Clinical Study Protocol: DEF-315

Study Title:	A Phase III, Open-Label, Multicenter Trial to Evaluate Ejection Fraction, End-Diastolic and End-Systolic Volumes, by Unenhanced and DEFINITY®-enhanced 2D-Echo and Magnetic Resonance Imaging
Study Number:	DEF-315
Study Phase:	Phase 3
Product Name:	DEFINITY®
IND Number:	048626
Indication:	DEFINITY® is indicated to improve the accuracy of left ventricular ejection fraction measurements with echocardiography.
Investigators:	Multicenter
Sponsor:	Lantheus Medical Imaging, Inc.
Sponsor Contact:	331 Treble Cove Road North Billerica, MA 01862 +1 (978)-667-9531
Medical Monitor:	Cesare Orlandi, MD

	Date
Original Protocol	07 February 2018
Amendment 1	02 May 2018
Amendment 2	12 June 2018
Amendment 3	15 August 2018

SYNOPSIS

Sponsor: Lantheus Medical Imaging, Inc.

Study Title:

A Phase III, Open-Label, Multicenter Trial to Evaluate Ejection Fraction, End-Diastolic and End-Systolic Volumes, by Unenhanced and DEFINITY®-enhanced 2D-Echo and Magnetic Resonance Imaging

Study Number: DEF-315

Study Phase: Phase 3

Primary Objective(s):

The primary objective of this study is to demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography.

Secondary Objective(s):

The secondary objectives of this study are to:

1. Demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms.
2. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
3. Demonstrate a reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
4. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms.
5. 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.

Study Design:

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.

Subjects will be enrolled over approximately 10 months at approximately 10 centers located in the United States. 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) to achieve an approximately even distribution within four pre-defined LVEF groups (>50, 41-50, 30–40, <30%). Subjects with optimal and sub-optimal echocardiograms will be enrolled.

Each patient will undergo an unenhanced ultrasound examination and a DEFINITY® contrast-enhanced examination on the same day at Day 0. Subjects will remain at the clinical site for at least 30 minutes of observation after DEFINITY® administration. The site investigator will determine whether an echocardiogram is optimal or sub-optimal based on the unenchanced echocardiogram performed on Day 0.

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.

In addition, a safety follow-up phone call will be conducted at 72±24 hours after DEFINITY® administration.

All echocardiography images will be interpreted by 3 experienced independent blinded readers at a central imaging core laboratory. 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 a single reader. Echocardiographic imaging data will be compiled, analyzed, and stored without knowledge of the CMR findings.

Study Population:

Approximately one hundred and fifty (150) evaluable subjects will be enrolled at approximately 10 US centers. Subjects who have had a 2D echo with or without contrast or other methods (e.g. CMR, MUGA scan) to evaluate screening LVEF within 6 months prior to enrollment (Day 0) may be enrolled. The study population will consist of males and non-pregnant and non-lactating females 18 years-of-age or older.

Duration of Treatment:

Subjects will receive DEFINITY® treatment once as a part of a single day imaging session.

Study Treatment:
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.

Efficacy Assessments:

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

The secondary endpoints are to:

1. Demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms.
2. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
3. Demonstrate a reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
4. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms.
5. 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.

Safety Assessments:

Enrolled subjects will be followed for adverse events (AEs), serious adverse events (SAEs) and changes in concomitant medications from the time the Informed Consent (IC) is signed through 72±24 hours after completion of DEFINITY® administration. All AEs will be recorded on the electronic case report form (eCRF). The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0 will be utilized for Adverse Event (AE) reporting of severity.

Statistical Methods for Primary Objective:

The primary objective of the study is to demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography, using CMR as the truth standard.

The following analyses will be carried out for each of the three blinded readers. 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 vs. 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:

$$H0: \mu D = \mu U \text{ vs. } H1: \mu D \neq \mu U$$

Where μD is the mean of the absolute value of the differences between the DEFINITY®-enhanced echo LVEF and the CMR LVEF. Similarly, μU is the mean of the absolute value of the differences between the unenhanced echo LVEF and the CMR LVEF. A sample of 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 with a standard deviation of 10 or less and allows for approximately 5% premature withdrawal. If the null hypothesis is rejected in favor of DEFINITY® for at least two of three blinded readers, the primary analysis will be considered a success.

The following are additional analyses to be carried out separately for each blinded reader to achieve the primary objective:

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 of the bias, the precision (standard deviation of the per-subject differences) and the root mean square error (RMSE) will be calculated for DEFINITY®. 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 unenhanced echocardiography vs. 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 per-subject actual difference between DEFINITY®-enhanced echocardiography and CMR LVEF will be plotted vs. the per-subject sum of the two measurements. Limits of agreement (defined as the mean of the DEFINITY® vs. CMR difference) +/- 2 SD will be shown on the plot. Similar analyses will be carried out for the unenhanced echocardiography LVEF again using CMR as the reference standard.

Deming regression plots of LVEF vs. 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% confidence intervals 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% confidence interval will be calculated for DEFINITY®-enhanced echo vs. CMR and for unenhanced echo vs. CMR.

Statistical Methods for Secondary Objectives:

A secondary objective of this study is to demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms. For this, the above analyses for the primary objective will be repeated on subjects with suboptimal echocardiograms.

An additional secondary objective is to demonstrate a reduction in inter-reader variability for the assessment of LVEF and End-Diastolic/Systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography. The inter-observer variability among each pair of readers within each imaging modality will be estimated using an intra-class correlation coefficient (ICC); descriptive comparisons of the ICC between DEFINITY® and unenhanced echocardiography will be carried out. Similar analyses will be conducted for the final secondary objective of assessing inter-reader variability within subjects with suboptimal echocardiograms.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
A2C	apical 2-chamber
A3C	apical 3-chamber
A4C	apical 4-chamber
AE	adverse event
BMI	body mass index
CABG	coronary artery bypass graft
CAD	coronary artery disease
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
cm	centimeter
CMR	cardiac magnetic resonance
CRO	Contract Research Organization
DMC	Data Monitoring Committee
ECG	electrocardiogram
eCRF	electronic case report form
ED	effective dose
Echo	Echocardiogram
EEG	electroencephalography
EF	ejection fraction
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GSM	Group sequential method
HEENT	head, ears, eyes, nose and throat
IB	Investigator's Brochure

Abbreviation	Definition
IC	informed consent
ICC	intra-class correlation coefficient
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Institutional Ethics Committee
IRB	Institutional Review Board
ITT	intent-to-treat
LC	liquid chromatography
LCX	left circumflex
LMI	Lantheus Medical Imaging
LV	left ventricular
LVEF	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MI	myocardial infarction
mITT	modified intent-to-treat
MUGA	Multiple-gated acquisition
μg	microgram
mg	milligram
MI	mechanical index
min	minute
mL	milliliter
mmHg	millimeters of mercury
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
O_2 Sat	oxygen saturation

