Statistical Analysis Plan

Sponsor Name: Lantheus Medical Imaging, Inc.

Protocol Number: DEF-315
Protocol Title: A Phase III, Open-Label, Multicenter Trial to Evaluate Ejection Fraction, End-Diagnostic and End-Systolic Volumes, by Unenhanced and DEFINITY®-enhanced 2D-Echo and Magnetic Resonance Imaging

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I confirm that I have reviewed this document and agree with the content.

Approvals

Syneos Health Approval

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1. Glossary of Abbreviations

Abbreviation	Description
A2C	Apical 2-chamber
A3C	Apical 3-chamber
A4C	Apical 4-chamber
AE	Adverse Event
ANOVA	Analysis of Variance model
BMI	Body Mass index
BP	Blood Pressure
CI	Confidence Interval
cm	Centimeters
CP	Conditional Power
CMR	Cardiac Magnetic Resonance Imaging
FCS	Fully Conditional Specification
IA	Interim Analysis
ICC	Intra-class Correlation Coefficient
ITT	Intent-to-Treat
kg	Kilogram
LVEF	Left Ventricular Ejection Fraction
MedDRA	Medical Dictionary for Regulatory Activities
m	Meter
mL	Milliliter
mITT	Modified Intent-to-Treat
MRI	Magnetic Resonance Imaging
MUGA scan	Multiple-Gated Acquisition scan
PP	Per-Protocol
PT	Preferred Term
RMSE	Root Mean Square Error
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation

Abbreviation	Description
SOC	System Organ Class
SP	Safety Population
TEAE	Treatment Emergent Adverse Event
TLFs	Tables, Listings, and Figures
WHO	World Health Organization

2. Purpose

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures (TLFs) which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

The SAP for this study will provide a framework in which answers to the protocols' objectives may be achieved in a statistically rigorous fashion, without bias or analytical deficiencies. Specifically, the SAP has the following purpose: To prospectively outline the analyses and presentations of data that will form the basis for conclusions to be reached that will answer the studies' objectives outlined in the protocol, and to explain in detail how the data will be handled and analyzed, adhering to commonly accepted standards and practices of biostatistical analysis in the clinical trial industry.

This analysis plan is based on the protocol dated 15-Aug-2018, amendment 3, and the original SAP, dated 06-Oct-2018, approved by the FDA along with the protocol. As Syneos Health was commissioned to perform the statistical analysis, they were also asked to write a SAP following enrollment in the study.

2.1. Responsibilities

Syneos Health will perform the statistical analyses and is responsible for the production and quality control of all TLFs.

2.2. Timings of Analyses

The primary analysis of safety and efficacy will take place after all subjects complete the final study visit, or terminate early from the study, and the database has been locked.

An interim analysis to evaluate sample size assumptions will be conducted when a minimum of 75 subjects have been enrolled and imaged. An unblinded team from Syneos Health biostatistics will perform the analyses as described in Section 10.0 of this SAP to maintain the blinding of the study.

3. Study Objectives

3.1. Primary Objective

Demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography.

3.2. Secondary Objective(s)

- Demonstrate improvement in accuracy in LVEF assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms
- Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography
- Demonstrate a reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography
- Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms
- Demonstrate a reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms

3.3. Brief Description

This is a Phase 3, prospective, open-label, multicenter study to evaluate LVEF measurement accuracy and reproducibility of DEFINITY® contrast-enhanced and unenhanced echocardiography as compared with non-contrast cardiac magnetic resonance imaging (CMR) used as the truth standard. Approximately one-hundred fifty (150) subjects will be enrolled over approximately 10 months at approximately 10 centers located in the United States. The study population will consist of male and female subjects 18 years of age of older.

Subjects will be screened based on LVEF measurements obtained via 2D echo with or without contrast or other methods (e.g. CMR, MUGA scan) obtained within 6 months of enrollment (Day 0). Subjects will be stratified to achieve an approximately even distribution within four pre-defined LVEF groups (>50, 41-50, 30–40, <30%). LVEF for screening purposes will be determined by the investigator and based on measurements with 2D echo or other methods (e.g. CMR, MUGA scan) obtained within 6 months prior to enrollment (Day 0). The site investigator will determine whether an echocardiogram is optimal or sub-optimal based on the unenhanced echocardiogram performed on Day 0. Subjects with optimal and sub-optimal echocardiograms (based on investigator opinion) will be enrolled. An echocardiogram is considered sub-optimal if 2 or more segments of the ventricular border are classified as not adequately visualized.

Each subject will undergo an unenhanced ultrasound examination and a DEFINITY® contrast-enhanced examination on the same day at Day 0. A minimum of 360 seconds of images will be collected during both the unenhanced and the DEFINITY® contrast-enhanced examinations. Subjects will remain at the clinical site for at least 30 minutes of observation after DEFINITY® administration. A safety follow-up

phone call will be conducted for all subjects at approximately 72±24 hours after completion of the imaging sessions, including DEFINITY® administration.

Enrolled subjects will be followed for adverse events (AEs), serious adverse events (SAEs), and changes in concomitant medications from the time the Informed Consent is signed through the safety follow-up telephone call.

A non-contrast CMR will be used as the truth standard. CMR studies will be performed within ±30 days of the echocardiographic assessments at Day 0. Final assignment of each subject to an LVEF group will be determined by CMR.

