STATISTICAL ANALYSIS PLAN

Version 2.0, 2020-12-07

Protocol Title: A Multicenter, Open-Label, Dose Ascending Study to Evaluate the Safety of NH002 as a Contrast Agent in Cardiac Echocardiography

Protocol No.: NH002-LV
Protocol Version: 1.0

Trust Bio-sonics, Inc.
Room D105, No. 2, Sec. 2, Shengyi Rd.
Zhubei City, Hsinchu County 30261
Taiwan (R.O.C.)

Table of Contents

Version history

This Statistical Analysis Plan (SAP) for study NH002-LV is based on the protocol version 1.0.

Table 1 SAP Version History Summary

SAP Version	Approval Date	Change	Rationale
1	2020-08-31	Not Applicable	Original version
2		1. Remove Section 5.5.1 LVEBD Score for Subjects with Suboptimal LVEBD (LVEBD Score < 14 and at Least 4 Segments with a Delineation Rating of +1 or +2) at Baseline. 2. Add Section 7.2.2 External Data Transfer.	1. According to the protocol, the Suboptimal LVEBD at Baseline is as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view, as assessed by the blinded central reader(s). 2. The external data is transferred by [redacted] Inc. and Trust Bio-sonics, Inc.

1. Introduction

This Statistical Analysis Plan (SAP) contains a more technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes detailed procedures for executing the statistical analysis of the primary and secondary variables and other data. The SAP is finalized and signed prior to hard lock of the database.

1.1. Flow Chart and Visit Schedule

	Screening	**Dosing Period**								**Safety Follow-up**	
		Day 1								*Day 4*	*Day 7*
	≤ 28 Days before Day 1, unless otherwise specified	Before dosing (t)	-5 min	t=0	+10 min	+30 min	+60 min	+120 min	+240 min	72 hours after EOT	7 days after EOT
Time window	N/A	N/A	N/A	(+2 min[2])	(+2 min[2])	+5 min	+5 min	+5 min	+5 min	+3 days	+3 days
Informed consent	X										
Eligibility review	X	X									
Demography	X										
Medical history (signs/symptoms)	X	X									
Vital signs[1]	X	X	X----------X			X	X	X			
Height (cm)	X										
Weight (kg)	X	X									
Standard 12-lead ECG	X	X			X[2]	X	X				
Modified 12-lead ECG			X[3]---------- X[3]								
PE	X[4]	X[5]									
Contrast agent IV bolus injection				X							
Imaging			X[6]---------- X[6]								
Pregnancy test(s) (WoCBP only)	X[7]	X[7]									
CBC (with differential)[8]		X					X	X	X		
TT and aPTT		X					X	X	X		
Metabolic panel[9]		X					X	X	X		
Cardiac troponin I[10]		X							X		
Pulse oximetry: SpO_2		X	X----------X			X	X	X			
Telephone call										X	X
Adverse events		X----------X								X	X
Concomitant medications	X	X----------X								X	X

NOTES:
1 Vital signs to include body temperature, HR, RR, and BP.
2 The time window of +2 minutes only applies to the standard 12-lead ECG assessment(s) scheduled or conditionally triggered within 10 minutes after dosing.
3 If any clinically significant ECG changes indicative of arrhythmia or S-T segment change are identified after dosing from the modified 12-lead ECG monitoring, a standard 12-lead ECG examination will be performed subsequently (after the first ECG change occurs). The imaging will be temporarily stopped for the conditionally triggered standard 12-lead ECG examination. All the identified ECG changes from the modified 12-lead ECG monitoring will be recorded in a timely manner. The results of the modified 12-lead ECG will also be included into the subject's study file as reference. Further, safety assessments will be based on the results of the standard 12-lead ECG. If such ECG changes occur during both the modified 12-lead ECG monitoring and the subsequent confirmative standard 12-

lead ECG examination in 2 or more subjects during the study, the protocol will be revised to require a standard 12-lead ECG examination be performed for all subsequent subjects at an earlier post-injection time point when these early ECG changes may potentially occur.
4 Complete PE at screening to include assessments of general appearance, skin (turgor, pallor, cyanosis, erythema, lesions), head (eyes, ear, nose, and throat), neck, lungs and heart (observation, palpation, percussion, auscultation), abdomen, back, lymph nodes, and extremities and a neurological exam.
5 Focused PE (all PEs after screening) to include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments.
6 B-mode echocardiography and contrast-specific ultrasonography will be employed for all pre-injection and unenhanced images, while post-injection images will be acquired through contrast-specific ultrasonography only.
7 Urine or serum (β-HCG) pregnancy test is required for WoCBP at screening; it is *not* required for postmenopausal or surgically-sterilized women. For WoCBP, urine *and* serum pregnancy tests are *both* required before dosing on Day 1.
8 CBC to include HCT, Hgb, WBC with differential, ANC, and PLT.
9 Metabolic panel to include sodium, potassium, chloride, calcium, magnesium, phosphorus, glucose, BUN, creatinine, total protein, albumin, ALP, ALT, AST, total bilirubin, and bicarbonate.
10 Cardiac troponin I levels should be measured before dosing and once again at 4 hours after dosing in all subjects.

