CLINICAL STUDY PROTOCOL

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

Protocol Number:	NH002-LV
EudraCT Number:	Not Applicable
Investigational Product:	NH002 (Perflutren Lipid Microspheres) Injectable Suspension
Phase:	1
Sponsor:	Trust Bio-sonics, Inc. Room D105, No. 2, Sec. 2, Shengyi Rd. Zhubei City, Hsinchu County 30261 Taiwan (R.O.C.)
Protocol Date:	13 Mar 2019
Protocol Version:	1.0

1 PROTOCOL APPROVAL SIGNATURES

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

Protocol Number: NH002-LV

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (cGCP) and applicable regulatory requirements.

Sponsor Signatory

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Signature

Date

2 SYNOPSIS

Protocol Number:

NH002-LV

Title:

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

Investigational Product:

NH002 (Perflutren Lipid Microspheres) Injectable Suspension

Phase:

Phase 1

Objectives:

Primary Objective:

To evaluate the safety and tolerability of 3 different ascending doses of NH002.

Secondary Objective:

To evaluate the preliminary diagnostic efficacy of NH002 as a contrast agent in echocardiography.

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 Section 7.5.1.2 in 30% of subjects (within any 1 of the 3 dose levels). See Section 7.5.1.2 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 (ie, 4 hours) and evaluated for AEs. Subjects will be contacted by phone after the end of treatment at 72 hours (ie, 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.

Number of Subjects:

Up to 36 subjects will be enrolled. If a subject is withdrawn from the study, except for those subjects who will be terminated early due to AEs, the subject may be replaced as necessary with another subject assigned to the same dose. Further subjects may be enrolled at a given dose level if additional data are necessary to establish safety and tolerability prior to dose escalation.

Treatment:

The contrast agent will be administered by IV bolus injection within 30 to 60 seconds, followed by a 10 mL saline flush over 10 seconds. The following dose regimens will be tested: 2.5 µl/kg, 5.0 µl/kg, 10.0 µl/kg.

Subjects will be enrolled with sequential allocation to the NH002 doses. Each patient will undergo an unenhanced ultrasound examination and an NH002 contrast-enhanced examination on the same day at Day 1. Each subject will receive 1 of the following 3 NH002 doses: 2.5 µl/kg, 5.0 µl/kg, 10.0 µl/kg IV bolus. Sequential allocation is as follows, if all 3 dose levels exhibit acceptable toxicity: the first 12 subjects will receive 2.5 µl/kg, the next 12 will receive 5.0 µl/kg, and the final 12 will receive 10.0 µl/kg. (See Section 7.5.1.2 for further details, including the definition of unacceptable toxicity for this trial.) The 12 subjects in each ascending cohort will be enrolled only if no serious adverse reactions or toxicity reactions have been observed within 7 days from completion of the safety observation period for the last subject in the preceding cohort.

A dose escalation committee (DEC) will evaluate clinical, laboratory, and electrocardiographic safety data before advancing to the next higher dose level. If stopping criteria for dosing as defined in Section 7.5.1.2 is observed in a specific cohort, the previous dose level will be considered to be the maximum tolerated dose and no further increases in dose will be included in the study.

Duration of Treatment:

The duration of treatment is expected to last between 4 to 5 hours during 1 day, barring any unexpected adverse reactions.

Study Population:

To be eligible for study entry, subjects must satisfy all of the following inclusion criteria:

1. Male or female subjects 20 years of age or older
2. Ability to understand and the willingness to provide written informed consent
3. Suspected of having cardiac disease or undergoing evaluation of cardiac anatomy for congenital heart disease
4. Negative pregnancy test within 24 hours of the procedure if subject is a woman of childbearing potential

Subjects will be excluded from the study if one or more of the following exclusion criteria is applicable:

1. Known right-to-left, bidirectional, or transient right-to-left cardiac shunt(s)
2. Chronic obstructive pulmonary disease
3. Ejection fraction at screening or baseline < 40%
4. Uncontrolled serious ventricular arrhythmias or at high risk for arrhythmias due to prolongation of the QT interval (QT corrected by Fridericia's formula > 450 msec in males and > 470 msec in females)
5. Clinically significant abnormality in renal and liver function tests (alanine aminotransferase/aspartate aminotransferase > 2 × upper limit of normal [ULN]; serum creatinine > 1.5 × ULN)
6. Known or suspected hypersensitivity to one or more of the ingredients of NH002, perflutren, or other ultrasound contrast agents
7. Received an investigational compound within 30 days before enrolling in the study
8. Received any contrast agent either intravascularly or orally within 48 hours before NH002 administration
9. Pregnant or lactating female (conception during the study should be avoided)
10. Serious medical or psychiatric illness/condition likely, in the judgment of the investigator, to interfere with compliance to protocol treatment/research
11. Clinically unstable cardiopulmonary conditions considered not suitable for participation in the trial, in the judgment of the investigator

12. 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)

Primary Endpoint(s):

- Adverse event reporting and changes in physical examination findings, vital signs, clinical laboratory evaluations, oxygen saturation, and electrocardiograms (ECGs).

Secondary Endpoint(s):

- The percentage of subjects with moderate or complete left ventricular opacification (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 left ventricular (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).

Statistical Analysis:

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.