Abbreviation	Definition
pH	potential of hydrogen
PFP	phospholipids-encapsulated perfluoropropane
PT	preferred term
QC	quality control
QT [interval]	interval on the ECG from the onset of the QRS complex to the end of the T-wave
QTcB	Bazett-Corrected QT interval
RCA	right coronary artery
RMSE	root mean square error
RR	respiratory rate
SAE	serious adverse event
SAF	Safety Evaluable Population
SAS®	Statistical Analysis System
sec	second(s)
SOC	system organ class
SP	safety population
TE	echo time
TEAE	treatment emergent adverse event
TIA	transient ischemic attack
TR	repetition time
US	United States
WOCBP	women of childbearing potential

1 INTRODUCTION

1.1 Background

Left ventricular (LV) volumes and ejection fraction (EF) are important clinical parameters for the diagnosis, management, and prognosis of subjects with cardiac diseases. Important treatment decisions and the evaluation of therapeutic effects are based on these parameters. Several techniques have been used for the analysis of LV volumes and EF, among them cine ventriculography, echocardiography, cardiac magnetic resonance (CMR) and computed tomography (Foley 2012).[1] CMR has evolved into the preferred reference technique because of its high spatial resolution and ability to obtain complete volumetric data sets, allowing very accurate determinations of regional and global LV function. Although the most frequently used modality in clinical practice, echocardiography has been hampered by its moderate reproducibility and accuracy to define LVEF. Poor acoustic windows and inadequate discrimination of the endocardial border are the main reasons for the poor reproducibility and accuracy of the test, besides geometric assumptions resulting from the two-dimensional approach (Foley 2012).[1] In a few studies, contrast echocardiography has been shown to allow improved assessment of LV volumes and EF, especially in subjects with difficult imaging conditions (Hoffman 2005, 2014,).[2,3] Recent innovations in contrast-specific ultrasound techniques have further enabled improvements in visualization of the LV endocardial border above the level already shown in previous trials with the use of contrast-enhanced ultrasound imaging.

DEFINITY® (perflutren injectable suspension) is a sterile, non-pyrogenic suspension of phospholipids-encapsulated perfluoropropane microbubbles that is activated by shaking with the aid of the Vialmix™. In subjects with suboptimal echocardiograms, the addition of DEFINITY® improves the assessments of cardiac structure (ventricular chambers and endocardial borders) and function (regional wall motion).

The objective of this multicenter study is to determine the accuracy and inter-reader agreement of DEFINITY® contrast-enhanced and unenhanced 2D echocardiography for the assessment of LV volumes and EF in comparison with non-contrast CMR.

This trial will be conducted strictly according to the protocol, Good Clinical Practice (GCP), the sites' Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs), and other related laws and regulations as applicable.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography.

2.2 Secondary Objectives

The secondary objectives of the study are to:

1. Demonstrate improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms.
2. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
3. Demonstrate a reduction in inter-reader variability for the assessment of end-diastolic/systolic volumes using DEFINITY® contrast-enhanced versus unenhanced echocardiography.
4. Demonstrate a reduction in inter-reader variability for the assessment of LVEF using DEFINITY® contrast-enhanced versus unenhanced echocardiography in subjects with suboptimal echocardiograms.
5. 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 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

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) evaluable subjects will be enrolled and will undergo unenhanced and DEFINITY®-enhanced echocardiograms and CMR.

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 prior to enrollment. Subjects will be stratified to achieve an approximately even distribution within four pre-defined LVEF groups (>50, 41-50, 30–40, <30%) as measured by CMR. Subjects with optimal and suboptimal echocardiograms will be enrolled.

Each patient will undergo an unenhanced ultrasound examination and a DEFINITY® contrast-enhanced examination on the same day. A minimum of 360 seconds of images will be collected during both the unenhanced and the DEFINITY® contrast-enhanced examinations.

The site investigator will determine whether an echocardiogram is optimal or sub-optimal based on the unenchanced echocardiogram performed 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.

Subjects will remain at the clinical site for at least 30 minutes of observation after the end of the administration of DEFINITY®. A safety follow-up telephone call will be conducted for all subjects at approximately 72±24 hours after completion of the imaging sessions.

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

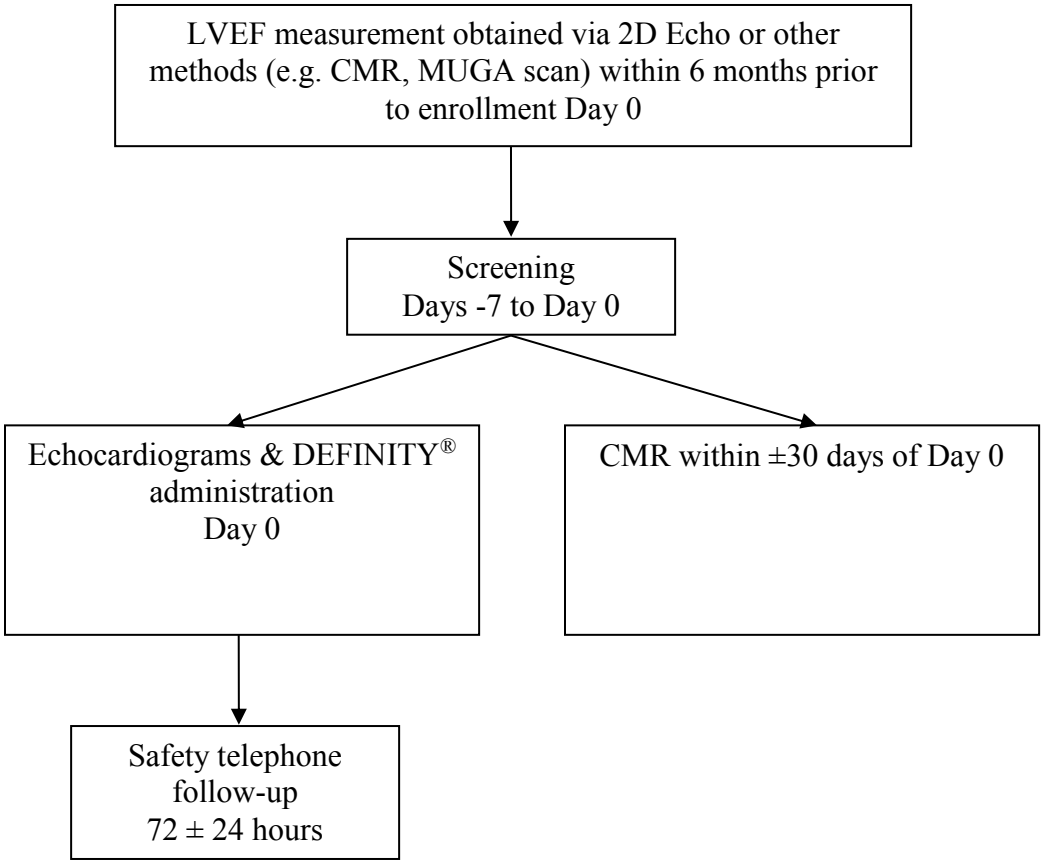
Unenhanced and DEFINITY®-enhanced echocardiograms will be performed with standard apical 2 chamber, apical 3 chamber, and apical 4 chamber, (A2C, A3C, and A4C, respectively) views. DEFINITY®-enhanced echocardiograms will be performed using low or very low mechanical index (MI) imaging. Images will be recorded in standard digital format, masked to patient identifiers, and sent to a central echocardiography facility for analysis. There, 3 experienced independent blinded readers will interpret the results according to the Image Review Charter; additional analyses will also be performed as described in the Image Review Charter. Each reader's independent interpretation will be recorded in a database.