1.2. Study Design

This is a phase 1, multicenter, open-label clinical study to evaluate the safety and tolerability of 3 ascending doses of NH002. Up to 36 eligible subjects will be enrolled with sequential allocation to 1 of 3 cohorts with the following intravenous (IV) doses of NH002: 2.5 µl/kg, 5.0 µl/kg, or 10.0 µl/kg. Each patient will undergo an unenhanced ultrasound examination and a NH002 contrast-enhanced examination on the same day at Day 1. Subjects will remain at the clinical site for 240 minutes of observation after NH002 administration.

All AEs will be evaluated during the successive groups of subjects, per dose level. In general, enrollment will be halted and safety data reviewed for termination or continuation with the occurrence of any serious adverse event (SAE) considered at least possibly related to study agent administration (in any 1 subject) or the occurrence of clinically significant toxicity listed in the study protocol in 30% of subjects (within any 1 of the 3 dose levels). See Section 7.5.1.2 in the study protocol for details on stopping criteria for dosing for this trial.

There will be a screening period (28 days before NH002 administration), a dosing period (during Day 1) which will include a single dose administration of NH002, and a 7-day follow-up period for each cohort. Continuous echocardiographic imaging will be performed at specified time points before, during, and after dose administration. The contrast agent will be prepared and administered by designated pharmacy technician(s), study nurse(s), sonographer, or radiologist. Subjects will be observed at a clinical site for at least 240 minutes (i.e., 4 hours) and evaluated for AEs. Subjects will be contacted by phone after the end of treatment at 72 hours (i.e., Day 4 [+3]) and at Day 7 (+3), for a follow-up safety assessment. Any drug-related AE or SAE will be followed until resolution.

High ultrasound mechanical index (MI) values may cause microsphere cavitation or rupture and lead to ventricular arrhythmias. Additionally, end-systolic triggering with high MIs has been reported to cause ventricular arrhythmias. The safety of NH002 at MIs greater than 0.8 will not be evaluated.

1.3. Randomization

This is a phase 1 dose ascending study without the use of an active control as well as a placebo control. No dose or treatment randomization is applicable.

1.4. Objectives and Endpoints

Objectives	Endpoints
Primary	
• To evaluate the safety and tolerability of 3 different ascending doses of NH002.	• Treatment Emergent Adverse event (TEAE) reporting and changes in physical examination findings, vital signs (VS), clinical laboratory evaluations, other laboratory evaluations, oxygen saturation, and electrocardiograms (ECGs).
Secondary	
• To evaluate the preliminary diagnostic efficacy of NH002 as a contrast agent in echocardiography.	• The percentage of subjects with moderate or complete LVO, defined by an LVO grade of 2 (moderate) or 3 (complete), as assessed by the blinded central reader(s); • The percentage of subjects with complete LVO, defined by an LVO grade of 3 (complete), as assessed by the blinded central reader(s); • The change from baseline on the left ventricular endocardial border delineation (LVEBD) score, defined using a standard 12-segment model, as assessed by the blinded central reader(s). The LV endocardium of the 4- or 2-chamber apical views are divided into 6 segments, with 2 basal, mid- and apical segments in each view. For each segment, LVEBD is graded as follows: 0 = inadequate border (border not visible); 1 = sufficient (border barely visible); 2 = good (border clearly visible). A total delineation score (0-24) is obtained by adding the scores from the 6 individual segments in each of the 2 views; • The changes from baseline on LVEBD score of subjects with suboptimal LVEBD at baseline, as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view, as assessed by the blinded central reader(s);

	• The duration of clinically useful contrast calculated by measuring the time between the disappearance of shadowing effect (useful effect starts) and the time when moderate or full LV enhancement and contrast enhancement are no longer adequate (useful effect ends), as assessed by the blinded central reader(s).

2. Statistical Hypotheses

Not applicable.