A Dose Escalation Committee

To enhance the safety and integrity of the study data, a DEC will evaluate clinical, laboratory, and electrocardiographic safety data, routinely before advancing to the next higher dose level/cohort. The DEC 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. 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 (ie, Cohort 2 and Cohort 3), 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.

Statistical Analysis:

The safety assessments, including AEs, ECGs, and clinical laboratory evaluations, where indicated, will be presented using descriptive statistics for each dose of NH002. Data will be summarized for each dose group and overall.

Safety Evaluations

Each subject must be carefully monitored for the development of any AEs throughout the study from the signing of the informed consent form to 7 days after receipt of the dose of study agent (ie, Day 7 Follow-up call). Safety evaluations will be based on 12-lead ECGs, changes in physical examinations performed, including vital signs and changes in laboratory parameters. These safety evaluations will also be assessed and recorded at specified time points: prior to injection, during injection and imaging, and up to 7 days post-injection.

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4 LIST OF ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BP	blood pressure
BUN	blood urea nitrogen
CBC	complete blood count
cGCP	current good clinical practice
CRF	case report form
DEC	Dose Escalation Committee
[redacted]	[redacted]
EBD	endocardial border delineation
ECG	electrocardiogram
FDA	Food and Drug Administration
HB	heart block
HCT	hematocrit
HED	human equivalent dose
HIPAA	Health Insurance Portability and Accountability Act
HR	heart rate
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IRB	institutional review board
IV	intravenous
LV	left ventricle [ventricular]
LVEBD	left ventricular endocardial border delineation
LVO	left ventricular opacification
MCH	mean cell hemoglobin
MI	mechanical index
N	number of subjects in the dataset or population
N/A	not applicable
PE	physical examination
PICC	peripherally inserted central catheter
PLT	platelet count
SpO_2	peripheral oxygen saturation
RR	respiratory rate
SAE	serious adverse event
SUSAR	suspected unexpected serious adverse reaction
TT	thrombin time
TTE	transthoracic echocardiogram
UCA	ultrasound contrast agent
ULN	upper limit of normal
VS	vital signs
WBC	white blood cell

WoCBP	woman/women of childbearing potential

5 INTRODUCTION

5.1 Background

Echocardiography serves as one of the first-line imaging modalities in the evaluation of cardiac disease, mainly due to its ability to evaluate both the anatomy and function of the heart.[1] Echocardiography remains a standard of care due to its portability, accuracy, and cost-effectiveness in the rapid diagnosis and follow-up of most cardiac diseases. Even so, suboptimal left ventricular (LV) cavity visualization and endocardial border delineation (EBD) often compromise the clinical utility of echocardiography.[2]

Improved delineation of endocardial border is associated with more accurate assessments of LV systolic function. The use of contrast enhancement improves echocardiographic assessment of wall motion at rest, and increases the accuracy of LV volume and ejection fraction measurements.[3]

In echocardiography, ultrasound contrast agents (UCAs), when used with contrast-specific imaging techniques have an established role for diagnostic cardiovascular imaging.[4] The clinical need for such agents has risen, as more than 30% of certain populations use echocardiograms that are considered technically difficult or uninterpretable.[5] Ultrasound contrast agents are composed of acoustically-active gas-filled microspheres (microbubbles) which optimize their detection by increasing the signal strength of ultrasound waves.[6]

In October 2007, the US Food and Drug Administration (FDA) imposed additional product label warnings that included serious cardiopulmonary reactions, several new disease-state contraindications, and a mandated 30-minute post-procedure monitoring period for UCAs.[7] These additional warnings were prompted by reports of cardiopulmonary reactions that were temporally related, but were not clearly attributable. Subsequent published reports over the following months established the safety and the improved efficacy of clinical ultrasound applications with UCAs. Ultimately, in October 2011, the FDA further downgraded the warnings after hearing the results of the post-marketing data, which revealed continued safety and improved efficacy.[8]

Since then, UCAs have demonstrated their ability to reduce variability in echocardiography interpretation and also to reduce exposure to the ionizing radiation that is associated with other imaging modalities.[9] There remains an important opportunity to improve the clinical safety and efficacy of UCAs in association with echocardiography. Providing investigators with the option of a more appropriate UCA will potentially improve diagnostic decisions and better serve patients with cardiac conditions.

5.2 **NH002**

NH002 is a UCA developed for use in patients with suboptimal echocardiograms to opacify the LV chamber, and to improve the delineation of the left ventricular endocardial border (LVEBD). Relative to the approved UCAs on the market, NH002 is expected to have a longer half-life, an improved safety profile, and effectiveness at a lower dose.

NH002 is formulated as a microbubble injectable suspension in single-use, 1-mL lipid solution in [redacted] in headspace for intravenous (IV) administration. The active pharmaceutical ingredients API in NH002 are the gas core: perflutren (C_3F_8), [redacted]
For more details regarding the manufacturing process, also refer to the current version of the Investigator's Brochure for NH002.

NH002 also requires an activation process for the perflutren (C_3F_8) and lipids to be mixed and assembled into microbubbles. The activation method required utilizes agitation with the TRANSMIX™ device. TRANSMIX™ is [redacted] and a mechanical agitator for the [redacted] mixing of the lipids solution and C_3F_8 for gas encapsulation.