CMR images will also be collected and independently read at a central laboratory by a single reader.

The schedule of events is provided in Appendix 1.

Figure 3-1 displays an overview of study events.

Figure 3-1 Study Event Overview



3.2 Rationale for Study Design

The study is intended to demonstrate the utility of DEFINITY® contrast-enhanced echocardiography in providing accurate and reproducible measurements of LVEF and LV volumes as compared with unenhanced 2D echocardiography using non-contrast CMR as the truth standard.

A broad-based population with known or suspected cardiac disease with either optimal or suboptimal 2D echocardiograms or other imaging methods from which LVEF can be derived (e.g. CMR, MUGA scan) will be included.

The primary endpoint of improved accuracy in LVEF measurement will be assessed from the results of an independent assessment and subsequent analysis conducted by the central imaging laboratory. Secondary endpoints will also be assessed, including accuracy in the suboptimal echocardiogram subset and inter-reader variability in the overall population and the suboptimal echocardiogram subset.

Interpretation of images will be conducted with a blinded read by three experienced physicians at a central imaging laboratory who are not associated with patient recruitment. In addition, 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 performed at Day 0. The independent readers will employ a prospectively defined and standardized methodology described in the Image Review Charter, which will reduce the potential for variation in the criteria used for image reads.

CMR images will also be collected and read independently at a central imaging laboratory, using a methodology that will also be described in the Image Review Charter.

3.3 Study Duration

All screening assessments will occur within 7 days prior to enrollment/DEFINITY® administration (Day 0). Non-contrast CMR studies will occur within ± 30 days of DEFINITY® administration. Safety monitoring will begin with the signing of the informed consent and continue up to 72±24 hours post-DEFINITY® administration. The expected duration of subject participation is not more than 41 days. Subjects are expected be enrolled over a 10 month period.

4 STUDY POPULATION

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.

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

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 patient.
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)

5 STUDY TREATMENT(S)

5.1 Description of Treatment(s)

5.1.1 Investigational Product

DEFINITY® is an ultrasound contrast agent consisting of phospholipids-encapsulated perfluoropropane (PFP), also known as perflutren, microspheres, which is designed to enhance echocardiographic ultrasound images. DEFINITY® (perflutren injectable suspension) will be provided in a 2-mL clear vial containing a 1.5 mL fill volume.

DEFINITY® contains no preservative. Bacterial contamination with the risk of post-administration septicemia can occur following the puncture of the elastomeric septum. It is essential to follow directions for preparation of DEFINITY® carefully and to adhere to strict aseptic procedures during preparation.

The chemical, and pharmacologic properties and formulation of DEFINITY® are described in detail in the Investigator's Brochure (IB).

5.2 Treatments Administered

All treatments will be administered as described in the Dose Preparation and Administration Guide. Appendix 2.

5.2.1 Administering Study Drug

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 – Appendix 2.

5.3 Selection and Timing of Dose for Each Patient

All subjects will receive administration of DEFINITY® as described above and in the Dose Preparation and Administration Guide – Appendix 2.

5.4 Method of Assigning Subjects to Treatment Groups

There is only one treatment group in this study.

5.5 Blinding

This is an open-label study; therefore, no blinding procedures are required for investigators and subjects.

5.6 Concomitant Therapy

Concomitant medications will be collected from the screening visit to the 72±24 hour safety follow-up phone call, and will be recorded on the eCRF.

5.7 Treatment Compliance

The investigator will comply with the protocol and all applicable regulatory requirements in assuring that the study drug is dispensed to subjects by authorized personnel only.

5.8 Packaging and Labeling

The labeling of the study drug will meet local regulatory requirements, but at a minimum will provide the investigational product name, expiry, lot number, manufacturer name and address, storage conditions, and caution warnings for investigational product use.

5.9 Storage and Accountability

The investigator is responsible for ensuring that deliveries of study drug are safely and properly received, recorded, handled, and stored in accordance with the national and/or local laws and are used in accordance with this protocol.

DEFINITY® should be stored in a refrigerator (2-8° C) upon receipt of the vials. Prior to activation, the vial should be warmed to room temperature. Following activation, DEFINITY® can be stored at room temperature (do not store above 30°C) and should be used within 12 hours of preparation. Refer to DEFINITY® Dose Preparation and Administration Guide (Appendix 2) for full storage details.

5.10 Investigational Product Retention at Study Site

The site will document the administration of the investigational product for each subject. All investigational product, used and unused, will be accounted for by a study monitor.

All unused or residual study drug must be properly destroyed at the study site in accordance with national and/or local laws and institutional policies and properly documented.

6 STUDY PROCEDURES

6.1 Screening

The timing of study procedures is provided in Appendix 1. All Screening/Baseline assessments will occur within 7 days prior to administration of study drug (Day 0).

6.2 Informed Consent

Informed consent (IC) must be obtained from each patient prior to the initiation of any study related procedures unless those procedures are considered standard of care for the diagnosis and/or treatment of the underlying disease process. The IC must meet the requirements as defined by International Conference on Harmonization (ICH) - Good Clinical Practice (GCP) guidelines, US Federal Regulations and/or conform to national or regional regulatory agency requirements, or local laws, whichever provides the greatest level of protection. Each patient must be provided, in an understandable manner, a written informed consent form (ICF) that describes the nature and duration of the study. Additionally, the patient must be allowed adequate time to consider the potential risks and benefits associated with his/her participation in the study. The IC must also contain language that allows the Sponsor (or designated representative), regulatory authorities, and the Institutional Review Board (IRB)/ Institutional Ethics Committee (IEC) direct access to the patient's medical records for the purpose of review.