3. Sample Size Determination

It is anticipated that 36 subjects will be enrolled (with up to 12 subjects per NH002 dose). Each subject will receive 1 dose of NH002. If a subject is withdrawn from the study, the subject may be replaced with another subject assigned to the same dose, except for those who are discontinued due to AEs, as necessary. Further subjects may be enrolled at a given dose level if additional data are necessary to establish safety and tolerability prior to dose escalation. As this is a dose-finding study, a formal sample size calculation was not required

4. Populations for Analysis

In accordance with ICH recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

The Full Analysis Set and Per Protocol Set will be used for summaries of demographic and baseline characteristics and efficacy variables. The Safety Set will be used for summaries of demographic and baseline characteristics and all safety variables.

4.1. Full Analysis Set (FAS)

The Full Analysis Set (FAS) will consist of all enrolled subjects who have completed all study evaluation periods, and received 1 dose injection of NH002.

4.2. Per Protocol Set (PP)

The Per Protocol Set will be a subset of the Full Analysis Set and will include all subjects who completed all study evaluation periods, received 1 dose injection of NH002 and had no major protocol deviations.

4.2.1. Major Protocol Deviations

The protocol deviations defined in Protocol Deviation Handling Plan[1] (PDHP) Version 1.0 will be recorded. All protocol deviations will be discussed between CRO (Medical Monitor, Data Manager, Biostatistician) and the Sponsor and classified as “minor” or “major”. Major protocol deviations leading to the exclusion of a patient from the PP Set will be discussed between CRO (Medical Monitor, Data Manager, Biostatistician) and the Sponsor. Protocol Deviations will be assessed prior to database lock.

4.3. Safety Set (SS)

The Safety Set (SS) will consist of all subjects receiving at least 1 injection of NH002.

5. Statistical Analyses

5.1. General Considerations

The safety and efficacy variables of each dose of NH002 will be presented using descriptive statistics. For continuous variables, the descriptive statistics (N, mean, standard deviation, median, minimum, and maximum) will be presented for safety variables, and the descriptive statistics (N, mean, standard deviation, median, minimum, maximum, and 95% CI) will be presented for efficacy variables. For categorical variables, the number and percentage of subjects in each category will be presented. Data will be summarized for each dose group and overall.

All data processing, summarization, and analyses will be performed using SAS Version 9.4.

5.1.1. Baseline Definition

For each analysis, the baseline value will be a non-missing value obtained from each assessment before dosing on Day 1. If baseline data is missing no derivation will be performed and will be set to missing.

5.2. Participant Dispositions and Demographics

5.2.1. Disposition of Subjects

The following subject data will be summarized and presented:

- Number of subjects screened for overall
- Number of subjects re-screened for overall
- Number of subjects received injection for each dose group and overall
- Number and percentage of subjects who received injection completed the study phase
- Number and percentage of subjects who received injection did not complete the study phase
- Number and percentage of subjects in each analysis set.
- Number and percentage of the reason for subjects excluded from PP analysis set.
- Number and percentage of Major Protocol Deviations leading or not leading to exclusion from PP.
    - Table 01.1: Summary of Subject's Disposition, All Screened Subjects
    - Table 01.2: Incidence of Major Protocol Deviations, All Screened Subjects
    - Listing 01.1: Subject's Disposition, All Screened Subjects
    - Listing 01.2: Discontinued Patients, All Screened Subjects
    - Listing 01.3: Inclusion/Exclusion Criteria Not Met, All Screened Subjects
    - Listing 02: Protocol Deviations, All Screened Subjects

- Listing 03: Exclusion from Analysis Population, Safety Set

5.2.2. Demographic and Other Baseline Characteristics

Demographic and baseline characteristics include age, sex, height, weight, BMI and subjects with suboptimal LVEBD. Age, sex, and height will be collected once at screening visit, and weight will be measured twice at screening visit and before dosing on Day 1. The baseline weight will be the measurement on Day 1. The Body Mass Index (BMI) will be derived by weight at baseline and height. These variables data will be summarized by each cohort and overall for the SS, the PP and the FAS population.

Age=(Date of ICF signed-Date of Birth)/365.25, rounded down to the integer.

BMI (kg/m^2)=Body Weight (kg)/(Height (cm)/100)^2, rounded to the first decimal place.

Descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be presented for continuous variables and the number and percentage of subjects in each category will be presented for categorical variables. No formal testing of demographic or baseline characteristics will be performed.

The number and percentage of subjects with at least one medical history will be tabulated by each dose group and overall. The medical history will be summarized by system organ class and preferred terms in Medical Dictionary for Regulatory Activities (MedDRA) Version 22.0. Also, medical history data will be listed with start date and ongoing at the start of study drug.