Prior to activation, the initial C_3F_8 content of NH002 is [redacted]. After activation, the C_3F_8 content encapsulated in microbubble suspension of NH002 is [redacted]

The shell coat of other clinically available microspheres (eg, DEFINITY®) is composed of biodegradable materials (such as galactose, albumin, phospholipids, or polymers) which slow down gas diffusion and dissolution to improve the circulation time in vivo. Currently, phospholipids are the most commonly used shell material. Additionally, lipid coatings oscillate flexibly in response to ultrasound exposure, and thus exhibit excellent acoustic properties.[7]

The formulation of NH002 differs in that its lipid shell is composed of [redacted] According to the *Handbook of Pharmaceutical Excipients*, these lipid materials exhibit relatively low toxicity and good biological tolerance (at doses of g/kg range).[10] Moreover, NH002 contains [redacted], and is thus believed to have minimal additional safety concerns (Table 1).

Table 1: The Phospholipid Materials of NH002

[redacted]

The design of NH002 is focused on the advantage of the lipid shell. The major lipid component used in NH002, [redacted] This could increase the [redacted] It could also [redacted] in vivo by stabilizing the microbubbles.[11]

[redacted]

[redacted] is expected to facilitate the formation and stabilization of smaller bubbles in NH002.[12] This leads to a higher percentage of small bubbles (0.9–1.5 μm) with resonance frequencies falling within the frequency range of clinical diagnostic ultrasound (1–21 MHz) as well as a lower percentage of large particles (> 10 μm) that pose a risk of gas embolism in circulation. A similar imaging performance can therefore be potentially obtained by NH002 at a lower dose and at a lower total gas content when compared to listed agents indicated to market.[13]

Table 2 provides product features of NH002.

Table 2: Product Features of NH002

		NH002
API content (Headspace)		C_3F_8 [redacted]
Volume		1-mL solution in a 2-mL vial
Appearance	*before activation*	clear colorless liquid
	after activation	homogenous, opaque, milky white injectable microsphere suspension
Activation method		Agitation (using TRANSMIX™)
[redacted]		

5.2.1 Nonclinical studies

Nonclinical studies have been conducted to evaluate the pharmacology, toxicology, and genotoxicity of NH002 (Table 3). NH002 appears to have an acceptable safety profile to support the first-in-human study. Preliminary imaging results of the dog studies showed significant enhancement with low-dose NH002 as compared to saline. See the following figures (Figure 1 and Figure 2) for comparison. A pre-injection image in B-mode is provided for additional consideration (see Figure 3).

Figure 1: Imaging Results with Saline in Dog Contrast Mode

Figure 2: Imaging Results with NH002 in Dog (Contrast Mode)

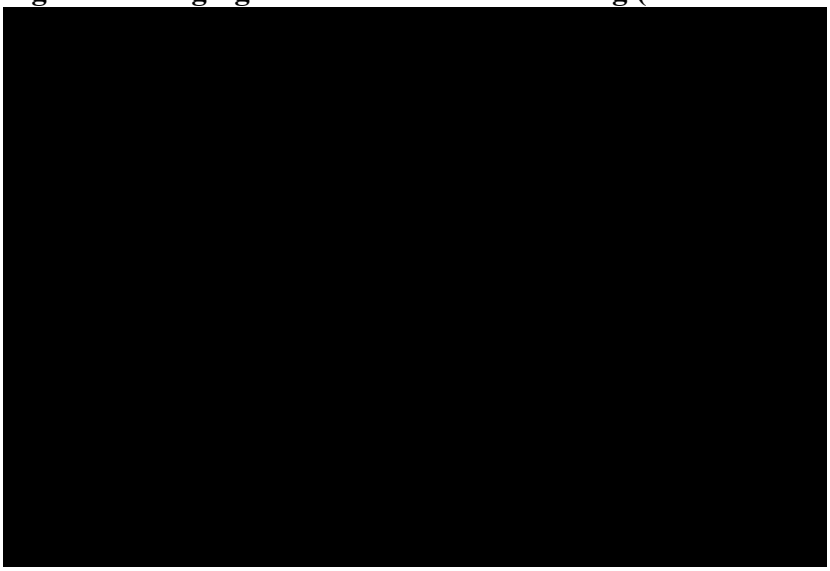


Figure 3: Pre-injection Imaging Results in Dog (B-mode)

For more details regarding the study protocols for the nonclinical studies, refer to the current version of the Investigator's Brochure for NH002.

Table 3: Nonclinical Studies with NH002 Microbubble Injectable Suspension

Study No.	Study Type	Strain/Species	Route/Doses	Study Outcome
Pharmacology				
[redacted]				
Pharmacokinetics				
[redacted]				

[redacted]

Toxicology

[redacted]

Other Studies (Hemolysis)

[redacted]

[redacted]

5.2.2 Clinical studies

The clinical development plan for NH002 relies on the established safety and efficacy for the listed drug, DEFINITY. A perflutren pharmacokinetic comparison of NH002 and DEFINITY showed the concentration of NH002 perflutren (C_3F_8) in blood is expected to be between [redacted] that of DEFINITY, in the same injection volume. Pharmacodynamic studies also show low dose NH002 (Human Equivalent Dose [HED]: 2.5 µL/kg) provides strong contrast enhancement of the LV with an effective life-time imaging of [redacted] The suggested starting dose is 2.5 µL/kg, which is [redacted] of DEFINITY's injection volume (10 µL/kg).