In situations where study subjects are not legally competent to provide consent (i.e., cognitively incapacitated patient), written consent must be obtained from an authorized guardian or representative. In these situations, the ICF must be signed and dated by a witness and, if possible, assent must be obtained from the patient. The ICF must also be signed and dated by the individual obtaining the IC.

The Investigator, as part of the study documentation, must retain the original signed and dated ICF and must provide a signed copy to each patient or authorized representative. The Investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

6.3 Medical History

A general medical history for each patient will be obtained by interview and through a review of available medical records. Data to be collected and recorded on the eCRF will include demographics (e.g. age, gender, race/ethnicity) and an overview of significant past or present illnesses, including previous administration of DEFINITY®. Known drug allergies, other allergies, or history of hypersensitivity reactions will also be recorded on the eCRF.

Information obtained via interview should be supplemented with data from hospital records or clinic charts as available.

All concomitant medications will be recorded on the eCRF. This information will be obtained by interview and through a review of available medical records.

6.4 Physical Examination

A physical examination will be performed at screening and will include height, weight, general appearance, and assessment of appearance (head, ears, eyes, nose and throat [HEENT]); neck; cardiovascular; lungs; abdomen; lymph nodes; extremities; neurological; skin; musculoskeletal; and other). All physical examination abnormalities will be recorded on the eCRF.

6.5 Vital Signs

All vital sign measurements should be obtained with the patient lying supine, having rested quietly for at least 2 minutes, as feasible. Vital signs (including heart rate, respiratory rate, and systolic and diastolic blood pressure) will be collected at the Screening visit only.

6.6 Pregnancy Test

A urine sample for pregnancy testing will be obtained for all women of childbearing potential at screening and within 24 hours prior to dosing the study drug.

6.7 Cardiac Magnetic Resonance Imaging

6.7.1 Image Acquisition

Subjects will receive a non-contrast enhanced CMR examination. MRI examinations will be performed by a qualified technologist supervised by a physician experienced with cardiac MRI. Steady-state free precession (SSFP) MRI will be performed using a 1.5 T (or above) imaging system and a standardized and harmonized pre-specified method in accordance with practice guidelines for images that are to be used for LVEF evaluation. Cardiac slices relative to single apical 2- and 4-chamber views as well as multi-slice short-axis images running from the apex to the base will be obtained for each patient. The apical 2-chamber view should be parallel to the interventricular septum and intersect the cardiac apex and mid-mitral valve; whereas, the apical 4-chamber view should be perpendicular to the interventricular septum, maximizing ventricular size, and should intersect the apex and mid-mitral valve. The left ventricular outflow tract and aorta must not be visualized in this view. Parasternal short-axis images of the left ventricle will be acquired, perpendicular to the long axis of the ventricle spanning apex to base.

Consecutive breath held short-axis cine images will be obtained with full ventricular coverage.

All images will then be transferred to an Imaging Core Laboratory for further analysis. No site analysis will be performed. Instructions for MRI data transfer and storage at the central imaging laboratory will be provided in the study Imaging Manual.

6.7.2 Imaging Core Laboratory

To identify the LV end-diastolic and end-systolic images, the full stack of short axis images will be evaluated. The LV end-diastolic image will be chosen as the phase with the largest LV blood volume. The LV end-systolic image will be chosen as the phase with the smallest LV blood volume. The Simpson's method of summation of discs will then then applied (sum over all slices of cross sectional area multiplied by the sum of the slice thickness and the inter-slice gap) to determine LV end-diastolic (LVEDV) and LV end-systolic (LVESV) volumes for entire LV. Quantification of LVEF will then be calculated based on volumes using the formula: $LVEF=(LVEDV-LVESV)/LVEDV$.[4,5]

A single central MR reader will verify and correct if necessary the identification of the LV end-systolic and diastolic images provided by a trained and blinded technologist to determine the LV end diastolic and end-systolic volumes. Similarly a trained and blinded technologist will provide initial ventricular contours which will be verified and corrected if necessary by the central MR reader prior to final calculation of the volumes.

6.8 Echocardiography Imaging Procedures

6.8.1 Image Acquisition

A bedside resting transthoracic echocardiogram will be performed by personnel trained in study procedures according to standardized instructions provided here and in the study Imaging Manual. Unenhanced echocardiography will be performed in each of the standard A2C, A3C, and A4C views after optimization of these views. At least 3 consecutive cardiac cycles should be recorded in standard digital format in each view. After unenhanced imaging has been conducted, study drug will be administered as described above and in the Dose Preparation and Administration Guide. DEFINITY®-enhanced images will be obtained with low or very low MI imaging using a standardized and harmonized method consistent with practice guidelines. After study drug administration and subsequent image optimization, the same A2C, A3C, and A4C views should be obtained as with unenhanced imaging over at least 3 consecutive cardiac cycles.

Subjects will remain at the site for observation until at least 30 minutes after the end of the administration of study drug.

All images will then be transferred to an Imaging Core Laboratory for further analysis. No site analysis will be performed. Instructions for echo data transfer and storage at the central imaging laboratory will be provided in the study Imaging Manual.

6.8.2 Imaging Core Laboratory

Left ventricular end-diastolic and end-systolic volumes will be measured using the modified biplane Simpson's method in the apical 4 chamber and apical 2 chamber views. End-diastole will be identified as the frame in which the left ventricular cavity size is visually largest. End-systole will be identified as the frame in which the left ventricular cavity size is visually smallest, just before mitral valve opening. The inner contour of the LV cavity (interface between left ventricular cavity and compacted myocardium) will be traced according to the recommendations of the American Society of Echocardiography (ASE), leaving the papillary muscles and trabeculations within the cavity. Left ventricular ejection fraction will be calculated from the biplane volumes using the formula: $LVEF=(LVEDV-LVESV)/LVEDV$.[6]

A blinded echocardiography technologist at the Imaging Core Laboratory will provide an initial contour of the LV cavity for the blinded read. The blinded echocardiography readers will adjust contours based on their judgement and will provide independent LV measurements. The same method for LVEF calculation will be used for all echocardiograms, including suboptimal and non-suboptimal and both enhanced and non-enhanced.

6.9 Efficacy Assessments

Three blinded independent readers will perform all of the efficacy assessments following the methodology described in the Imaging Review Charter.

6.9.1 Primary Efficacy Assessments

The primary efficacy assessment will be the measurement of LVEF.

6.9.2 Secondary Efficacy Assessments

Secondary efficacy assessments will be the end-systolic and end-diastolic LV volumes.

6.10 Adverse Event Assessments

6.10.1 Performing Adverse Event Assessments

In this clinical trial, an AE is defined as any new untoward medical occurrence or worsening in severity or frequency of a pre-existing medical condition in a study patient, which does not necessarily have a causal relationship with investigational product. AEs occurring from the

time the informed consent is signed until the 72±24 hour telephone follow-up will be reported. Treatment-emergent adverse events (TEAEs) are a safety endpoint and are discussed in Section 8.4.1. The NCI-CTCAE version 4.0 will be utilized for Adverse Event (AE) reporting of severity by grade.

