON dose levels.

- Diagnostic usefulness, evaluated as Non-diagnostic (the image quality is not sufficient to confidently visualize the endocardial border) or Diagnostic (sufficient or excellent image quality for EBD assessments), 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 4-point scale (0 = no confidence, 1 = low confidence, 2 = moderate confidence, 3 = high confidence) will be described for non-contrast and OPTISON dose levels.

  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 in comparison to non-contrast echocardiographic images will be summarized as a categorical variable by OPTISON dose level.

- The diagnostic confidence of the evaluation of LVEF, assessed using a 4-point scale (0 = no confidence, 1 = low confidence, 2 = moderate confidence, 3 = high confidence) will be described for non-contrast and OPTISON dose levels.

The change in diagnostic confidence will be derived using the same method as for change diagnostic confidence of the assessment of LV EBD and wall motion and summarized as a categorical variable by OPTISON dose level.

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

- 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.

6.4.4 Handling of Uninterpretable Images

In the FAS analysis of the primary endpoint, the uninterpretable images will be categorized as none for contrast intensity and 0 visualization of EBD. In the secondary analyses, the uninterpretable images will be considered as 0 enhancement duration, poor for image quality, and non-diagnostic for diagnostic usefulness. Additionally, the number of uninterpretable images will be summarized by non-contrast and dose level.

6.4.5 Reader Differences

All efficacy summaries described in Section 6.4 will be presented by reader.

Data from re-read will be listed.

6.5 Safety Analysis

6.5.1 General Considerations

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. Safety data will be summarized overall and for each weight group (≤40, >40 kg).

6.5.2 Analysis of Adverse Events

6.5.2.1 Treatment-Emergent 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 weight group (≤40, >40 kg) and overall for the Safety Population. AEs will be mapped to System Organ Class (SOC) and preferred terms using the MedDRA version 24.0. All AEs will be listed.

An overall summary of TEAEs will present the number (%) of subjects and number of events for the following events:

- TEAE
- Severe TEAE
- Serious TEAE
- TEAE considered related to IMP
- Serious TEAE considered related to IMP
- TEAE leading to discontinuation of study drug
- TEAE leading to death

The number and percentage of subjects experiencing TEAEs, and the number of events will be summarized by SOC and preferred term in alphabetical order. Summaries of TEAEs by maximum severity and TEAEs considered related to the study drug will also be presented by SOC and preferred terms.

6.5.2.2 SAEs (Including Deaths) and Adverse Events Leading to Study Drug Discontinuation

The number and percentage of subjects, with the corresponding number of events, experiencing treatment-emergent SAEs (including deaths) and adverse events leading to discontinuation of study drug will be tabulated by SOC and preferred term, overall and by weight group. Deaths, SAEs and TEAEs leading to discontinuation of study treatment will be listed.

6.5.3 Analysis of Vital Signs and Weight

6.5.3.1 Variables and Criteria Defining Abnormality

Vital sign variables are systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and pulse oximetry. These will be measured at pre- and post-treatment time points as specified in Table 2.

Table 4 presents the criteria for vital sign normal limits in a pediatric population by age group for respiratory rate, heart rate, systolic blood pressure, and weight. Besides, pulse oximetry < 95% will be considered as abnormal in this study.

Table 4 Pediatric Vital Signs Normal Ranges

Age Group	Respiratory Rate	Heart Rate	Systolic Blood Pressure	Weight in kilos	Weight in pounds
School Age (6-12 yrs.)	20 – 30	70 – 110	80 – 120	20 – 42	41 – 92
Adolescent (13+ yrs.)	12 – 20	55 – 105	110 – 120	>50	>110

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

6.5.3.2 Statistical Methods

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. Results will be presented by weight group (≤40, >40 kg) and overall.

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. The number of subjects in the safety population missing either baseline or post-baseline vital sign information will also be presented. Results will be presented by weight group and overall. All vital signs results will be listed. Classification (below normal, normal, or above normal) will be presented in the listing for respiratory rate, heart rate, systolic blood pressure, and pulse oximetry.