Tested dose levels of NH002 [redacted]) as compared to DEFINITY ([redacted]) showed that all doses could enhance all the endocardial borders significantly. See Figure 4. For details, also refer to the current version of the Investigator's Brochure for NH002.

Figure 4: Enhanced EBD by NH002 as Compared to DEFINITY

[redacted]

Abbreviation: EBD= endothelial border delineation.

The proposed phase 1 study is expected to provide supporting evidence of the safety and efficacy of NH002.

5.3 **Risk/Benefit Assessment**

Perflutren has been used as a UCA in the US since 1997 (Optison™, GE Healthcare, NDA 020899). The safety of perflutren contrast agents for echocardiography has been established in the published literature. A retrospective study in 63,189 adults undergoing transthoracic echocardiograms (TTEs) over 5 years was conducted to examine mortality within 1 day of the procedure. Of the 2518 TTEs that a perflutren contrast agent was used, 10 deaths occurred, and the investigator concluded that the use of contrast enhancement was not associated with increased mortality.[14]

Other studies have concluded both the safety of contrast agents for enhancing imaging during echocardiography, and the outweighing benefit of improved diagnostic capabilities over the relatively low risk of adverse reactions.[14,15,16] In 2013, Platts et al. performed a multicenter retrospective analysis on consecutive patients in Australia who received DEFINITY and were monitored for serious adverse events (SAEs) over a 5-year period.[15] A total of 5956 studies with UCAs were performed, most of which were outpatient stress tests. There were 16 SAEs related to UCAs (0.27%), all of which were mild and transient, with the most common reactions being back pain and rash. There were no cases of serious anaphylaxis or death within 30 minutes of the contrast administration.[15,16]

Since the time of the most recent change to the package labels for both Optison™ and DEFINITY, there have also been several published articles and reviews detailing both the safety and efficacy of UCAs in a variety of patient populations. In 2012, Wever-Pinzon et al. published a retrospective series on 1513 consecutive inpatients with documented pulmonary hypertension who had received UCAs. These patients were followed for 24 hours post-UCA administration for SAEs, including respiratory decompensation, hypotension, arrhythmias, syncope, convulsions, anaphylactic reactions, or death. Out of the 1513 patients, only 3 patients had an SAE after the administration of the UCA, none of these events was directly attributed to the UCA itself.[17]

Kitzman et al, demonstrated adequate-to-full LV opacification (LVO) in the apical 4-chamber view in 87% of their patients given 5 ml/kg of perflutren, 91% of patients given 10 ml/kg of perflutren, and 0% of patients given placebo (p, 0.01 for both perflutren dose groups vs placebo). Similar results were seen in the apical 2-chamber view. Overall, 91% of all patients who received perflutren also showed improved EBD after the injection compared with 12% of patients who received placebo (p, 0.001).[2]

Fifty-six percent of the patients (30 of 53) who received perflutren and who had nondiagnostic echocardiographic images in the apical 4-chamber view were converted to diagnostic echocardiograms. In the overall group, salvage was demonstrated in 48% of patients with nondiagnostic echocardiograms. Improved confidence in image interpretation as determined by the site investigators also occurred in 86% of all patients receiving perflutren. The ability to

detect wall motion abnormalities was considered enhanced in 87% of all patients receiving perflutren. Site investigators judged that the [perflutren] injection provided new information that was likely to result in a change in patient management in 57% of all patients who received perflutren, and to eliminate the need for an additional test in 60% of all patients who received perflutren.[2]

This study has been designed to evaluate the safety and tolerability of NH002 in patients, while minimizing any potential risks. Although the potential benefits in patients are unknown at this time, prior early clinical signs, and nonclinical data provide a sound basis supporting the hypothesis that perflutren microspheres are a complementary diagnostic strategy. Thus, the benefit/risk assessment for this phase 1 study is anticipated to be favorable based on the lack of effective alternative treatments, the limited life expectancy due to malignant disease, and the strength of the scientific hypothesis under evaluation.

6 STUDY OBJECTIVES & ENDPOINTS

6.1 Primary Objective

To evaluate the safety and tolerability of 3 different ascending doses of NH002.

6.2 Primary Endpoint

Adverse event (AE) reporting and changes in physical examination findings, vital signs (VS), clinical laboratory evaluations, oxygen saturation, and electrocardiograms (ECGs).

6.3 Secondary Objective

To evaluate the preliminary diagnostic efficacy of NH002 as a contrast agent in echocardiography.

6.4 Secondary 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 left ventricular EBD (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).

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan: Description

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 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 up to 240 minutes of observation after NH002 administration.

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

There will be a screening period (28 days before NH002 administration), a dosing period (during Day 1) that 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 (4 hours) and evaluated for AEs. Subjects will be contacted by phone after the end of treatment at 72 hours (ie, 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.

In accordance with protocol guidelines for imaging, each study center will follow standard practice for (transthoracic) echocardiographic imaging, pre- and post-dosing. Efficacy evaluations will be based on blinded central echocardiographic imaging assessments.