An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal (investigational or marketed) product, whether or not considered related to the medicinal (investigational or marketed) product.

Note: For this clinical trial, observations and findings (previously unknown) discovered during the procedures and medically assessed as most-likely pre-existing (e.g., myocardial wall hypokinesis, thrombus, cardiac valve deficits, and so on) will not be considered AEs and will not be reported as such in the eCRF. However, these observations and any hospitalizations/treatments required to treat the new findings will be reported elsewhere in the eCRF. Any complications resulting from the study procedures (e.g., intravenous extravasations) *will* be recorded as an AE.

A medication error is defined as any event related to the administration of a medicinal or investigational product that may cause or lead to inappropriate use or patient harm. Medication errors can be actual or potential errors and may include misuse, administration error, overdose, under-dose, or abuse. For this clinical trial, any medication errors (e.g., administration error, overdose, etc., that occur at the time of study product administration) must be recorded as an AE.

All identified AEs must be recorded and described on the appropriate AE section of the eCRF by the investigator (or designee). If known, a diagnosis should be recorded, rather than multiple individual symptoms. In addition to the event term, the following information should be captured for all AEs:

- Date (and time) of onset and resolution
- Severity of the event (see definitions below)
- Investigator's opinion of the causal relationship to study drug or comparator, procedure, or underlying disease (see definitions below)
- Treatment action taken
- Information regarding resolution/outcome

6.10.2 Timing

Timely and complete reporting of safety information helps to identify any untoward medical occurrence, thereby allowing:

- Protection of safety of study subjects,
- Greater understanding of the overall safety profile of the investigational product,
- Appropriate modification of study protocols, and
- Adherence to worldwide regulatory requirements.

Adverse event monitoring will be initiated at the time the informed consent is signed and will continue until completion of all echo assessments and patient discharge. All subjects will be followed up by telephone assessments for AEs at 72±24 hours following DEFINITY® administration.

6.10.3 Severity

The Investigator (or designee) will assess the severity of the AE using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0 will be utilized for Adverse Event (AE) reporting. The NCI-CTCAE definitions of severity are below.

Grade	Degree of Severity
1	Mild, with mild or no symptoms; no interventions required
2	Moderate; minimal intervention indicated; some limitation of activities
3	Severe but not life threatening; hospitalization required; limitation of patient's ability to care for him/herself
4	Life threatening; urgent intervention required
5	Death related to adverse event

This assessment is subjective and the Investigator should use good clinical judgment to compare the reported AE to similar type experiences observed in clinical practice.

6.10.4 Relationship

The Investigator will assess the relationship of the AE to DEFINITY®. The following categories and definitions of causal relationship should be used for this study:

- **Not related:** indicates that there is little or no chance that the study treatment or procedure caused the reported AE; other conditions, including concomitant illnesses, progression or expression of the disease state, or a reaction to a concomitant medication, appear to explain the reported AE. For the purposes of determining expedited reportability, AEs categorized as unlikely related to investigational product will not be considered associated with the investigational product.
- **Possibly related:** indicates that the association of the AE with the study treatment or procedure is unknown; however, the AE is not reasonably attributed to any other condition
- **Related:** indicates that a reasonable temporal association exists between the AE and study treatment or procedure and, based upon the investigator's clinical experience, the association of the event with the study treatment seems likely

6.10.5 Clinical Significance

Any abnormality in physical examinations or vital signs not present at Baseline should be judged for clinical significance by the Investigator.

6.10.6 Serious Adverse Events

6.10.6.1 Definition

A serious adverse event (SAE) is defined by regulation as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening AE
- In-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity, or
- Congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include

allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in inpatient hospitalization.

Note: Hospitalization for scheduled elective procedures or hospitalization from clinically indicated patient management decisions based on pre-study conditions should not be classified as SAEs. Examples of this would include a scheduled coronary intervention or CABG procedure within the SAE reporting period. Additionally, a treatment procedure scheduled due to diagnosis during the investigational procedure would not be classified as an SAE. An example of this would be angioplasty to treat a patient's underlying CAD. Events resulting from the investigational procedure or a worsening of a pre-study condition which satisfy the definition of a SAE must be reported.

6.10.6.2 Reporting Serious Adverse Events

All SAEs, whether related or unrelated to the investigational product, must be reported within 1 calendar day to LMI Drug Safety by confirmed facsimile transmission, email or phone. Overnight express mail may also be used in lieu of a facsimile transmission. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

Collection of complete information concerning SAEs is extremely important. Thus, follow-up information that becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

The contact information for reporting SAEs and follow-up SAE information is:

Contact	Telephone Number	Fax Number	Email Address
LMI Safety	1-800-343-7851 + 978-677-9531	FAX: 1-978-436-7296	lantheussafety@lantheus.com

If the investigator needs to discuss an SAE or any other aspect of the protocol with the Medical Monitor, s/he can be reached at the following numbers:

LMI Medical Monitor
24-Hour phone number: +1-240-481-8818
Fax: +1-978-436-7296

In the event of AEs that are serious, unexpected, and possibly related or related to DEFINITY® administration, LMI will contact the Investigator and will provide a MedWatch and/or Council for International Organizations of Medical Sciences (CIOMS) narrative of the case, which will include an Analysis of Similar Events. The Investigator is responsible for notifying the appropriate IRB in accordance with institutional requirements. The Investigator and IRB will determine if the IC requires revision. The Investigator should also comply with the IRB procedures for reporting any other safety information.

6.11 Removal of Subjects from the Trial or Investigational Product

The investigator may withdraw a patient from the study for any of the following reasons:

- Patient withdraws consent
- Patient is lost to follow up
- Patient has an AE that, in the opinion of the Investigator, requires the patient's discontinuation
- Discretion of the Investigator
- The Sponsor or Investigator terminates the study

All events that result in discontinuation of study treatment will be appropriately recorded and reported. In addition, for all subjects who discontinue prematurely, an evaluation which reflects the status of the patient at premature termination, along with a final assessment and the reasons for termination, will be recorded on the eCRF.

7 QUALITY CONTROL AND ASSURANCE

The sponsor has implemented and maintains Quality Assurance and Quality Control systems with written procedural documents (e.g. SOPs, work instructions, monitoring plans, etc.) to ensure that the study is conducted, and data are generated, recorded, and reported in compliance with the protocol, ICH-GCP, and all applicable regulatory requirements.

These quality systems include monitoring (see Section 9.6); data collection, review and entry (see Section 9.11); training; and audit by the sponsor's Quality group or representative. Audits are independent of routine monitoring and quality control functions, and are conducted to evaluate trial conduct and compliance with the protocol, procedural documents, ICH-GCP and applicable regulatory requirements.