- Table 02.1.1: Summary of Demographics and Baseline Characteristics, Safety Set
- Table 02.1.2: Summary of Demographics and Baseline Characteristics, Per Protocol Set
- Table 02.1.3: Summary of Demographics and Baseline Characteristics, Full Analysis Set
- Table 02.2: Summary of Medical History, Safety Set
- Listing 04.1: Demographics and Baseline Characteristics, All Screened Subjects
- Listing 04.2: Medical History, All Screened Subjects

5.2.3. Concomitant Medication

Concomitant medication will be listed by subject identity, drug name, indication, dose, unit, frequency, route, start date and end date.

- Listing 05.1: Concomitant Medications, All Screened Subjects

5.2.4. Exposure

The study drug administration will be listed by subject identity, dosing date, drug activation time, start dosing time, end dosing time and study drug dose.

- Listing 05.2: Study Drug Administration, Safety Set

5.3. Primary Endpoints Analysis

This is a Phase 1 study to evaluate the safety and tolerability of 3 ascending doses of NH002. Therefore, the primary endpoints are all safety assessment.

All safety assessments, including Treatment Emergent Adverse event (TEAEs), physical examination findings, vital signs (VS), clinical laboratory evaluations, other laboratory evaluations, oxygen saturation and electrocardiograms (ECGs) where indicated, will be presented using descriptive statistics for each dose of NH002. Data will be summarized for each dose group and overall.

5.3.1. Adverse Events

Adverse events recorded before injection, during injection and imaging, and post-injection. Treatment emergent adverse events (TEAEs) are those with start date on or after dosing or those worsen after dosing.

If the minutes of onset time is missing and the onset date and hour of onset time are on or after that of dosing date, the adverse event will be considered as a TEAE.

If the onset time is missing and the onset date is on or after that of dosing date, the adverse event will be considered as a TEAE.

If the onset date is a partial date (month/year) and month/year occurs on or after that of dosing date, the following cases will be considered:

- If month/year of the onset date is greater than the month/year of dosing date, the adverse event will be considered as a TEAE;
- If month/year of the onset date is equal to the month/year of dosing date, and the stopped date is on or after dosing date, the adverse event will be considered as a TEAE;
- If month/year of the onset date is equal to the month/year of dosing date, and the stopped date is a partial date, the adverse event will be considered as a TEAE.

For safety analyses, the number and percentage of subjects with at least one TEAE will be tabulated by each dose group and overall. The TEAEs will be summarized by system organ class and preferred terms in Medical Dictionary for Regulatory Activities (MedDRA) Version 22.0. Similar summaries will also be provided for related TEAEs, serious TEAEs, treatment related serious TEAEs, TEAEs that lead to death and institution of concomitant medication.

For the summary of TEAEs by severity, if a subject experienced more than one episode of an adverse event, the subject is counted only once within a preferred term by using the most severe episode. If a subject experienced more than one adverse event within a system organ class, the subject is counted once for each preferred term and once for the system organ class.

The related TEAEs which are defined as the causal relationship in “Very Likely/Certain”, “Probably”, and “Possible” will also be summarized by each dose group and overall.

- Table 03.1.1: Overview of Treatment Emergent Adverse Events, Safety Set
- Table 03.1.2: Summary of Treatment Emergent Adverse Events, Safety Set
- Table 03.1.3: Summary of Treatment Emergent Adverse Events with Severity, Safety Set
- Table 03.1.4: Summary of Related Treatment Emergent Adverse Events, Safety Set
- Table 03.1.5: Summary of Treatment Emergent Adverse Events Leading to Death, Safety Set
- Table 03.1.6: Summary of Treatment Emergent Serious Adverse Events, Safety Set
- Table 03.1.7: Summary of Related Treatment Emergent Serious Adverse Events, Safety Set
- Table 03.1.8: Summary of Treatment Emergent Adverse Events Institution of Concomitant Medication, Safety Set
- Table 03.2.1: Listing for Treatment Emergent Adverse Events Leading to Death, Safety Set
- Table 03.2.2: Listing for Treatment Emergent Serious Adverse Events Safety, Set
- Table 03.2.3: Listing for Treatment Emergent Adverse Events Institution of Concomitant Medication, Safety Set
- Listing 07: Adverse Events All Screened Subjects

5.3.2. Laboratory Evaluation

5.3.2.1. Clinical Laboratory Evaluation

The clinical laboratory evaluations including hematology and clinical chemistry will be collected before dosing, 60 minutes, 120 minutes and 240 minutes after dosing at dosing period.

- **Hematology**

Hematology include Hematocrit, Hemoglobin, RBC, MCV, MCH, MCH concentration, ANC, WBC, Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes, and Platelet. ANC will be derived by the following formula:

ANC=WBC (*10^3/mm3)* Neutrophils (%)*10

- **Clinical Chemistry**

Clinical Chemistry include creatinine, BUN, AST, ALT, ALP, total bilirubin, albumin, total protein, sodium, potassium, chloride, glucose, bicarbonate, calcium, magnesium, and phosphorus.