To enhance the safety and integrity of the study data, a dose escalation committee (DEC) will evaluate clinical, laboratory, and electrocardiographic safety data, routinely before advancing to the next higher dose level/cohort. The DEC 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. 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 (ie, Cohort 2 and Cohort 3), 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. If treatment stopping criteria, as defined in Section 7.5.1.2, is observed in a specific dosing

group, the previous dose will be considered to be the maximum tolerated dose and no further increases in dose will be included in the study.

Proceeding to the next higher dose level is permissible before completion of a cohort when no visible contrast effect and no safety concerns are observed in 4 subjects in a cohort. Such decision will be triggered by the sponsor in conjunction with the DEC, and as approved by the appropriate regulatory agencies.

A more conservative dose assignment, evaluation of an intermediate dose, and/or expansion of an existing dose level is permissible if such measures are needed for patient safety or for a better understanding of NH002 dose-related toxicity, and/or exposure. Such changes will be determined by the sponsor in conjunction with the DEC, and as approved by the appropriate regulatory agencies.

Further, safety assessments will be based on the results of the standard 12-lead ECG assessments. During imaging, modified 12-lead ECG (with repositioning of leads V5-V6 electrodes upward) will also be performed (ie, from the time before dosing to approximately 10 minutes after dosing) for continuous monitoring. If early ECG changes indicative of arrhythmia or S-T segment change 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. See Section 9.1.2.2.7.

7.1.1 Imaging Guidelines

In accordance with protocol guidelines for imaging, each study center will follow standard practice for (transthoracic) echocardiographic imaging and subject preparation. This includes subject positioning and comfort measures, pre- and post-dosing. [redacted]

Refer to Image Evaluation Guidelines for details.

The 2008 American Society of Echocardiography consensus statement on contrast agents in echocardiography advocates a team approach for the successful introduction and implementation of contrast protocols into an echocardiography laboratory.[4] The team would primarily consist of the sonographer, physician echocardiographer, and where available, nursing staff who are competent and certified in obtaining IV access and administration of IV agents.

It is recommended that the sonographers be appropriately qualified and credentialed in echocardiography and the responsible investigators be independent and competent echocardiographers with skills in basic and advanced life support.

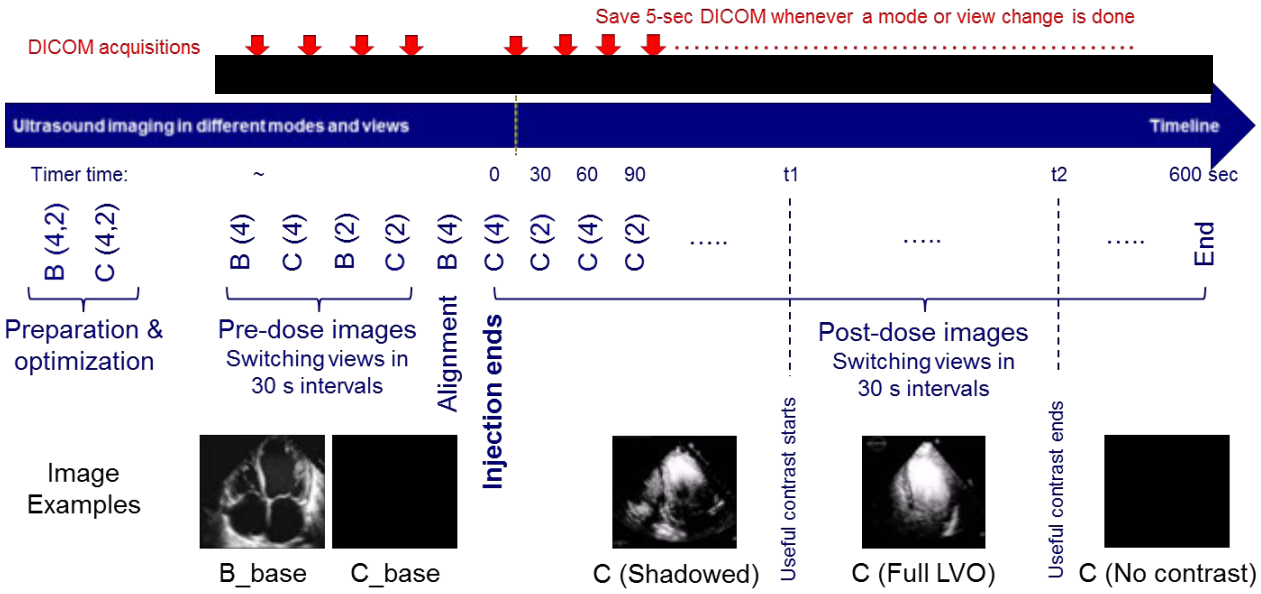
Each subject will undergo an unenhanced ultrasound examination. After unenhanced imaging, subjects will then receive 1 dose of NH002 at the assigned cohort dose level. Before contrast administration, the instrument settings on the ultrasound imaging system, including gain, MI,

focal depth, time gain compensation, and dynamic range will be optimized for each subject and kept constant during the imaging.

For each dose, two-dimensional transthoracic echocardiography will be performed using both B-mode ultrasonography and contrast-specific ultrasonography before agent administration and using only contrast-specific ultrasonography after agent administration. The pre-injection imaging will be performed once in both apical 4- and 2- chamber views, first using B-mode ultrasonography and then contrast-specific ultrasonography. Switch between both apical views or imaging modes will be performed in 30-second intervals. Upon NH002 administration, the post-injection imaging will be subsequently performed in both apical 4- and 2- chamber views using only contrast-specific ultrasonography. During this period, alternation of both apical views will be performed in 30-seconds until 10 minutes after NH002 administration. In both imaging sessions, the 4-chamber view will be performed first.