6.5.4 Analysis of ECG Parameters

6.5.4.1 Variables and Criteria Defining Abnormality

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 an interpretation based on ECG intervals (heart rate, PR, RR, QRS, QT, QTcF [Fridericia]). QTcF will be defined as follows:

- Fridericia's correction (QTcF): $QT_CF = QT / \sqrt[3]{RR}$
- Bazett's correction (QTcB): $QT_CB = QT / \sqrt{RR}$

Normal ranges for the observed values and change from baseline by age group are defined in Table 5, Table 6 and Table 7.

Table 5 Mean and Percentiles of ECG Cardiac Measures Among Pre-pubertal Children

ECG Variable	Mean (sd)	1st %ile	5th %ile	Median	95th %ile	99th %ile
Heart Rate (bpm)	83.0 (12.1)	59	65	82	104	114
RR Interval (ms)	738.5 (107.8)	526.3	576.9	731.7	923.1	1040
PR Interval (ms)	132.8 (17.3)	100	108	130	162	180
QRS Interval (ms)	80.0 (9.0)	64	70	80	96	102
QT Interval (ms)	354.7 (24.3)	300	318	352	396	416
QTc Bazett (ms)	414.0 (19.6)	367	383	414	443	460
QTc Data-derived (ms)	399 (17.3)	357.4	372	398.7	424.1	439.6
QTc Fridericia (ms)	393.2 (17.1)	353.3	366.3	392.9	419	433

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

Table 6 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	60	76	98	109
RR Interval (ms)	792.9 (121.1)	555.6	612.2	780	1000	1132
PR Interval (ms)	136.8 (18.5)	100	112	136	170	190
QRS Interval (ms)	83.0 (9.1)	68	70	80	96	106
QT Interval (ms)	368.8 (27.0.)	310	330	368	412	440
QTc Bazett (ms)	415.6 (21.3)	362	381	416	448	468
QTc Data-derived (ms)	403.9 (19.1)	357	373.3	404	433.1	450.1
QTc Fridericia (ms)	399.4 (19.0)	354.5	369.4	399.6	429	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). Source: Child and Adolescent Psychiatry and Mental Health 2007;1:11

Table 7 Mean and Percentiles of ECG Cardiac Measures Among Post-pubertal Children

ECG Variable	Mean (sd)	1st %ile	5th %ile	Median	95th %ile	99th %ile
Heart Rate (bpm)	70.8 (11.4)	48	52	70	91	102
RR Interval (ms)	870.3 (144.3)	588.2	659.3	857.1	1153.8	1250
PR Interval (ms)	140.8 (18.9)	104	116	140	176	192
QRS Interval (ms)	87.8 (9.9)	70	72	88	104	112
QT Interval (ms)	382.9 (29.0)	328	340	380	432	470
QTc Bazett (ms)	412.2 (20.7)	350	376	413	444	457
QTc Data-derived (ms)	405.0 (18.5)	357.2	371.6	406.5	433	447.5
QTc Fridericia (ms)	402.1 (18.5)	354.5	371.1	402.6	430.9	446.5

Note: Post-Pubertal group (N = 737) defined as females whose age was >13 yrs (n = 240) and males whose age was > 14 yrs (n = 497). Source: Child and Adolescent Psychiatry and Mental Health 2007;1:11

6.5.4.2 Statistical Models

All subjects in the safety population who have a baseline and a post-baseline assessment of ECG data will be included in the presentation of the results. Overall ECG investigator interpretation will be categorized as normal, abnormal (Not Clinically Significant), and abnormal (Clinically Significant). Number and percentage of subjects in each category at each timepoint will be presented.

Consistent with ICH E14 guidance, ECG data will be presented both as analyses of central tendency (e.g., means, medians) and categorical analyses. ECG data will be summarized overall and by weight group (≤40, >40 kg).

Twelve-lead ECG data (observed values and changes from baseline) will be presented using descriptive statistics by weight group (≤40, >40 kg) and overall for each time point (baseline, 10 minutes, and 30 minutes post-dose).