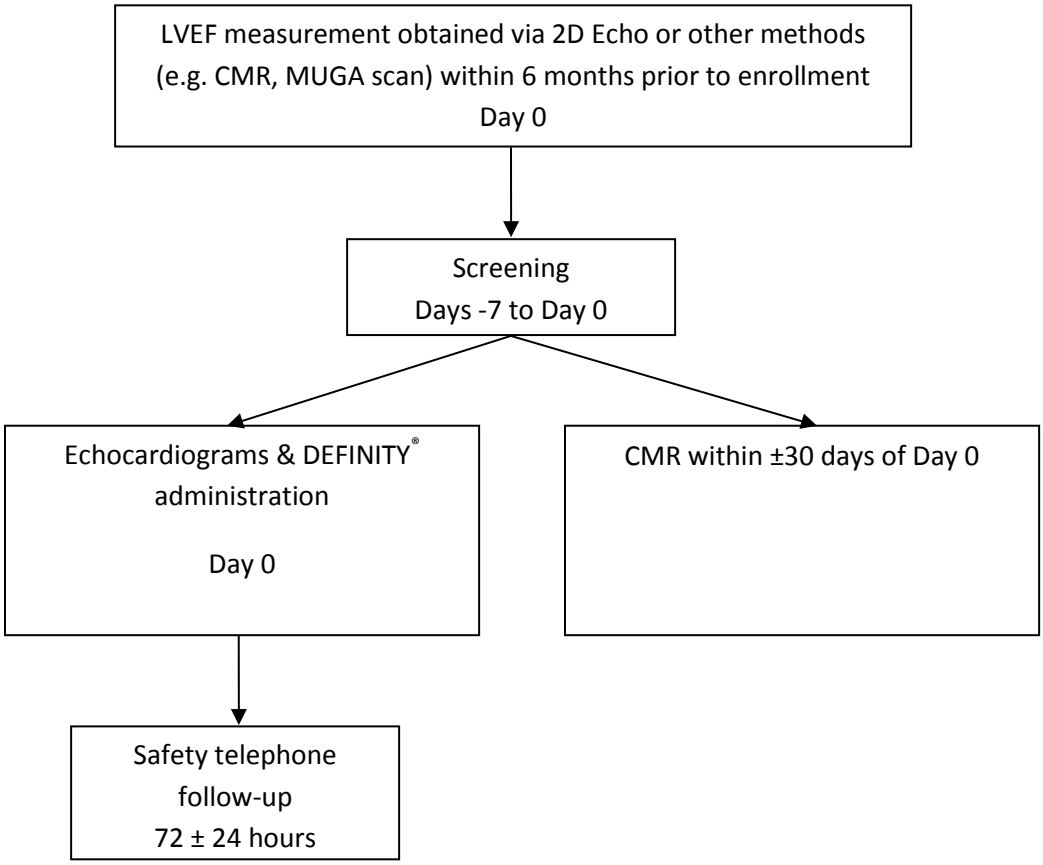
Unenhanced and DEFINITY®-enhanced echocardiograms will be performed with standard apical 2 chamber, apical 3 chamber, and apical 4 chamber (A2C, A3C, and A4C, respectively) views using harmonic imaging. Images will be recorded in standard digital format, masked to subject identifiers, and sent to a central imaging core laboratory for analysis.

At the central imaging core laboratory, 3 experienced independent blinded readers will interpret the results according to the Image Review Charter. A single, independent, blinded echocardiologist will read each subject's unenhanced echocardiogram as either optimal or suboptimal. This will be independent of the site investigator determination. All CMR images will be read centrally by 1 experienced independent blinded reader.

Echocardiographic imaging data will be compiled, analyzed, and stored without knowledge of the CMR findings.

All screening assessments will occur within 7 days prior to enrollment/DEFINITY® administration (Day 0). CMR studies will occur within ±30 days of DEFINITY® administration. Safety monitoring will continue up to 72±24 hours post-DEFINITY® administration. The expected duration of subject participation is not more than 41 days (see Figure 1).

Figure 1. Schedule of Events



3.4. Subject Selection

Approximately one hundred and fifty (150) evaluable subjects who have previously undergone a 2D echocardiogram with or without contrast or other assessment (CMR, MUGA scan) will be enrolled in this trial. The study population will consist of male and female subjects 18 years of age or older.

Enrollment will be stratified according to CMR LVEF to achieve an approximately even distribution across four subgroups (LVEF >50, 41-50, 30–40, <30%). LVEF for screening purposes will be determined by the investigator and based on measurements with 2D echo or other methods (e.g. CMR, MUGA scan) obtained within 6 months prior to enrollment (Day 0). Subjects with optimal and sub-optimal echocardiograms will be enrolled. Suboptimal echocardiograms will be based on the site investigator's evaluation of the unenhanced echocardiography images obtained on Day 0. An echocardiogram is considered suboptimal if 2 or more segments of the ventricular border are classified as not adequately visualized in any one of the 3 apical views.

3.4.1. Inclusion Criteria

Potential study subjects must meet the following inclusion criteria to be enrolled in this study.

1. Men and women ≥ 18 years of age in sinus rhythm
2. Able to communicate effectively with trial personnel
3. LVEF measurements obtained via 2D Echo with or without contrast or other methods (e.g. CMR, MUGA scan) obtained within 6 months prior to enrollment (Day 0)
4. Has provided signed informed consent after receiving a verbal and written explanation of this clinical trial

3.4.2. Exclusion Criteria

Potential study subjects who meet any of the following criteria will be excluded from the study.