8 PLANNED STATISTICAL METHODS

8.1 Statistical Analyses

8.1.1 General Considerations

The contract research organization (CRO) will be responsible for data management and statistical analysis in this trial. All statistical analyses will be performed using SAS (SAS Institute, Inc., Cary, NC) version 9.4 or higher. Patient data listings and tabular presentations of results will be provided. Presentation of summary statistics for continuous variables will include N, mean, median, and standard deviation, as well as the minimum and maximum values. For categorical variables, the number and percent of each category within a parameter for non-missing data will be calculated. All statistical tests will be two-sided employing a significance level of 5% unless otherwise specified. Further details of the criteria and conduct of the statistical analyses are below and will be included in the Statistical Analysis Plan for this study.

8.1.2 Analysis Populations

8.1.2.1 Safety Population

The Safety Population (SP) will include all subjects who have signed informed consent and who have received any amount of DEFINITY® in the study. This is the primary analysis population for the safety analysis.

8.1.2.2 Intent-to-Treat

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

8.1.2.3 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 MR studies. This is the primary analysis population for the efficacy endpoints.

8.1.2.4 Per-Protocol Population

The Per-Protocol (PP) population will include all subjects in the mITT population who a) did not violate inclusion and 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; (d) have CMR LVEF.

Efficacy analyses will be conducted for both mITT and PP population sets. Differences in results using the two populations will be carefully examined and the results of that evaluation presented in the study report.

8.2 Efficacy Endpoint(s)

8.2.1 Primary Efficacy Endpoint

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 patient, 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 patient. 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 vs. 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:

$$H0: \mu D = \mu U \text{ vs. } H1: \mu D \neq \mu U$$

where μD and μU are the mean of the DEFINITY® and unenhanced echo absolute value of the difference vs. CMRs, respectively. A sample of size 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.

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 the 3 blinded readers. CMR LVEF is interpreted by a single reader; this is the CMR LVEF that will be used as the comparator for each of the three blinded readers.

The primary analysis will be performed on mITT subjects with non-missing LVEF for DEFINITY®, unenhanced echocardiography, and the reference 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 outlined below and as will be further detailed in the Statistical Analysis Plan.

The following are additional analyses to be carried out separately for each blinded reader to achieve the primary objective:

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 of the bias,

the precision (standard deviation of the per-subject differences) and the root mean square error (RMSE) will be calculated for DEFINITY®. 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 unenhanced echocardiography vs. 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 difference between DEFINITY®-enhanced echocardiography and CMR LVEF will be plotted vs. the per-subject sum of the two measurements. Limits of agreement (defined as the mean of the DEFINITY® vs. CMR difference) +/- 2 SD 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 vs. 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% confidence intervals 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% confidence interval will be calculated for DEFINITY®-enhanced echo vs. CMR and for unenhanced echo vs. CMR. The ICCs and their two-sided confidence intervals 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).[7]

Assessment Across Study Centers: The mean "absolute value of the difference vs. CMR" for each 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 blinded reader. Within each blinded reader, an assessment of study center effect on the mean treatment difference will be carried out using one-way analysis of variance. 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 enhanced echocardiography, will support pooling subjects across sites for the primary analysis for that reader.

Missing Data: Within each blinded reader, the primary analysis will be performed on subjects with non-missing LVEF for DEFINITY®, unenhanced echocardiography, and the reference standard. A supportive analysis will be run where, within each blinded reader, missing LVEF is multiply imputed using the fully conditional specification (FCS) multiple regression. The covariates in the imputation model will be fully specified in the formal

statistical analysis plan and will include baseline and demographic variables and will also include imaging method (DEFINITY®, unenhanced, or CMR reference standard). A total of 50 imputations will be generated; the paired t-test comparing mean bias between DEFINITY® and unenhanced 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.

8.2.2 Other Primary Endpoint(s)

None

8.3 Secondary Endpoint(s)

8.3.1 Secondary Efficacy Endpoint(s)

8.3.1.1 Improvement in accuracy in left ventricular ejection fraction (LVEF) assessment using DEFINITY® contrast-enhanced over unenhanced echocardiography in subjects with suboptimal echocardiograms

The above primary analyses will be repeated here, but on the subgroup of subjects with suboptimal echocardiograms. There will be no imputation of missing data for this analysis.

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

The inter-observer variability among each pair of readers within each imaging modality will be estimated using an intra-class correlation coefficient (ICC). 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 formula:

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

The mean percentage of error and its 95% confidence interval will be calculated for each pair of readers within each imaging modality.

There will be no imputation of missing data for this analysis.

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

The above analyses used to assess inter-reader variability on LVEF will be carried out, but on end-diastolic/systolic volumes. There will be no imputation of missing data for this analysis.

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

The above analyses used to assess inter-reader variability on LVEF will be carried out, but on the subgroup of subjects with suboptimal echocardiograms. There will be no imputation of missing data for this analysis.

8.3.1.5 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 above analyses used to assess inter-reader variability on LVEF in subjects with suboptimal echocardiograms will be carried out, but on end-diastolic/systolic volumes. There will be no imputation of missing data for this analysis.

8.3.2 Other Secondary Endpoint(s)

None.

8.4 Safety Endpoint(s)

8.4.1 Primary Safety Endpoint(s)

Safety analyses will include tabulations of the incidence (number and percent of subjects) of treatment-emergent adverse events (TEAEs) and procedure-related AEs by MedDRA system organ class (SOC) and preferred term (PT). This will be repeated for serious adverse events, for adverse events leading to study withdrawal and for adverse events at least possibly related to study procedures. This will be performed on the safety population.

8.4.2 Secondary Safety Endpoint(s)

None.

8.5 Demographics and Baseline Characteristics

Demographic profiles including age, gender, race, ethnicity, height, weight and BMI will be summarized for all Safety evaluable subjects. Descriptive statistics (n, mean, median, standard deviation, minimum and maximum) will be provided for each quantitative variable, and frequencies and percentages will be provided for each categorical variable.