In addition to the observed values and change from baseline results, the shifts from baseline to post-baseline time points will be summarized in tabular form for each ECG parameter and the overall interpretation. The overall interpretation will be based on the categories of the interpretation listed previously, while the ECG parameters will be listed based on being below normal (<1st percentile), normal, or above normal (>99th percentile) compared to the reference ranges in Table 5, Table 6 and Table 7. The number and percentage of subjects in each shift table cell will be displayed. The number of subjects who are missing either baseline or post-baseline ECG data will also be provided for each timepoint.

All ECG results will be listed including overall interpretation and status compared to normal ranges (below normal / normal / above normal).

In addition, changes from baseline in the QTc interval will be displayed according to the Committee for Medicinal Products for Human Use (CHMP) criteria (absolute QTc interval prolongation, of >450, >480, >500 ms and change from baseline in QTc interval >30 and >60 ms).

6.5.5 Analysis of Physical Examination

Physical examination findings at baseline and after OPTISON administration will be summarized for the safety population in the form of a shift table. Findings for each body system will be categorized as normal, abnormal (non-clinically significant), and abnormal (clinically significant). The number and percentage of subjects in each shift table cell will be displayed. The number of subjects who are missing either baseline or post-baseline Physical examination data will also be provided for each timepoint. Data will be presented by weight group (≤40, >40 kg) and overall.

7 REFERENCES

[ICH E14]
International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. The clinical evaluation of QT/QTC interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs–E14. Step 4 version; May, 2005

[Cohen et al. 1998]
Cohen JL, Cheirif J, Segar DS, et al. Improved left ventricular endocardial border delineation and opacification with OPTISON (FS069), a new echocardiographic contrast agent.
JACC 1998;32:746-752

8 LIST OF TABLES, FIGURES AND DATA LISTINGS THAT ARE TO BE PROGRAMMED

Whenever applicable, tables listed below will present results overall and by weight group and will be repeated by reader.

TABLE NUMBER	TABLE TITLE	POPULATION FOR ANALYSIS
14.1.1	Summary of Subject Disposition	All Subjects
14.1.2.x	Summary of Subject Demographics	Safety, FAS, PP
14.1.3	Summary of Medical History	Safety
14.1.4.1	Summary of Prior Medications	Safety
14.1.4.2	Summary of Concomitant Medications	Safety
14.1.5	Summary of Exposure to OPTISON	Safety
14.2.1.1a	Summary of Visualization of LV Wall by Segments	ITT
14.2.1.1b	Summary of Visualization of LV Wall by Segments	PP
14.2.1.2a	Summary of Visualization of LV Wall Overall	ITT
14.2.1.2b	Summary of Visualization of LV Wall Overall	PP
14.2.2a	Summary of Peak Contrast Intensity in LV	ITT
14.2.2b	Summary of Peak Contrast Intensity in LV	PP
14.2.3a	Summary of Peak Contrast Filling in LV	ITT
14.2.3b	Summary of Peak Contrast Filling in LV	PP
14.2.4a	Summary of Contrast Enhancement Duration in LV	ITT
14.2.4b	Summary of Contrast Enhancement Duration in LV	PP
14.2.5a	Summary of Image Quality	ITT
14.2.5b	Summary of Image Quality	PP