1. Female subjects who are pregnant or lactating. All women of child bearing potential [WOCBP] must have a negative urine pregnancy test at screening regardless of contraceptive use history.
2. Women of child-bearing potential are excluded unless they:
   a. are post-menopausal defined as amenorrhea ≥ 12 consecutive months, **OR**
   b. have undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or bilateral oophorectomy), **OR**
   c. have been using an adequate and medically approved method of contraception to avoid pregnancy for at least 1 month prior to DEFINITY® dose administration and be willing to continue using the same method for the duration of the study.
3. Current illness or pathology that would prevent undergoing investigational product administration due to a significant safety risk to the subject.
4. Uncontrolled arterial hypertension (defined as systolic blood pressure ≥ 200 mmHg or diastolic blood pressure ≥ 110 mmHg) or arterial hypotension (defined as systolic blood pressure ≤ 90 mmHg).
5. Unstable cardiovascular status defined as:
   a. myocardial infarction or unstable angina pectoris within 6 months prior to enrollment/DEFINITY® dose administration day
   b. transient ischemic attack or stroke within 3 months prior to DEFINITY® dose administration
   c. symptomatic valvular heart disease or moderate to severe stenotic valvular heart disease
   d. clinically significant congenital heart defects
   e. current uncontrolled cardiac arrhythmias causing symptoms or hemodynamic compromise
   f. acute pulmonary embolus or pulmonary infarction
   g. acute myocarditis or pericarditis
   h. acute aortic dissection
   i. atrial fibrillation
6. Any major surgery within 4 weeks prior to screening
7. Known contraindications to undergoing CMR (e.g. implanted pacemakers, cardioverter, defibrillators) or claustrophobia
8. Participation in any investigational drug, device, or placebo study within 30 days prior to screening
9. Known hypersensitivity to perflutren, or any of the excipients in DEFINITY®
10. Prisoners or those who are subject to compulsory detention or involuntary incarceration for treatment of either a psychiatric or physical illness (e.g., infectious disease)

3.5. Determination of Sample Size

The primary analysis is to demonstrate an improvement in LVEF accuracy from unenhanced imaging to imaging with DEFINITY® contrast enhancement using CMR as the truth standard.

For each subject, the absolute value of the difference of DEFINITY® LVEF minus CMR LVEF will be calculated. Similarly, the absolute value of the difference of unenhanced imaging LVEF minus CMR LVEF will be calculated for each subject. The primary analysis is to assess the significance of the difference between DEFINITY® and unenhanced echo with respect to the mean "absolute value of the difference versus CMR". These means will be compared with a paired t-test at a two-sided 0.05 level of significance. Specifically, the null and alternative hypotheses are: H0: μD = μU versus H1: μD ≠ μU, where μD and μU are the mean of the DEFINITY® unenhanced echo absolute value of the difference versus CMRs, retrospectively. A sample size of 150 enrolled subjects has 90% power to reject the null hypothesis in favor of the alternative if the true difference μD - μU is at least 2.75 (in favor of DEFINITY®) with a standard deviation of 10 or less and allows for approximately 5% premature withdrawal. Power was calculated using the PASS 15 software (NCSS,LLC).

3.6. Treatment Assignment & Blinding

There is only one treatment group in this open-label study.

To allow the statistical team to be able to work on the interim analysis (IA), without unblinding to the IA results, the statistical team will remain blinded to the reader and CMR reported results until all subjects complete the final study visit, or terminate early from the study, and the database has been locked. A separate unblinded statistical team will create the unblinded results to be reviewed during the IA.

3.7. Administration of Study Medication

DEFINITY® will be administered as a diluted bolus injection. 1.3 mL of activated DEFINITY® will be diluted with 8.7 mL of preservative-free saline to evenly distribute microspheres. An initial injection of up to 3 mL of diluted DEFINITY® will be administered with subsequent injections of 1 to 2 mL, as needed. Full details are available in the Dose Preparation and Administration Guide in the protocol.

3.8. Study Procedures and Flowchart

The schedule of events for this study is shown in Figure 1. A schedule of procedures is displayed in Appendix 13.1. Below is a description, by visit, of all activities.

3.8.1. Screening

All Screening/Baseline assessments will occur within 7 days prior to administration of study drug (Day 0).

- Determine eligibility according to inclusion/exclusion criteria
- Informed consent completed
- General medical history information collected
- Concomitant medications collected
- Physical examination conducted
- Vital signs collected
- Urine pregnancy test (as applicable)
- Adverse event collection

3.8.2. Echocardiography Imaging Session

Subjects providing informed consent and meeting the inclusion/not meeting the exclusion criteria will be enrolled into the study and undergo the following assessments on Day 0:

- Urine pregnancy test
- Concomitant medications collected
- Bedside resting transthoracic echocardiogram
- Following the unenhanced imaging scan, the study drug (DEFINITY®) will be administered
- Following study drug administration and subsequent image optimization, the same A2C, A3C, and A4C views will be obtained
- After completion of all assessments, subjects will remain on site for at least 30 minutes of observation
- Adverse event collection

3.8.3. Telephone Assessment

A safety follow-up call will be conducted for all subjects at approximately 72±24 hours after completion of the imaging sessions. Concomitant medications and adverse events will be collected during this call.

3.8.4. Cardiac Magnetic Resonance Imaging

Within ±30 days of the Day 0 imaging session, subjects will receive a non-contrast enhanced CMR examination as described in the Study Imaging Manual.

4. Endpoints

4.1. Primary Efficacy Endpoint

The primary endpoint of this study is improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography. The primary analysis is to compare LVEF accuracy from unenhanced imaging to imaging with DEFINITY® contrast enhancement using cardiac magnetic resonance imaging (CMR) as the truth standard.

4.2. Secondary Efficacy Endpoints

The secondary endpoints are as follow:

- Improvement in accuracy in LVEF assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms
- Reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography
- Reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography
- Reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms
- Reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms

4.3. Safety Endpoints

Enrolled subjects will be followed for AEs, SAEs, and changes in concomitant medications from the time the informed consent is signed through 72±24 hours after completion of DEFINITY® administration.