The results of clinical laboratory evaluations will be summarized using mean, standard deviation, median, minimum and maximum for each dose group and overall at each time point during

dosing period. Additionally, a within-subject change will be calculated as the post-baseline measurement minus the baseline measurement and summarized after dosing. Therefore, the change from baseline will also be summarized.

The abnormal laboratory result will be noted as [L] and [H] for lower and for higher than the reference ranges, respectively, which supplied by the local laboratories. It will be displayed in listing. Shift tables will be produced by the non-missing results to compare the baseline with post-injection.

Abnormal laboratory results will be listed in Table 03.3.1 and Table 03.3.2.

- Table 03.3.1: Listing for Abnormal Complete Blood Count Values, Safety Set
- Table 03.3.2: Listing for Abnormal Metabolic Panel Values, Safety Set
- Table 04.1: Summary of Complete Blood Count, Safety Set
- Table 04.2: Shift Table of Complete Blood Count, Safety Set
- Table 05.1: Summary of Metabolic Panel, Safety Set
- Table 05.2: Shift Table of Metabolic Panel, Safety Set
- Listing 08.1: Complete Blood Count Part 1, Safety Set
- Listing 08.2: Complete Blood Count Part 2, Safety Set
- Listing 08.3: Metabolic Panel Part 1, Safety Set
- Listing 08.4: Metabolic Panel Part 2, Safety Set
- Listing 08.5: Metabolic Panel Part 3, Safety Set

5.3.2.2. Other Laboratory Variables

Screening for pregnancy will be performed (serum or urine β-HCG at screening and serum and urine β-HCG before dosing on Day 1, for WoCBP only). A Detailed listing of all pregnancies will be provided.

Other laboratory variables collected are:

- **Coagulation studies of TT and aPTT**

Coagulation studies including Thrombin Time (TT) and Activated Partial Thromboplastin time (aPTT) will be measured before dosing, 60 minutes, 120 minutes and 240 minutes after dosing at dosing period.

- **Cardiac troponin I/Cardiac troponin T**

Cardiac troponin I/Cardiac troponin T will be measured before dosing and 240 minutes after dosing at dosing period.

The results of Coagulation studies and Cardiac troponin I/Cardiac troponin T will be summarized by the descriptive statistics (n, mean, standard deviation, median, minimum and maximum) for

each dose group and overall at each time point during dosing period. Also, the change from baseline will be summarized.

The abnormal laboratory result will be noted as [L] and [H] for lower and for higher than the reference ranges, respectively, which supplied by the local laboratories. It will be displayed in listing. Shift tables will be produced to compare the baseline results with post-injection. Abnormal laboratory results will be listed in Table 03.3.3, Table 03.3.4 and Table 03.3.5. The results of pregnancy tests will be shown in Listing.

- Table 03.3.3: Listing for Abnormal Coagulation Studies Values, Safety Set
- Table 03.3.4: Listing for Abnormal Cardiac Troponin I Values, Safety Set
- Table 03.3.5: Listing for Abnormal Cardiac Troponin T Values, Safety Set
- Table 06.1: Summary of Coagulation Studies, Safety Set
- Table 06.2: Shift Table of Coagulation Studies, Safety Set
- Table 07.1.1: Summary of Cardiac Troponin I, Safety Set
- Table 07.1.2: Summary of Cardiac Troponin T, Safety Set
- Table 07.2.1: Shift Table of Cardiac Troponin I, Safety Set
- Table 07.2.2: Shift Table of Cardiac Troponin T, Safety Set
- Listing 08.6: Coagulation Studies, Safety Set
- Listing 08.7.1: Cardiac Troponin I, Safety Set
- Listing 08.7.2: Cardiac Troponin T, Safety Set
- Listing 08.8: Pregnancy Test, Safety Set

5.3.3. Vital Signs

Vital signs (BP, body temperature, RR, and HR) will be recorded at screening, before injection, during injection and imaging (beginning approximately 5 minutes prior to and continuing until 10 minutes after injection), and 30, 60, and 120 minutes post-injection, in a standardized manner. Blood pressures (SBP and DBP) will be measured only at 5 time points for every 2 minutes within 10 minutes after injection. Body temperature will be only collected twice at 5 minutes and 10 minutes within 10 minutes after injection.

The results of vital signs will be summarized using mean, standard deviation, median, minimum and maximum for each dose group and overall at screening visit and each time point during dosing period. The baseline for the vital signs will be the last measurement before dosing. Also, the change from baseline of the vital signs will be calculated as the post-baseline measurement minus the baseline measurement and summarized after dosing.