8.6 Interim Analysis

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 biostatistician following a pre-specified plan using the method of by Mehta and Pocock (2011).[1] 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 DEFINITY® -unenhanced difference is the true population difference. Specifically, the calculation of conditional power will be as follows:

Let μD be the true mean of the absolute value of the LVEF differences between the DEFINITY®-enhanced echo LVEF and the CMR LVEF, and let μU be the true mean of the absolute value of the LVEF differences between the unenhanced echo LVEF and the CMR LVEF. After 75 patients are enrolled and followed (which should lead to approximately 71 evaluable patients), 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 patients 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 df 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 value observed at the interim (the calculation of Δ is described below; it is not correct to calculate it as the interim point estimate of μU minus the interim point estimate of μD).
d. I_1 is the observed "information" at the interim analysis based on the interim observed standard deviation of μD minus μE; its calculation is described below.
e. I_2 is the anticipated "information" at the final analysis; its calculation is described below.
f. t_1 is the interim paired t-statistic testing the null hypothesis, calculated from the data observed at the interim

Further information on the above parameters is as follows:

Assuming the Definity-CMR raw LVEF difference (not absolute value) is normally distributed with mean μ and sd σ, then its absolute value, D, follows the *folded normal* distribution with true mean and sd as follows:

$$\mu_D = \sqrt{\frac{2}{\pi}}\sigma e^{-\frac{\mu^2}{\sigma^2}} + \mu\left[1 - 2\Phi\left(-\frac{\mu}{\sigma}\right)\right] \qquad \sigma_D = \sqrt{\mu^2 + \sigma^2 - \mu_D^2}$$

where ϕ is the standard normal cumulative distribution function. A similar algorithm can be applied to the unenhanced-CMR LVEF raw difference and its absolute value U to determine μ_U and σ_U. In the above CP formula, Δ will be set to the estimate of μ_D - μ_U, where the estimates of μ_U and μ_D are each calculated from the above equation using point estimates of the relevant μ and σ. The standard deviation of the difference between the absolute value of *D* minus the absolute value of *U* is

$\sqrt{\sigma_D^2 + \sigma_U^2 - 2 * Cov(D,U)}$ where $2 * Cov(D,U) = \rho * \sigma_D * \sigma_U$

where ρ is the correlation between *D* and *U*. This standard will be used in the calculation of the "information", as further described below.

So, in the CP formula for this example,

a. Δ will be set to the estimate of μ_D - μ_U
b. $I_1 = \frac{1}{s^2/n_1}$ where *s* = the interim estimate of the above standard deviation between the absolute values D and U
c. $I_2 = \frac{1}{s^2/n_2}$ where *s* - the interim estimate of the above standard deviation between the absolute values D and U and n_2 is the planned final sample size = 143.

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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),[1] the following algorithm will be carried out: (1) 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; otherwise (2) 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; (3) if 36% < CP <90% (the Mehta and Pocock "promising zone") for at least two of the three readers based on the final sample size, then the sample size will be increased so that the CP is at least 90% for every reader whose CP fell in the promising zone. However, if there is a reader who does not fall in the promising zone, then that reader's primary results will be based only on the patients enrolled under the original protocol-specified sample size. Further, for every reader for whom the sample size is increased, that reader's results will only be based on the first X images, where X is the number of images that yielded CP of 90%. 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. If for a given reader that falls into the promising zone, the maximum increase to 300 enrolled subjects still does not yield 90% CP, the sample size may still be increased to 300 for this reader to maximize conditional power, at the discretion of the independent statistician). If (1), (2), and (3) above are not satisfied, the study will continue to its original planned sample size of 150 enrolled subjects. This approach to sample size increase does not inflate the overall Type I error for a given reader.

The 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 only be to keep sample size as is or to increase enrolled sample size to a given value; the reason for the recommendation will not be given to the sponsor.

8.7 Determination of Sample Size

See Section 8.2.1 on the primary efficacy endpoint for the determination of sample size.

9 ADMINISTRATIVE CONSIDERATIONS

9.1 Investigators and Study Administrative Structure

Each Investigator is responsible for conducting the study in accordance with the protocol, all applicable laws, regulations and ICH-GCP.

Lantheus Medical Imaging is the Sponsor of this study and is responsible for overseeing its conduct. Study-specific information regarding the imaging procedures will be described in the Imaging Manual.

9.2 IRB or IEC Approval

It is the Investigator's responsibility to ensure that all aspects of the ethics review are conducted in accordance with the current ICH and GCP guidelines, and/or in accordance with local laws, whichever provides the greatest level of protection for the study subjects. The protocol and any information supplied to the patient to obtain informed consent, including written ICF(s), patient recruitment procedures (e.g., advertisements) and written information (e.g., information leaflets), must be reviewed and approved by a qualified IRB/IEC prior to enrolling subjects in the study. Before study initiation, the Sponsor must receive documentation of the IRB/IEC approval, which specifically identifies the study/protocol, and the assurance number or a list of the committee members.

Amendments to the protocol and revisions to the IC must also be submitted to and approved by the IRB/IEC.

At intervals required by the IRB/IEC, but not less than annually, the Investigator must submit to the IRB/IEC a progress report with a request for re-evaluation and re-approval of the study. A copy of the progress report and re-approval of the study must be sent to the Sponsor.

When the Sponsor provides the Investigator with a Safety Report, the Investigator must promptly forward a copy to the IRB/IEC.

After completion or termination of the study, the Investigator must submit a final report to the IRB/IEC and forward a copy to the Sponsor. As part of the record retention requirements for the study, the Investigator must maintain documentation of all submissions, correspondence and approvals to and from the IRB/IEC.

9.3 Ethical Conduct of the Study

This study will be conducted in conformance with all applicable country or local requirements regarding ethical committee review and informed consent using the guidelines

for the protection of the rights and welfare of subjects participating in biomedical research, as described in the following documents:

- US Code of Federal Regulations (CFR) dealing with clinical studies (21 CFR including parts 50 and 56 concerning informed consent and IRB US regulations)
- Good Clinical Practice: Consolidated Guidance (ICH-E6 R2)

By signing the protocol, the investigator(s) will agree to adhere to the instructions and procedures described in it and thereby to adhere to the principles of GCPs to which it conforms.

9.4 Patient Information and Consent

Informed consent must be obtained from each patient prior to entering the study, and must meet the requirements as defined by the ICH GCP guideline, US Federal Regulations and/or conform to local laws, whichever provides the greatest level of protection. Each patient must be provided, in an understandable manner, written and verbal information that describes the nature and duration of the study. Additionally, the patient must be allowed adequate time to consider the potential risks and benefits associated with his/her participation in the study. The IC must also contain language that allows the Sponsor (or designated representative), regulatory authorities, and the IRB/IEC direct access to the patient's medical records for the purpose of review and copying.

The ICF must be signed and dated by the study patient. In situations where a study patient is not legally competent to provide consent (i.e., a mentally incapacitated patient), written consent must be obtained from an authorized guardian or representative. In these situations, the consent form must be signed and dated by a witness and, if possible, assent must be obtained from the patient. The ICF must also be signed and dated by the individual obtaining the IC.

The Investigator, as part of the study documentation, must retain the original signed and dated ICF and must provide a signed copy to each patient or authorized representative. The Investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

9.5 Patient Confidentiality

The Sponsor affirms and upholds the principle of the patient's right to protection against invasion of privacy.