5. Analysis Sets

There will be 5 populations for this study. They are listed below.

5.1. Screened Population

The Screened Set will include all subjects screened in the study. Unless specified otherwise, this set will be used for subject listings and summaries of subject disposition.

5.2. Safety Population

The Safety population (SP) will include all subjects who have signed an informed consent and who have received any amount of DEFINITY® in the study. This is the primary analysis population for the safety analysis. Unless otherwise stated, the SP will be used for all analyses of safety endpoints and for the presentation of subjects in all subject listings.

5.3. Intent-to-Treat Population

The Intent-to-Treat (ITT) population will include all subjects who have signed an informed consent.

5.4. Modified Intent-to-Treat Population

The Modified Intent-to-Treat (mITT) population will include all ITT subjects who complete unenhanced imaging, DEFINITY®-enhanced imaging, and cardiac MRI assessments. This is the primary analysis population for the efficacy endpoints.

5.5. Per-Protocol Population

The Per-Protocol (PP) population will include all subjects in the mITT population who

a) Did not violate inclusion and/or exclusion criteria that would likely have an effect on the primary outcome;
b) Do not have major protocol violations;
c) Have LVEF data on both unenhanced and DEFINITY®-enhanced for at least one reader; and
d) Have CMR LVEF.

Efficacy analyses will be conducted for both the mITT and PP populations. Differences in results between the 2 populations will be carefully examined.

5.6. Protocol Deviations

Deviations from the protocol, as defined in the protocol, will be documented and monitored on an ongoing basis by the Sponsor, study monitors, and project manager throughout the study.

Prior to database lock, the study team will forward all relevant protocol violation documentation in a pre-specified format to the study statistician. These violations will be used to determine eligibility for the PP population and will be listed in a separate report to be included in the clinical study report.

6. General Aspects for Statistical Analysis

6.1. General Methods

Syneos Health will be responsible for data management and statistical analysis. All statistical analyses will be performed using SAS version 9.4 or higher. Subject data listings and tabular presentations will be provided. Summary statistics for continuous variables will include, unless otherwise stated, the following values: N, mean, median, standard deviation, minimum, and maximum. For categorical variables, the number and percent of subjects in each category will be calculated, while the number of subjects with missing data will be presented as a 'Missing' category. Unless otherwise stated, subjects with missing values will be included in the denominator count when computing percentages.

Results will be presented to 1 decimal place when applicable. All statistical tests will be two-sided employing a significance level of 5% unless otherwise specified. Further details of each analysis will be described in the appropriate section.

Demographic and efficacy analyses will be conducted on the mITT population as the main analysis population. Efficacy analyses will also be conducted on the PP population. The safety analyses will be conducted on the SP.

The primary analysis will be performed on subjects with non-missing LVEF for DEFINITY®-enhanced echocardiography imaging, unenhanced imaging, and the truth standand CMR. A supportive analysis will be conducted where missing LVEF is multiply imputed using the fully conditional specification (FCS) multiple regression, as detailed in section 6.2. Otherwise, there will be no imputation of missing efficacy data.

6.2. Missing Data

There will be no imputations for missing efficacy data except the supportive primary endpoint paired t-test. For all other analyses, the number of subjects with missing data will be presented under a 'Missing' category. Unless otherwise stated, subjects with missing values will be included in the denominator count when computing percentages. When continuous data are being summarized, only the non-missing values will be evaluated for computing summary statistics.

6.2.1. Primary Supportive Analysis

A supportive analysis will be run where, within each blinded reader, missing LVEF is multiply imputed using the FCS multiple regression prior to carrying out the primary endpoint analysis. The covariates in the imputation model will be study center, age, gender, body weight (kg), race, and ethnicity. This procedure will be done separately for each imaging method (DEFINITY® enhanced, unenhanced, and CMR truth standard). A total of 50 imputations will be generated. The paired t-test comparing mean bias between DEFINITY® and unenhanced (see primary endpoint null and alternative hypotheses in section 3.5) will be performed separately on each of the 50 imputed datasets, and the t-test results will be combined across datasets using the usual multiple imputation techniques to create one overall paired t-test results on imputed data.

6.2.2. Concomitant Medications

In cases of a missing start or end date, the following methods will be implemented:

Missing start date:

- Missing entire date: estimate the date of DEFINITY® administration
- Missing day and month:
  - If the year is the same as the year of the DEFINITY® administration date, it will be estimated by the date of DEFINITY® administration
  - If the year is different to the year of the DEFINITY® administration date, it will be estimated as 1st January of that year
- Missing day only:
  - If the month/year are the same as the month/year of the DEFINITY® administration date, it will be estimated by the date of DEFINITY® administration
  - If the month/year is different to the month/year of the DEFINITY® administration date, it will be estimated as the first day of the month

Missing end date:

- Missing entire date: estimate the End of Study date
- Missing day and month:
  - If the year is the same as the year of the End of Study date, is will be estimated by End of Study date
  - If the year is prior to the year of End of Study date, it will be estimated by as 31st December of that year
- Missing day only:
  - If the month/year are the same as the month/year of End of Study date, it will be estimated by the End of Study date
  - If the year or month is different from the year or month of End of Study date, it will be estimated by the last day of the month

6.2.3. Adverse Events

If any part of the start date is missing for an AE, and it cannot be determined whether it occurred prior to the start of DEFINITY® administration, a conservative approach will be taken and the AE start date will be estimated to be the day of DEFINITY® administration.