- Table 08: Summary of Vital Signs, Safety Set

- Listing 08.9: Vital Signs, Safety Set

5.3.4. Pulse oximetry

SpO_2 will be assessed pre-injection, during injection and imaging (beginning approximately 5 minutes prior to and continuing until 10 minutes after), and at 30, 60, and 120 minutes post-injection.

The result of SpO_2 will be summarized using mean, standard deviation, median, minimum and maximum for each dose group and overall at each time point during dosing period. Also, the change from baseline of SpO_2 will be summarized after dosing.

- Table 11: Summary of Pulse Oximetry, Safety Set
- Listing 08.12: Pulse Oximetry, Safety Set

5.3.5. Physical Examination

At screening, a complete physical examination will be performed. A focused physical examination including skin and cardiopulmonary assessments will be performed before dosing and during dosing at the following time points after contrast injection: 30, 60, and 120 minutes post-injection. Each focused PE assessment will be scored as either normal or abnormal. The baseline will be the last assessments before dosing. Any changes with respect to the baseline will be recorded and assessed.

Number and percent of subjects with normal and abnormal skin and cardiopulmonary conditions in physical examination will be tabulated. Shift tables for all categories of non-missing physical examination results will be produced to compare baseline results with post-injection results.

Physical examination with abnormal findings will be provided in a listing. No statistical analyses will be performed.

- Table 09.1: Summary of Physical Examination, Safety Set
- Table 09.2: Shift Table of Physical Examination, Safety Set
- Listing 08.10.1: Physical Examination Part 1, Safety Set
- Listing 08.10.2: Physical Examination Part 2, Safety Set
- Listing 08.10.3: Physical Examination Abnormal Findings, Safety Set

5.3.6. Electrocardiograms (ECGs)

Standard 12-lead ECGs will be performed at screening, before dosing on Day 1, and at 10, 30, and 60 minutes post-injection. Safety assessments will be based on the results of the standard 12-lead ECGs. The ECG parameters include QTc, QT, and HR and ECG interpretation will be classified as either normal, abnormal but not clinically significant (NCS), or abnormal clinically significant (CS).

In addition, modified 12-lead ECG monitoring will be performed during imaging to prevent the ECG electrodes from affecting the accessibility of the optimal imaging windows of echocardiography. Imaging can be performed with a modified 12-lead ECG, by repositioning the V5-V6 leads upward. If any clinically significant ECG changes indicative of arrhythmia or S-T segment change are identified within 10 minutes after dosing from the modified 12-lead ECG monitoring, an additional standard 12-lead ECG examination will be performed subsequently when the earliest ECG change occurs.

The descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) of the ECG parameters at screening visit and each time point during dosing period including changes from baseline will be summarized and presented by each dose group and overall. The baseline will be the last measurements before dosing.

Number and percent of subjects with normal, abnormal, NCS and abnormal, CS results for the 12-lead ECG interpretation will be tabulated by each dosing group and overall. A shift table showing the changes of non-missing results in normal, abnormal, NCS and abnormal, CS from baseline to each post-baseline measurement will be displayed.

- Table 10.1: Summary of 12-Lead ECG Parameters, Safety Set
- Table 10.2: Summary of 12-Lead ECG Interpretation, Safety Set
- Table 10.3: Shift Table of 12-Lead ECG Interpretation, Safety Set
- Listing 08.11: 12-Lead ECG Test, Safety Set

5.4. Secondary Endpoints Analysis

5.4.1. Secondary Efficacy Endpoints

5.4.1.1. Definition of endpoints

- The percentage of subjects with moderate or complete LVO, defined by an LVO grade of 2 (moderate) or 3 (complete), as assessed by the blinded central reader(s);
- The percentage of subjects with complete LVO, defined by an LVO grade of 3 (complete), as assessed by the blinded central reader(s);
- The change from baseline on the LVEBD score, defined using a standard 12-segment model, as assessed by the blinded central reader(s). The LV endocardium of the 4- or 2-chamber apical views are divided into 6 segments, with 2 basal, mid-, and apical segments in each view. For each segment, LVEBD is graded as follows: 0 = inadequate border (border not visible); 1 = sufficient (border barely visible); 2 = good (border clearly visible). A total delineation score (0-24) is obtained by adding the scores from the 6 individual segments in each of the 2 views.
- The changes from baseline on LVEBD score of subjects with suboptimal LVEBD at baseline, as defined by 2 or more contiguous segments of 6 segments that cannot be

visualized reliably in either the apical 4- and/or the 2-chamber view, as assessed by the blinded central reader(s);

- The duration of clinically useful contrast calculated by measuring the time between the disappearance of shadowing effect (useful effect starts) and the time when moderate or full LV enhancement and contrast enhancement are no longer adequate (useful effect ends), as assessed by the blinded central reader(s).