DICOM cineloop sequences will be stored. Each cineloop sequence will be acquired for a duration of 5 seconds whenever the imaging mode or the imaging view is changed. [redacted] See Figure 5 for details.

Figure 5: Imaging Guidelines



Note: B=B mode; C=Contrast mode; 4=Apical 4-chamber view; 2=Apical 2-chamber view

Each subject should have 24 DICOM file(s) for the NH002 dosing and the unenhanced imaging. These files will be exported to the USB flash drive or CD from the ultrasound machine.

The video for NH002 dosing will be recorded by an external video recorder in mp4 format. The sponsor will provide each site with a recording instrument confirmed to be compatible with [redacted]. All clinical sites will use the same model of the recording instruments. The recording duration will be at least 15 minutes for each dosing period (starting from approximately 5 minutes before

and continuing until 10 minutes after dosing). Each subject should have at least 1 video file stored in a USB flash drive or CD (for the NH002 dosing and the unenhanced ultrasound imaging). The sponsor will also provide USB flash drives or CDs to sites.

Central image evaluations will occur offsite to ensure that the blinded reader(s) perform their image evaluations independently. The blinded central reader(s) will not have any knowledge of subject identifiers nor dose level received to obtain an image. DICOM image groups obtained in each subject will be merged and presented to the blinded reader(s) in a random sequence. Each blinded evaluation will be locked in the dataset shortly after it is obtained and before additional types of image evaluations are performed, if indicated *and applicable*.

If indicated *and applicable*, consensus image evaluations (or consensus reads) can be performed after the individual readings (if applicable) are completed and locked. (These consensus reads will NOT serve as the primary image evaluation used to demonstrate any preliminary efficacy of NH002.) ***If used***, the consensus reads will also be locked once obtained and before additional types of blinded readings are performed.

The blinded central reviewer(s) with appropriate training, education, and qualifications will assess the echocardiographic images for this study. The blinded central reader(s) will be provided with redacted USB flash drive(s) or CD(s) in order to access and score randomized video image groups without knowledge/information of dosing or subject identifiers. Refer to Image Evaluation Guidelines for more information.

7.1.2 Ventricular Arrhythmia Related to High Mechanical Index

High ultrasound 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 MI (transmit power) control is important to adjust for penetration needs while limiting bubble destruction. Mechanical index settings vary by patient. The MI should be set at **0.8 or below**.

Therefore, NH002 is *not* recommended for use at MIs greater than 0.8.

7.2 Schedule of Assessments

	Screening	**Dosing Period**								**Safety Follow-up**	
	≤ 28 Days before Day 1, unless otherwise specified	*Day 1*								*Day 4*	*Day 7*
		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]				X[5]	X[5]	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

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANC = absolute neutrophil count; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; β-HCG = beta-human chorionic gonadotropin; BP = blood pressure; BUN = blood urea nitrogen; CBC = complete blood count; cTnI = cardiac troponin I; ECG = electrocardiogram; EOT = end of treatment; HCT = hematocrit; HgB = hemoglobin; HR = heart rate; kg = kilogram; IV = intravenous; min = minutes; N/A = not applicable; PE = physical examination; PLT = platelet count; RR = respiratory rate; TT = thrombin time; WBC = white blood cells; WoCBP = women of childbearing potential.

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. See Section 9.1.2.2.7. 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. See Section 7.1.1 for details.

[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.

7.3 Discussion of Study Design

7.3.1 Study Design

The objectives of this study are to evaluate the safety and tolerability of 3 ascending doses of NH002. This will be done through this phase 1, multicenter, open-label, dose escalation study.

The selected endpoints for demonstrating safety of NH002 are in accordance with current standards.[7,14-17] Prespecified safety follow-up evaluations will monitor subjects for any AEs and potential adverse allergic reactions during and after dosing.

The evaluable population of eligible subjects with potential cardiac conditions are well suited for exploration. Subjects with clinically unstable cardiopulmonary conditions, considered unsuitable as determined by the investigator(s), will be excluded from trial participation. These include conditions such as, marked ventricular ectopy, atrial fibrillation with variable ventricular response, myocardial infarction or unstable angina pectoris (within 6 months prior), acute decompensated heart failure, acute renal failure and respiratory failure of any cause, moderate to severe pulmonary hypertension, and acute pulmonary embolus or pulmonary infarction. A dose-finding strategy will be employed, which concentrates patient dosing assignments at and around the dose which has the best efficacy/tolerability profile based on a utility function (ie, LVO).[18]

The rationale for the design of this phase 1 trial is to appropriately evaluate and explore the selected doses of NH002. NH002 is expected to provide improved ultrasound image quality in subjects with sub-optimal image(s) at baseline, and better product potency, in addition to decreasing the risk of gas embolism in pulmonary circulation.[13] Stopping guidelines will also be used to adapt to any perceived changes in safety and tolerability. See Section 7.5.1.2 and Section 9.1.2.1.