7. Demographic, Other Baseline Characteristics and Medication

7.1. Subject Disposition

Subject disposition will include the number and percentage of ITT subjects who were enrolled in the trial, who received DEFINITY®, who completed the trial, and who discontinued from the trial. The number and percentage of ITT subjects who discontinued from the trial will also be presented by reason of discontinuation. The number and percentage of subjects in each analysis population will be presented. All percentages will be based on the total number of ITT subjects.

7.2. Demographic and Other Baseline Characteristics

All baseline and demographic characteristics will be summarized overall on the mITT. This will include age at screening, age group at screening, gender, ethnicity, race, height (cm), weight (kg), and body mass index (BMI). Descriptive statistics will be provided for each continuous variable; whereas, frequencies and percentages will be provided for categorical variables. Age, Age Group, and BMI calculations are described below:

- Age Group = two age groups will be created: (1) < 65 years, (2) ≥ 65 years
- BMI = weight(kg)/height^2(m)

7.3. Medical History

Medical History will be summarized overall on the mITT population. Medical history data will be collected by body system for all enrolled subjects. The details of history by body system are collected as open text. Medical history will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) version 21.0 or higher. The number and percentage of subjects within each system organ class (SOC) and preferred term (PT) will be presented. A subject experiencing a medical history within more than one SOC and PT will be counted only once within that SOC and PT, respectively.

Active medical history is defined as histories marked as ongoing at time of screening. Past medical histories are defined as histories marked as resolved at time of screening. A summary table will be provided for both active and past medical history. A listing of combined medical history will also be provided.

7.4. Medication

Medications will be coded using the World Health Organization (WHO) Drug dictionary, version Q3-2016. The number and percentage of subjects will be presented overall, by WHO therapeutic area and WHO preferred drug name. Both a table and listing will be created independently for the prior and concomitant medications for the SP. Methods for handling and categorizing missing medication data are reported in section 6.2.2.

7.4.1. Prior Medication

Prior medications are those that started and stopped before exposure to DEFINITY®.

7.4.2. Concomitant Medication

Concomitant medications are all medications taken during the study period, including those which started before administration, but were reported ongoing at the first administration.

8. Efficacy

All efficacy parameters will be summarized and presented in tables based on the mITT and PP populations. Note, approximately 10% of imaging scans will be re-read by each reader. Unless otherwise specified, the first/original read is what will be used for all reporting and statistical analyses.

8.1. Primary Efficacy Endpoint and Analysis

The primary endpoint is LVEF accuracy. The primary analysis is to demonstrate an improvement in LVEF accuracy from unenhanced imaging to that derived from imaging with DEFINITY® contrast enhancement using CMR as the truth standard.

For each subject, the absolute value of the difference of DEFINITY® LVEF minus CMR LVEF will be calculated. Similarly, the absolute value of the difference of unenhanced imaging LVEF minus CMR LVEF will be calculated for each subject. The primary analysis is to assess the significance of the difference between DEFINITY® and unenhanced echo with respect to the mean "absolute value of the difference versus CMR".

The null and alternative hypotheses for this study are:

$$H_0: \mu D = \mu U \text{ versus } H_1: \mu D \neq \mu U; \text{ or, that is}$$

$$H_0: \mu U - \mu D = 0 \text{ versus } H_1: \mu U - \mu D \neq 0$$

where μD and the μU are the mean of the DEFINITY® and unenhanced echo absolute value of the difference versus CMR, retrospectively. The null hypothesis will be tested at a two-sided 0.05 level of significance using a paired t-test derived from the sample point estimates of the mean and standard deviation of the difference between unenhanced echo's and DEFINITY's® "absolute value of the difference versus CMR".

The analyses will be conducted separately for each of the three blinded readers of the DEFINITY® enhanced and unenhanced images. The criterion for success is that the null hypothesis is rejected in favor of DEFINITY® for at least 2 of 3 blinded readers for all subjects. CMR LVEF will be used as the comparator for each of the 3 blinded readers, and is interpreted by a single reader.

The primary analysis will be performed on mITT subjects with non-missing LVEF for DEFINITY®, unenhanced echocardiography, and the truth standard. A supportive analysis will be run where a missing echocardiography or CMR LVEF is multiply imputed using the fully conditional specification (FCS) multiple regression as described in section 6.2.1.

8.1.1. Other Analyses for the Primary Endpoint

The following will be carried out separately for each of the 3 blinded readers without imputation of missing LVEF.

Bias is the mean of the actual (not absolute value) per-subject differences between the imaged LVEF and CMR LVEF. The bias, the two-sided 95% confidence interval (CI) of the bias, the precision (standard deviation of the per-subject differences) and the root mean square error (RMSE) will be calculated for the LVEF derived from the DEFINITY®-enhanced images. The RMSE is the square root of the bias-squared + precision-squared and is considered a measure of overall accuracy. These analyses will be repeated for

LVEF values derived from the unenhanced echocardiography versus those from CMR. It is anticipated that the RMSE will be smaller for DEFINITY®-enhanced LVEF than for unenhanced LVEF.

DEFINITY®-enhanced and unenhanced echocardiography LVEF will each be assessed for measurement accuracy against the reference CMR using Bland-Altman analysis and Deming regression analysis. For the Bland-Altman analysis, a plot of the per-subject actual difference between DEFINITY®-enhanced echocardiography and CMR LVEF will be plotted versus the per-subject sum of the two measurements. Limits of agreement (defined as the mean of the DEFINITY® versus CMR difference) ±2 standard deviations will be shown on the plot. The same analyses will be performed for unenhanced echocardiography using CMR as the reference standard.

Deming regression plots of LVEF versus CMR LVEF will be generated for each of DEFINITY®-enhanced and unenhanced echocardiography. Unweighted Deming regression will be employed to estimate the regression slope and intercept with two-sided 95% CI of each assuming the measurement error is the same for CMR and each of the echocardiography error techniques.