9.6 Study Monitoring

Each study will be monitored by qualified representatives of the Sponsor. Monitoring visits provide the Sponsor with the opportunity to: evaluate the progress of the study; verify the accuracy and completeness of eCRFs; assure that all protocol requirements, applicable laws and/or regulations and Investigator obligations are being fulfilled; and resolve any inconsistencies in the study records. The Investigator must allow the Sponsor's representatives direct access to periodically review, at mutually convenient times during the study and after the study has been completed, all eCRFs and office, hospital, and laboratory records supporting the participation of each patient in the study. In the case where medical records are stored electronically, direct access to the relevant records relating to the clinical trial including past medical history must be provided to the study monitor, auditors or other representatives of the sponsor. eCRFs and supporting documentation of the study conduct must be kept up-to-date and available for each monitoring visit.

Deviations from the protocol with regard to patient enrollment, study conduct or in any other category will be recorded. The Investigator is responsible for abiding by the IRB/EC's rules and regulations for reporting protocol deviations. The Sponsor may adjust the monitoring schedule for more frequent visits to allow more prompt collection of the data; provide continued training to the study center personnel on an as needed basis; and/or to follow up on any issues identified at the study center.

Details of the monitoring activities will be described in the Clinical Monitoring Plan.

9.7 Case Report Forms and Study Records

Data reflecting the patient's participation in the study and experiences with the study treatment must be reported by the Investigator to the Sponsor. These data must be recorded on eCRFs or other media approved by the Sponsor. For rules regarding completion and correction of eCRFs, see the eCRF completion guidelines that accompany the eCRFs. eCRFs must be reviewed, electronically signed and dated by the Investigator or Sub-investigator and must be submitted in a timely manner to the Sponsor. Significant delays in submission of study data to the sponsor may result in a site being placed on enrollment hold.

Study documentation includes all eCRFs; Safety Reports received from the Sponsor; SAE reports sent to the Sponsor; data correction forms; source documents; monitoring logs; Sponsor-Investigator correspondence; protocols and amendments; clinical supplies receipts; dispensing and final disposition records; IRB/IEC correspondence and approvals; signed consent forms; and Statement of Investigator forms.

Source documents include all original records of observations, results and activities necessary to reconstruct and evaluate the study. Source documents include, but are not limited to, laboratory reports, electrocardiogram tracings, X-ray films, ultrasound

photographs, patient diaries, patient progress notes, hospital charts, appointment books, radiology reports or pharmacy records, and any other records or reports of procedures performed during the study. Source documents also may include copies of the eCRF or Sponsor supplied worksheets when original information is recorded directly onto these forms.

Whenever possible, an original recording of an observation must be retained as the source document. However, a photocopy of a record is acceptable provided it is legible and is a verified copy of the original document.

9.8 Data Monitoring Committee

No DMC will be formed for this study.

9.9 Protocol Violations/Deviations

The Investigator will not intentionally deviate from the protocol without prior written agreement from the Sponsor and the IRB/IEC except when necessary to eliminate an immediate hazard(s) to study subjects. The investigator must take all possible steps to avoid unintentional deviations.

All deviations, whether intentional or unintentional, must be documented and submitted to the appropriate parties (Sponsor, IRB/IEC, etc.) as required by applicable regulation or local practice.

9.10 Access to Source Documentation

In compliance with international and federal (US) regulations relative to monitoring clinical studies and the fulfillment of its obligation to verify compliance with this protocol, the Sponsor will require the investigator to permit the Sponsor's representatives and, when necessary, representatives of the FDA and other regulatory authorities, to review and/or copy any or all portions of the patient medical records relevant to this study. The potential for disclosure of patient's confidential medical information will be included in the informed consent form signed by all subjects in the study.

At some point during the study, individuals from the Sponsor's Quality group or their authorized representative may visit the Investigator's site to conduct an audit of the study. The purpose of this visit will be to determine the Investigator's adherence to the protocol, applicable regulations and the Sponsor's procedures, in addition to assessing the accuracy of the study data. Before initiating this audit, the Investigator will be contacted by the Sponsor to arrange a convenient time for this visit. The Investigator and staff are expected to cooperate with the auditors and allow access to all patient records supporting the eCRFs and other study-related documents.

9.11 Data Generation and Analysis

All database and data management activities will be handled by a CRO contracted by LMI using qualified and validated database systems. At scheduled monitoring visits, eCRFs will be verified against source documentation and submitted as final data. Any subsequent changes to the eCRFs must be performed in accordance with the CRO's standard operating procedures for editing and clarifying eCRFs. Data entry will be performed by the sites using an electronic data capture (EDC) system. Comment fields on the eCRFs will be used as a means of clarification and communication between the investigator and the sponsor; however, comments entered in these fields will not be edited or clarified.

9.12 Retention of Data

The Investigator must ensure that all records pertaining to the conduct of the clinical study, including signed eCRFs, informed consent forms, drug accountability records, source documents and other study documentation are adequately stored for the required time period to allow for review and reconstruction of the study. This documentation must be retained for:

- 2 years following approval of the last marketing application that the data was used to support; or
- 2 years after formal discontinuation of the clinical development program; or
- a record retention period mandated by any national and/or local laws, regulations, and customs.

The Investigator must not destroy any records associated with the study without receiving written approval from the Sponsor. The Investigator must notify the Sponsor in the event of accidental loss or destruction of any study records. If the Investigator leaves the institution where the study was conducted, the Sponsor must be contacted to arrange alternative record storage options.

9.13 Financial Disclosure

All Clinical Investigators participating in clinical studies subject to FDA Regulation 21 CFR Part 54 – Financial Disclosure by Clinical Investigators are required before study initiation to submit a completed Clinical Investigator Financial Certification/Disclosure Request Form that sufficiently details any financial interests and arrangements that apply. For the purpose of this regulation, Clinical Investigator is defined as any Investigator or Sub-investigator who is directly involved in the treatment or evaluation of research subjects, including the spouse and any dependent child of each Investigator and Sub-investigator. These requirements apply to both US and foreign Clinical Investigators conducting covered clinical studies.

The Clinical Investigators will also be reminded that they must report any changes in their financial information regarding significant equity interests and significant payments for the duration of the trial, and for a period of 1 year after completion of their participation in the covered clinical study. At the conclusion of the study, the Clinical Investigators may, again, be required to submit a completed Clinical Investigator Financial Certification/Disclosure Request Form to document any changes in financial interests.

9.14 Publication and Disclosure Policy

All information collected during this study will be regarded as confidential. Investigators must obtain written permission from the Sponsor prior to disclosure or publication of data obtained during the conduct of this study. Manuscripts prepared for publication will be submitted to the Sponsor for review and comment prior to submission to a publisher, in accordance with policies set forth in the Clinical Trail Agreement signed by the Principal Investigator.

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Appendix 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]Patient 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.

Appendix 2 Dose Preparation and Administration Guide