TABLE NUMBER	TABLE TITLE	POPULATION FOR ANALYSIS
14.2.6a	Summary of Diagnostic Usefulness	ITT
14.2.6b	Summary of Diagnostic Usefulness	PP
14.2.7a	Summary of Diagnostic Confidence of LV EBD and Wall Motion Between Contrast and Non-Contrast Enhanced Images	ITT
14.2.7b	Summary of Diagnostic Confidence of LV EBD and Wall Motion Between Contrast and Non-Contrast Enhanced Images	PP
14.2.8a	Summary of Diagnostic Confidence of LVEF Between Contrast and Non-Contrast Enhanced Images	ITT
14.2.8b	Summary of Diagnostic Confidence of LVEF Between Contrast and Non-Contrast Enhanced Images	PP
14.2.9a	Summary of LV Function Assessed by LVEF	ITT
14.2.9b	Summary of LV Function Assessed by LVEF	PP
14.2.10a	Summary of Dose Suitability	ITT
14.2.10b	Summary of Dose Suitability	PP
14.3.1	Overall summary of Treatment Emergent Adverse Events	Safety
14.3.1.1	Summary of Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.2	Summary of Treatment Emergent Adverse Events by maximum severity by System Organ Class and Preferred Term	Safety
14.3.1.3	Summary of Treatment Emergent Adverse Events considered related to IMP by System Organ Class, Preferred Term	Safety
14.3.2.1	Summary of Serious Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.2.4	Summary of Treatment Emergent Adverse Events Leading to Study drug Discontinuation by System Organ Class and Preferred Term	Safety
14.3.3.1	Summary and Change from Baseline – Vital Signs	Safety
14.3.3.2	Shifts from Baseline to Post-Administration Timepoints – Vital Signs	Safety
14.3.4.1	Summary and Change from Baseline – Electrocardiograms	Safety
14.3.4.2	Shifts from Baseline to Post-Administration Timepoints – Electrocardiograms	Safety
14.3.4.3	Summary of Overall Interpretation by Timepoint - Electrocardiograms	Safety
14.3.4.4	Shift from Baseline to Post-Administration Timepoints of Overall Interpretation - Electrocardiograms	Safety
14.3.4.5	Summary of QTc Interval Outlier Categories	Safety
14.3.5	Shift from Baseline to Post-Administration Timepoint – Physical Examination	Safety

LISTING NUMBER	LISTING TITLE	POPULATION FOR ANALYSIS
16.2.1	Subject Disposition	All Subjects
16.2.2	Protocol Deviations	All Subjects
16.2.3	Inclusion/Exclusion Criterion	All Subjects
16.2.4.1	Demographic Information	Safety
16.2.4.2	Medical History	Safety
16.2.4.3	Prior and Concomitant Medications	Safety
16.2.5	Exposure to OPTISON	Safety
16.2.6.1	Visualization of Segments	ITT
16.2.6.2	BIE Interpretations	ITT
16.2.6.3	Non-Contrast and Contrast Enhanced Comparisons	ITT
16.2.6.4	Dose Suitability	ITT
16.2.7.1	Adverse Events	Safety
16.2.7.2	Deaths	Safety
16.2.7.3	Serious Adverse Events	Safety
16.2.7.4	TEAEs leading to discontinuation of study treatment	Safety
16.2.8.1	Vital Signs	Safety
16.2.8.2	Electrocardiogram – Reader Results	Safety
16.2.8.3	Electrocardiogram – Overall Interpretation	Safety
16.2.8.4	Physical Examination	Safety

9 SUMMARY OF CHANGES

Changes from the statistical analyses planned in the protocol are summarized in the following table:

Protocol Amendment A01 Version 2.0	Changes
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. PP Population The PP population will include all subjects in the FAS population whose OPTISON-enhanced echocardiography images have adequate quality (sufficient contrast enhancement to visualize EBD).	For consistency with ICH E9 guidance, the efficacy population has been renamed from ITT population to Full Analysis Set. The PP Population definition has been updated to all subjects in the FAS population who do not have important protocol deviation impacting the study primary endpoint. A lack of contrast enhancement is not considered as a protocol deviation. Nevertheless, any important deviation from the Imaging Manual (e.g. wrong settings for echocardiographic imaging, standard apical 4-chamber and 2-chamber views not acquired...) will be reviewed during the Data Review Meeting and exclusion from the PP Population will be assessed.
All summary tables and data listings will be separated by dose levels.	Whenever relevant, summaries and listings will be presented by Weight group (≤40, >40 kg) and dose level.
Medications will be presented by ATC Class 1 and preferred term	Prior and concomitant medications will be tabulated by ATC Class 3 and preferred term
Primary endpoint analysis	Supportive analyses added in Section 6.4.2

Reference ranges for Vital signs	Adolescent (13+ yrs.) category added in Reference ranges table Table with reference ranges for Post Pubertal Children added
Reference ranges for ECG	Table with reference ranges for Post Pubertal Children added

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

10 APPENDIX

Not applicable