The intra-class correlation coefficient (ICC) and its two-sided 95% CI will be calculated for DEFINTIY®-enhanced echo versus CMR and for unenhanced echo versus CMR. The ICCs and their two-sided CIs will be calculated using between and within mean squares from an ANOVA model with method (echocardiography, CMR) and subject as the main effects and LVEF as the dependent variable. The ICC will be calculated using the SAS macro developed by Hamer (1990).

8.2. Secondary Efficacy Endpoint(s) and Analyses

8.2.1. Improvement in accuracy in LVEF assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms

The primary endpoint analysis will be repeated in the subset of subjects with suboptimal echocardiograms. This will include all primary analyses described in sections 8.1 and 8.1.1, excluding the supportive analyses at the end of section 8.1. The study is not powered to reject the primary endpoint null hypothesis, so the focus is more on the estimate of μD - μU, on Bland-Altman plots and on the Deming regression for each blinded reader. There will be no imputation of missing LVEF data for this analysis.

8.2.2. Reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography

The inter-reader variability among each pair of readers within each imaging modality will be estimated using an intra-class correlation (ICC) and its two-sided 95% CI. The ICC assesses rating reliability by comparing the variability of different ratings of the same subject with the total variation across all ratings and all subjects. The inter-observer variability in the assessment of LVEF between two readers will be determined by percentage of error. The percentage of error will be calculated using the following formula:

Percentage of error = (SD between 2 measurements / mean of the 2 measurements) x 100

The mean percentage of error and its 95% CI will be calculated for each pair of readers within each imaging modality. The pairwise ICCs and percentages of error differences will be compared descriptively between contrast-enhanced and unenhanced echocardiography.

In addition, specific to only section 8.2.2, there will also be a separate ICC analysis to assess intra-reader variability as approximately 10% of all echo results will be re-read by each reader.

8.2.3. Reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography

The same analysis used to assess inter-reader variability on LVEF (section 8.2.2) will be conducted for end-diastolic/systolic volumes.

8.2.4. Reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms

The same analysis used to assess inter-reader variability on LVEF (section 8.2.2) will be conducted for the subgroup of subjects with suboptimal echocardiograms.

8.2.5. Reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms

The same analysis used to assess inter-reader variability on LVEF (section 8.2.4) for the subgroup of subjects with suboptimal echocardiograms will be conducted for end-diastolic/systolic volumes.

9. Safety

The population used for safety analyses will be the Safety population (SP). Safety will be assessed on the basis of adverse event (AE) or serious adverse event (SAE) reports, physical examinations, and vital signs.

9.1. Exposure

DEFINITY® will be administered as a diluted bolus injection. 1.3 mL of activated DEFINITY® will be diluted with 8.7 mL of preservative-free saline to evenly distribute microspheres. An initial injection of up to 3 mL of diluted DEFINITY® will be administered with subsequent injections of 1 to 2 mL, as needed. Information about the administration of DEFINITY® is collected on the case report form. Exposure to DEFINITY® study drug will be summarized. The number and percentage of ITT subjects who are administered DEFINITY® will be presented. Summary statistics of the dose administered (mL) will be summarized for the safety population.

9.2. Treatment Compliance

Not applicable

9.3. Adverse Events

A treatment-emergent adverse event (TEAE) is an AE that started or worsened in severity following the start of administration of DEFINITY®. A table of AEs will be summarized for the following:

- Number of subjects having experienced at least one TEAE
- Number of subjects having experienced at least one TEAE related to DEFINITY®
- Number of subjects having experienced at least one TEAE related to study procedure
- Number of subjects having experienced at least one severe TEAE
- Number of subjects with study procedure interrupted due to a TEAE
- Number of subjects with study procedure interrupted due to a TEAE related to DEFINITY®
- Number of subjects with study procedure interrupted due to a TEAE related to the study procedure
- Number of subjects having experienced at least one serious TEAE
- Number of subjects having experienced at least one serious TEAE related to DEFINITY®
- Number of subjects having experienced at least one serious TEAE related to a study procedure
- Number of subjects having experienced at least one severe serious TEAE
- Number of subjects with study procedure interrupted due to a serious TEAE
- Number of subjects with study procedure interrupted due to a serious TEAE related to DEFINITY®
- Number of subjects with study procedure interrupted due to a serious TEAE related to the study procedure
- Number of subjects having experienced a fatal TEAE
- Number of subjects having experienced a fatal TEAE related to DEFINITY®
- Number of subjects having experienced a fatal TEAE related to the study procedure

If an AE is described as being 'related to DEFINITY®, unless otherwise stated, this will be defined as a relationship to DEFINITY® administration where the relationship is defined as either possibly related or related. If an AE is described as being 'related to study procedure', unless otherwise stated, this will be defined as a relationship to DEFINITY® enhanced echo and/or DEFINITY® administration and/or unenhanced echo and/or CMR where the relationship is defined as either possibly related or related.

A summary table of TEAEs (number and percentage of subjects who experienced an AE and number of events) grouped by primary SOC and PT will be presented for the following categories of events:

- All TEAEs
- All TEAEs related to DEFINITY®
- All TEAEs related to study procedure
- All serious TEAEs
- All serious TEAEs related to DEFINITY®
- All serious TEAEs related to study procedure
- All severe TEAEs
- All TEAEs leading to study discontinuation

A summary table of TEAEs by SOC and PT will also be presented by maximum severity.

A subject with more than one occurrence of the same AE in a particular SOC and PT will be counted only once in the total of subjects experiencing AEs in that particular SOC and PT, respectively. If a subject experiences the same AE at more than one severity, or with more than one relationship category, the most severe rating or the stronger causal relationship will be reported.