The clinical relevance for selecting the endpoints of LVO and duration of clinically useful contrast is founded upon multiple studies that LV cavity visualization and EBD are often compromised.[2,3,5,19] A recent report indicates that up to 20% of routine TTE in the clinic setting may have poor EBD and could be regarded as nondiagnostic. [19] Multiple studies also reported by Chong et al, have demonstrated that microbubble contrast combined with harmonic imaging for LVO improves the diagnostic accuracy, confidence, and interobserver agreement in assessment of regional systolic function or myocardial thickening in technically difficult-to-image cases. [19] Intravenous administration of microbubble contrast agents facilitates delineation of the EBD. This procedure has been shown to consistently increase the number of myocardial segments that can be interpreted, to improve accuracy of assessing regional and global left ventricular function, to decrease inter-interpreter variability, to increase interpreter confidence, and to be a cost-effective strategy.[20]

Patient factors further contribute to the significant challenges in poor image quality. It is recognized that the clinical utility of echocardiography, LVO, and EBD need to be improved to enhance overall endocardial visualization. Accurate assessments of LV volumes also require adequate EBD. Kitzman et al demonstrated that contrast-enhanced images resulted in the conversion of 48% of non-diagnostic examinations (defined as ≥ 4 of 6 nonevaluable segments in a single apical view) into “salvaged” studies (where ≤ 1 poorly visualized segment remained

on the same comparative view) following LVO.[2] By improving the quality of images acquired, it is expected to restore adequate diagnostic utility.

Refer also to Section 5 of the protocol.

7.3.2 Quality Management and Risk Evaluation

This protocol was evaluated to identify processes and data that were critical to assure human subject protection and reliability of study results.

Predefined quality tolerance limits have been established, taking into consideration the medical and statistical characteristics of the variables as well as the statistical design of the study, to identify systematic issues that could impact subject safety or data integrity. Detection of deviations from the predefined quality tolerance limits will trigger an evaluation to determine if action is needed. Any important deviations from the predefined quality tolerance limits and remedial actions taken will be described in the clinical study report.

Risk control measures will be periodically reviewed to ascertain whether the implemented quality management activities remain effective and relevant, taking into account emerging knowledge and experience.

7.4 Selection of Study Population

7.4.1 Number of Planned Subjects

It is estimated that 36 subjects will be enrolled in the study.

If a subject is withdrawn from the study, except for those subjects who will be terminated early due to AEs, the subject may be replaced as necessary 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.

Refer to the statistical considerations on which the numbers are based in Section 10.8.

7.4.2 Inclusion Criteria

To be eligible for study entry subjects must satisfy all of the following inclusion criteria:

1. Male or female subjects 20 years of age or older
2. Ability to understand and the willingness to provide written informed consent
3. Suspected of having cardiac disease or undergoing evaluation of cardiac anatomy for congenital heart disease
4. Negative pregnancy test within 24 hours of the procedure if subject is a woman of childbearing potential (WoCBP)

7.4.3 Exclusion Criteria

Subjects will be excluded from the study if one or more of the following exclusion criteria is applicable:

1. Known right-to-left, bidirectional, or transient right-to-left cardiac shunt(s)
2. Chronic obstructive pulmonary disease
3. Ejection fraction at screening or baseline < 40%
4. Uncontrolled serious ventricular arrhythmias or at high risk for arrhythmias due to prolongation of the QT interval (QT corrected by Fridericia's formula > 450 msec in males and > 470 msec in females)
5. Clinically significant abnormality in renal or liver function tests (alanine transferase [ALT]/aspartate aminotransferase [AST] > 2 × upper limit of normal [ULN]; serum creatinine > 1.5 × ULN)
6. Known or suspected hypersensitivity to one or more of the ingredients of NH002, perflutren, or other ultrasound contrast agents
7. Received an investigational compound within 30 days before enrolling in the study
8. Received any contrast agent either intravascularly or orally within 48 hours before NH002 administration
9. Pregnant or lactating female (conception during the study should be avoided)
10. Serious medical or psychiatric illness/condition likely, in the judgment of the investigator, to interfere with compliance to protocol treatment/research
11. Clinically unstable cardiopulmonary conditions considered not suitable for participation in the trial, in the judgment of the investigator
12. 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)

7.4.4 Removal of Subjects From Therapy or Assessments

Subjects may stop study agent and withdraw from the study for any of the following reasons:

- Subject request
- Use of nonpermitted concurrent therapy
- Non-compliance with the study agent or study schedule
- Lost to follow-up
- Occurrence of AEs not compatible with the continuation of subject participation in the study, in the investigator's opinion, or unacceptable to the subject to continue
- Investigator request
- Intercurrent illness
- Sponsor request
- Pregnancy

Subjects who do not comply with the protocol or who withdraw consent will be replaced. Subjects who stop study agent for any other reason (ie, AE) will not be replaced.

Subjects are free to withdraw from the study at any time without providing reason(s) for withdrawal and without prejudice to further research treatment. The reason(s) for withdrawal will be documented in the case report form (CRF).

Subjects withdrawing from the study will be encouraged to complete the same final evaluations as subjects completing the study according to this protocol, particularly safety evaluations. The aim is to record data in the same way as for subjects who completed the study.

Reasonable efforts will be made to contact subjects who are lost to follow-up. These efforts must be documented in the subject's file.