5.4.1.2. Main analytical approach

- LVO Grade

A pair of apical 4-chamber and 2-chamber view images in each of the pre-injection and post-injection imaging sessions will be subjected to LVO grade assessment by central reading.

The highest LVO Grade among the pair of apical 4-chamber and 2-chamber view images of pre-injection and post-injection will be summarized and presented by each dose group and overall. When the LVO grades of both apical 4-chamber and 2-chamber view images are the same, the LVO grade of the apical 4-chamber view image will be used.

The following descriptive statistics will be summarized and presented:

– Number and percentage of subjects in each LVO Grade.

– Number and percentage of subjects with moderate or complete LVO.

In addition, the bar chart for Cumulative Percent of LVO Grade will be presented by pre-dose and post-dose groups in each cohort and overall.

Two LVO grades obtained for each session along with the corresponding times of images used will be listed.

- Table 12.1.1: Summary of LVO Grade, Full Analysis Set
- Figure 12.1.2: Cumulative Percent of LVO Grade, Full Analysis Set
- Table 12.2.1: Summary of LVO Grade, Per Protocol Set
- Figure 12.2.2: Cumulative Percent of LVO Grade, Per Protocol Set
- Listing 06.1: Image Evaluation - LVO Grade, Safety Set

- LVEBD Score

The descriptive statistics (n, mean, standard deviation, median, minimum, maximum, and 95% CI) of LVEBD score for pre-injection, post-injection and the change from baseline will be summarized and presented by each dose group and overall. LVEBD scores for subjects with suboptimal LVEBD at baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view) will also be summarized.

Moreover, the boxplots of the LVEBD score for pre-injection, post-injection and the change of LVEBD score will be presented by each cohort and overall.

Likewise, for subjects with suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), the boxplots of the LVEBD Score for pre-injection, post-injection and the change of LVEBD score will be presented by each cohort and overall.

- Table 13.1.1.1: Summary of LVEBD Score, Full Analysis Set
- Figure 13.1.1.2: LVEBD Score, Full Analysis Set
- Figure 13.1.1.3: Change of LVEBD Score, Full Analysis Set
- Table 13.1.2.1: Summary of LVEBD Score, Per Protocol Set
- Figure 13.1.2.2: LVEBD Score, Per Protocol Set
- Figure 13.1.2.3: Change of LVEBD Score, Per Protocol Set
- Table 13.2.1.1: Summary of LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Full Analysis Set
- Figure 13.2.1.2: LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Full Analysis Set
- Figure 13.2.1.3: LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Full Analysis Set
- Table 13.2.2.1: Summary of LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Per Protocol Set
- Figure 13.2.2.2: LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Per Protocol Set
- Figure 13.2.2.3: LVEBD Score for Subjects with Suboptimal LVEBD at Baseline (as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view), Per Protocol Set
- Listing 06.2: Image Evaluation - LVEBD Score, Safety Set

- Duration of Clinically Useful Contrast Enhancement

The duration of clinically useful contrast enhancement is the number of minutes between start time point and end time point of useful contrast imaging, rounded to one decimal place.

The formula for duration calculation is as below.

Duration (Minutes)=(End Time (hh:mm:ss) of Useful Contrast Imaging - Start Time (hh:mm:ss) of Useful Contrast Imaging)/60, rounded to one decimal place.

The descriptive statistics (n, mean, standard deviation, median, minimum, maximum, and 95% CI) of the duration of clinically useful contrast enhancement will be presented by each dose group and overall. The unit of duration is minute.

Furthermore, the boxplots of duration of clinically useful contrast enhancement will be presented by each cohort and overall.

- Table 14.1.1: Summary of Duration of Clinically Useful Contrast Enhancement, Full Analysis Set
- Figure 14.1.2: Duration of Clinically Useful Contrast Enhancement, Full Analysis Set
- Table 14.2.1: Summary of Duration of Clinically Useful Contrast Enhancement, Per Protocol Set
- Figure 14.2.2: Duration of Clinically Useful Contrast Enhancement, Per Protocol Set
- Listing 06.3: Image Evaluation - Duration of Clinically Useful Contrast Enhancement, Safety Set

5.5. Analysis of Other Variables

5.5.1. Telephone Call

Telephone Call will be listed by subject identity, visit, day, date of contact, successfully reaching the subject, any new or changed symptoms experienced since last visit, and any new or changed medications used since last visit.