Any missing severity or relationship of an AE should be replaced by the worst case as follows:

- If severity is missing, then the AE will be included in the “severe” category
- If relationship is missing, then the AE will be included as “related to DEFINITY®”

Time to onset and duration of events in days will also be listed where:

- Time to onset is defined as (AE start date – date of DEFINITY® administration)
- Duration of event is defined as (AE stop date – AE start date + 1)

AEs with missing start dates will be included in the count of events, but a time will not be calculated. AEs will be coded using MedDRA version 21.0 or higher.

9.4. Laboratory Evaluations

A urine pregnancy test will be performed at screening and Day 0 as applicable. Results will be listed.

9.5. Vital Signs

The vital sign measurements are collected only at the screening/baseline visit. The following vitals will be summarized at baseline using descriptive statistics: systolic/diastolic blood pressure (BP), heart rate, and respiratory rate. In addition, all vital signs will be listed by subject.

The number and percentage of subjects with potentially clinically significant vital sign values at baseline will be tabulated. Potentially clinically significant vital sign values are detailed below and should be used to determine what is 'normal' and 'potentially clinically significant':

Parameter	Unit	Normal Range	Potentially Clinically Significant
Heart Rate	Beats per minute	60 – 100	1) <50 bpm 2) >100 bpm
Systolic BP	mmHg	90 – 139	1) >190 mmHg 2) <80 mmHg
Diastolic BP	mmHg	60 – 89	1) >110 mmHg 2) <50 mmHg

9.6. Physical Examination

Physical examination body system inspection results are collected at baseline and will be listed.

9.7. Study Center Effects

The mean "absolute value of the difference versus CMR" for each of DEFINITY®, and unenhanced echocardiography, as well as the difference between the two treatments with respect to this mean, will be presented for each study center and each blinded reader. Within each blinded reader, an assessment of study center effect on the mean treatment difference will be assessed using a one-way ANOVA. For each blinded reader, a site difference that is not significant at the 0.15 level of significance, or a site difference that is significant but where for every site the mean treatment difference is more favorable for DEFINITY® than unenhanced echocardiography, will support pooling subjects across sites for the primary analysis for that reader.

10. Interim Analyses

This study will utilize an adaptive design that allows one interim sample size re-calculation after a minimum of 75 subjects have been enrolled and followed. The pre-specified maximum allowable adjusted sample size following re-estimation will be 300 enrolled, or two times the initial planned sample size of 150 enrolled. The re-estimation of sample size will be conducted by an independent, unblinded biostatistician following a pre-specified plan using the method Mehta and Pocock (2011). Specifically, at the interim stage, the conditional power (CP) for obtaining a significant beneficial effect of DEFINITY® over unenhanced imaging with respect to the primary endpoint will be calculated for each blinded reader, using the protocol-specified planned sample size of 150 enrolled subjects (approximately 143 evaluable subjects assuming 5% premature withdrawal). This conditional power will be calculated under the assumption that the interim estimate of the mean unenhanced-DEFINITY® difference in LVEF accuracy is the true population mean difference. Specifically, the calculation of conditional power and sample size increase will be as follows:

Let μD be the true mean of the absolute value of the differences between the DEFINITY®-enhanced echo LVEF and the CMR LVEF, and let μU be the true mean of the absolute value of the differences between the unenhanced echo LVEF and the CMR LVEF. After a minimum of 75 subjects are enrolled and followed (which should lead to approximately 71 evaluable subjects), an unblinded interim analysis will be conducted to determine whether sample size should be increased to maintain adequate conditional power of up to 90% for each reader. Conditional Power (CP) for rejecting the null hypothesis in favor of DEFINITY® by the planned final sample size of 143 evaluable subjects is calculated as follows for each reader:

$$\text{CP} = P\left(Z > \frac{c_2\sqrt{I_2} - t_1\sqrt{I_1} - (I_2 - I_1)\Delta}{\sqrt{I_2 - I_1}}\right)$$

where,

a. Z is a random standard random variate
b. c_2 is the *t*-critical value to be used in the final analysis = 1.97681 (one-sided 0.025 level of significance; with 142 degrees of freedom assuming the final evaluable sample size is 143, which is the number of evaluable subjects expected for the final analysis with 150 enrolled subjects with 5% premature withdrawal).
c. Δ is the assumption of the true difference "μU minus μD" where μD is the mean of the absolute value of the differences between the DEFINITY®-enhanced echo LVEF and the CMR LVEF, and μU is the mean of the absolute value of the differences between the unenhanced echo LVEF and the CMR LVEF; Δ will be set to the interim sample's point estimate of μU minus μD; i.e., Δ will be set to $\bar{x}_U$ - $\bar{x}_D$, where $\bar{x}_U$ and $\bar{x}_D$ are the sample mean point estimates of μU and μD, respectively.
d. I_1 is the observed "information" at the interim analysis; specifically, $I_1 = \frac{1}{s^2/n_1}$ where *s* = the interim sample point estimate of the standard deviation of the difference between the unenhanced and DEFINITY® echo's "absolute value of the difference versus CMR" and n_1 is the interim sample size.
e. I_2 is the anticipated "information" at the final analysis; specifically, $I_2 = \frac{1}{s^2/n_2}$ where *s* is as defined in item 'd' directly above and n_2 is the planned final evaluable sample size = 143.
f. t_1 is the interim paired t-statistic testing the null hypothesis, derived from the interim sample point estimates of the mean and standard deviation of the difference between the unenhanced and DEFINITY® echo's "absolute value of the difference versus CMR".

The study will not be stopped for overwhelming efficacy or futility at this interim stage. Instead, based on Table 1 of Mehta and Pocock (2011), the following algorithm will be carried out:

- If the CP ≤ 36% for at least two of the three blinded readers based on the final sample size, the study will continue to its original planned sample size of 150 enrolled subjects.

- If the CP ≥ 90% for at least two of the three blinded readers based on the final sample size, the study will continue to its original planned sample size of 150 enrolled subjects.

- If 36% < CP <90% for exactly two out of the three readers, let n be the total protocol-specified enrolled sample size. Assume n_a is the total enrolled sample size for reader 1 to reach 90% CP, n_b is the total enrolled sample size for reader 2 to reach 90% CP, and n_c is the total enrolled sample size for reader 3 to reach 90% CP. Without loss of generality, assume both reader 1 and 2 had a CP within the range of 36% < CP <90%, reader 3 did not. Using this example, the final total number of enrolled subjects will be the sample size with which both readers have more than 90% CP but not exceeding $2n$; i.e., $\min(\max(n_a, n_b), 2n)$. All 3 readers' final analysis will be based on this $\min(\max(n_a, n_b), 2n)$ enrolled subjects.

- If 36% < CP <90% for all three readers, the final total number of enrolled subjects will be the sample size with which all readers have more than 90% CP but not exceeding $2n$; i.e., $\min(\max(n_a, n_b, n_c), 2n)$. Each reader's final analysis will be based on $\min(\max(n_a, n_b, n_c), 2n)$ enrolled subjects.

The number of enrolled subjects is not changed from the final protocol-specified sample size in any other cases. That is, in all such other cases, each reader's final analysis will be based on the n enrolled subjects.

The sample size may be increased up to 300 enrolled subjects (twice the protocol-planned sample size) for a given reader in order to obtain 90% CP. As stated above, if different sample size increases are specified for different readers, only the largest sample size increase will be shared with those blinded to the results. Thus, all readers will review the same number of scans and each reader's final analysis will be based on the number of subjects enrolled in the study.

The statisticians, sponsor, and investigators will remain blinded to the interim results for the duration of the ongoing study. After the interim analysis is carried out, the recommendation made to the sponsor will be either to keep sample size as is or to increase enrolled sample size to a given value not greater than 300. No other information derived from this interim analysis, including the reason for the recommendation, will be provided to the sponsor and other blinded personnel.

11. Changes from Planned Analysis

11.1. Changes from Analysis Planned in Protocol

There have been no changes in analyses from those defined in the protocol.

11.2. Changes from Analysis Planned in FDA Approved Statistical Analysis Plan (06Oct2018)

The variable 'Age' has been amended from being a derived value to being a reported value. No date of birth is collected for subjects in this study.

In section 3.6, a section was added to clarify that the study statistical team will remain blinded to the reader and CMR reported results until all subjects complete the final study, or terminate early from the study, and the database has been locked. A separate unblinded statistical team will create the unblinded results to be reviewed during the IA. The reason for this amended text is to ensure the study statistical team will also remain blinded to the IA study results along with the sponsor and investigators.

In section 8, it is noted that approximately 10% of the imaging scans will be re-read by each reader. Unless otherwise specified, the original read is what will be used for all reporting and statistical analyses. Further, in section 8.2.2, a separate ICC analysis was added to assess intra-reader variability. Any additional information regarding the re-reading of scans can be found in the Imaging Charter for this study.

In the vital signs table, section 9.5, the following updates have been made:

- For Heart Rate, the phrases 'and ≥25% decrease (increase) from baseline' were removed
- For Systolic blood pressure, the phrases '(entry is <160 mmHg' and 'decrease from baseline >30mmHg' were removed
- For Diastolic blood pressure, the phases '(entry is <90 mmHg)' and 'change from baseline of 20 mmHg (increase or decrease)' were removed; and a greater than sign was added in front of the Potentially Clinically Significant value of 110 mmHg as it was missing in the previous FDA approved SAP

Since the vitals signs are only collected once, at the screening/baseline visit, there will be no way to determine increases/decreases from baseline.

In section 10 (interim analysis), the two bullets to clarify how to proceed with the study in cases of CP ≤ 36% or CP ≥ 90% have been added for clarification, along with additional text regarding how to evaluate the final study results.

12. Reference List

1. Hamer RM. INTRACC.SAS Macro to Calculate Reliabilities for Intraclass Correlations [Internet]. SAS Institute;1990 [cited 15 January 2018]. Available from https://github.com/friendly/SAS-macros/blob/master/intracc.sas.

2. Mehta CR and Pocock SJ. Adaptive increase in sample size when interim results are promising: A practical guide with examples. Statist Med. 2011;30:3267-3284.

13. Appendices

13.1. Schedule of Procedures

Study Procedures	Screening/ Baseline[1]	Echo Imaging Session Day 0	Telephone Follow-up	CMR Imaging Session
Informed consent	X			
Inclusion/Exclusion	X			
Medical history	X			
Physical exam	X			
Body weight and height	X			
Vital Signs	X			
Urine pregnancy test[2]	X	X		
Resting unenhanced echocardiogram		X		
DEFINITY® administration and resting echocardiogram[3]		X	72 ± 24 hours after imaging session completion	Within ±30 days of imaging session
Concomitant medications	X	X	X	
Safety assessments (AEs, SAEs)[4]	◄	———	►	

[1]Procedures may be conducted up to 7 days prior to administration of study drug (Day 0).
[2]A urine pregnancy test will be performed at screening and within 24 hours prior to dosing study drug.
[3]Subject will remain at the clinical site for observation until at least 30 minutes after the end of study drug administration.
[4]AEs will be recorded from at the time the ICF is signed until the 72 ± 24 hour telephone follow-up.