- Listing 09: Telephone Call, Safety Set

5.6. Dose Escalation Committee (DEC)

To enhance the safety and integrity of the study data, a DEC will evaluate clinical, laboratory, and electrocardiographic safety before advancing to the next dose level. It will consist of sponsor personnel, the independent medical monitor, and the investigator(s) at the study site.

A sentinel review approach will be followed whereby the first review will be conducted at the end of the safety follow-up observation period for the first subject treated in Cohort 1 to determine if subsequent subjects may be dosed in the cohort. In order to review all cumulative safety data for Cohort 1, the next review will be conducted at the end of the safety follow-up observation period for the last subject treated in Cohort 1. This sentinel review approach will continue to be followed for all successive cohorts (i.e., Cohort 2 and Cohort 3), in order to review safety data and to provide a recommendation on study continuation, recommended dose, or early termination in case there is a concern regarding safety. This will be repeated until the highest dose level has been evaluated.

The tables and listings for DEC will follow the shells that only present the data of the dose in each cohort.

Further details regarding timelines and specific responsibilities of the DEC will be provided in a separate Charter[2].

5.7. Handling of Missing Data

As all treatments are scheduled in a single study visit, the level of missing data for the primary endpoint is anticipated to be small. Every attempt will be made to avoid missing data. All subjects will be used in the safety analysis, using non-missing data available. No imputation process will be used to estimate/substitute missing data.

6. References

1. Protocol Deviation Handling Plan (PDHP) Version 1.0.
2. The Dose Escalation Committee Charter Version 1.0.
3. NH002-LV_Protocol_v1.0.
4. Data Transfer Specification TRUS-10006193, ECHO Final 3.0, 16 Sep 2020.
5. Specification of Suboptimal Echocardiogram Data Transfer_20201116_TRUST.

7. Appendices

7.1. Appendix 1: List of Abbreviations

- **List of Abbreviations**

Abbreviations	Description of abbreviations
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BMI	Body Mass Index
β-HCG	Beta-Human Chorionic Gonadotropin
CBC	Complete Blood Count
CI	Confidence Interval
CS	Clinically Significant
DBP	Diastolic Blood Pressure
DEC	Dose Escalation Committee
EBD	Endocardial Border Delineation
ECG	Electrocardiogram
EOT	End of Treatment
FAS	Full Analysis Set
HCT	Hematocrit
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IV	Intravenous
LV	Left Ventricle [Ventricular]
LVEBD	Left Ventricular Endocardial Border Delineation
LVO	Left Ventricular Opacification
MCH	Mean Cell Hemoglobin
MedDRA	Medical Dictionary for Regulatory Activities
NCS	Not Clinically Significant
PD	Protocol Deviation
PDHP	Protocol Deviation Handling Plan
PE	Physical Examination
PLT	Platelet Count
PP	Per Protocol
RR	Respiratory Rate
SAE	Serious Adverse Event

Abbreviations	Description of abbreviations
SBP	Systolic Blood Pressure
SpO_2	Peripheral Oxygen Saturation
SS	Safety Set
TEAE	Treatment-Emergent Adverse Events
TT	Thrombin Time
VS	vital signs
WBC	white blood cell

7.2. Appendix 2: Adds to Protocol-Planned Analyses

7.2.1. Re-screened Subjects

For re-screened subjects, information is taken from the last re-screening visit instead of the screening visit.

The number of subjects re-screened are summarized in Table 01.1: Summary of Subject's Disposition.

7.2.2. External Data Transfer

The external data transfer process not described in the study protocol[3] is illustrated as below.

The efficacy data including LVO grade, LVEBD score, and the useful contrast imaging start time/ end time assessed by the blinded central readers (assigned by [redacted] Inc.) are not from the record on CRF but be transferred by [redacted] Inc. Further details are provided in the Data Transfer Specification[4].

For those subjects are suboptimal LVEBD at baseline, as defined by 2 or more contiguous segments of 6 segments that cannot be visualized reliably in either the apical 4- and/or the 2-chamber view, Trust Bio-Sonics, Inc. transfers the Suboptimal Echo Classification Data according to the Specification of Suboptimal Echocardiogram Data Transfer[5].

All external data is cleaned and reconciled to ensure the data correctness.

7.3. Appendix 3: Signatures

Prepared by: _____ Date: _____
Amy Lin (YYYY-MM-DD)
Data Analyst
[redacted] Inc.

Approved by: _____ Date: _____
Sky Chen (YYYY-MM-DD)
Director of Technical Operation
[redacted] Inc.

Reviewed by: _____ Date: _____
Shih-Tsung Kang (YYYY-MM-DD)
Tech Development Manager
Trust Bio-sonics, Inc.