The sponsor has the right to terminate the study at any time in case of SAEs or if special circumstances concerning the study agent or the company itself occur, making further research treatment of subjects impossible. In this event, the investigator(s) will be informed of the reason for study termination.

Pregnancy

Only female subjects with negative pregnancy tests will be allowed to enroll in the study. Subjects will be instructed that known or suspected pregnancy occurring during the study in female subjects should be confirmed and reported to the investigator, who will then withdraw the subject from the study without delay. Upon discontinuation from the study, only those procedures that would not expose the subject to undue risk will be performed.

The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study.

In the event that a subject is subsequently found to be pregnant after inclusion in the study, any pregnancy will be followed to term, and the status of mother and child will be reported to the sponsor after delivery.

Full details will be recorded on the withdrawal page of the CRF, or an SAE report will be completed if the subject has completed the study. Procedures to be followed if a pregnancy occurs are provided in Section 9.1.2.2.1.

7.4.5 Enrollment Guidelines

All eligibility requirements must be reviewed before the subject may enter the study. The following information must also be provided to the sponsor and/or their representative, as requested:

1. Completed and signed protocol-specific eligibility checklist.
2. All pages of the original signed informed consent forms (ICFs), including Health Insurance Portability and Accountability Act (HIPAA)[21] Form for US sites.

3. Relevant source documents such as: subject medical history and physical exam, admission or discharge notes, diagnostic reports, confirmation of diagnosis, and relevant subject-specific written communication.

7.5 Investigational Products

7.5.1 Investigational Products Administered

7.5.1.1 NH002

Each subject will receive 1 of 3 NH002 doses (2.5 µl/kg, 5.0 µl/kg, or 10.0 µl/kg) by IV bolus injection. The subjects will be allocated sequentially to each single dose as follows: initial 12 subjects receive 2.5 µl/kg, the next 12 receive 5.0 µl/kg, and final 12 receive 10.0 µl/kg. See Table 4.

Table 4: NH002 Administration

Agent	Subjects	Dose (µl/kg) IV bolus	Schedule
NH002	First set of 12	2.5	Day 1
	Next set of 12	5.0	
	Final set of 12	10.0	

7.5.1.2 Stopping Criteria for Dosing

All AEs will be evaluated during the successive cohorts of subjects, per dose level. Intrasubject dose escalations are not permitted.

Dose escalation will proceed successively with each cohort as described in previous sections (Section 7.5.1.1), unless any of the following criteria is/are exhibited:

- The occurrence of any SAE considered at least possibly related to study agent administration in any 1 subject who received study agent. The occurrence of such an SAE will supersede all other stopping rules.
- The occurrence of the following in 30% of subjects (within any one of the 3 dose levels):
  - moderate or severe clinically significant nonhematologic lab toxicity, including cardiac disorder(s)
  - moderate or severe clinically significant hematologic lab toxicity
  - mild, moderate, or severe clinically significant anaphylactic reaction

Supportive care is permissible at the discretion and clinical judgment of the treating investigator, until symptoms are resolved to baseline.

If any of the stopping criteria for dosing are observed, the study will be halted, and the risk to other subjects evaluated, prior to a decision as to whether to terminate the study (See also Section 9.1.2.1 for overall study stopping rules).

Proceeding to the next dose level is permissible before completion of a cohort when no visible contrast effect and no safety concerns are observed in 4 subjects in a cohort. Such decision will be triggered by the sponsor in conjunction with the DEC, and as approved by the appropriate regulatory agencies.

A more conservative dose assignment, evaluation of an intermediate dose, and/or expansion of an existing dose level is permissible if such measures are needed for patient safety or for a better understanding of NH002 dose-related toxicity, and/or exposure. Such changes will be determined by the sponsor in conjunction with the DEC, and as approved by the appropriate regulatory agencies.

7.5.1.3 Microspheres and Contrast Agent Activation

NH002 is a contrast agent which is intended for administration only after activation in the designated apparatus. Before injection, the product must be activated and prepared according to outlined instructions. The contrast agent will be prepared and administered by designated pharmacy technician(s), study nurse(s), sonographer, or radiologist.

An activation device provided by Trust Bio-sonics, Inc., TRANSMIX™, should be used as a companion to NH002 for microbubble activation. TRANSMIX™ is equipped [redacted] and a mechanical agitator for the enhanced mixing of the lipids solution and C_3F_8 for gas encapsulation.

[redacted] Refer to the TRANSMIX User's Guide for further details.

[redacted]

Immediately after activation, the activated contrast agent appears as a milky white suspension and should be used immediately. If the product is not used within 5 minutes after activation, the contrast agent should be resuspended by 10 seconds of hand agitation by inverting the vial, before the product is withdrawn in a syringe.

Invert the vial and withdraw the activated milky white suspension using an 18- to 20-gauge syringe needle. Withdraw the material from the middle of the liquid in the inverted vial. Do not inject air into the vial.

Use the product immediately after its withdrawal from the vial; do not allow the product to stand in the syringe.

For single use only: NH002 does not contain bacterial preservative(s). 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 activation carefully and to adhere to strict aseptic procedures during preparation.

7.5.1.4 Injection Volume(s)

The injection volume (µl) for each subject will be calculated based on their body weight (kg). See Table 5.

Table 5: NH002 Injection Volume(s)

NH002 Dose (µl/kg)	NH002 volume (µl) based on subject weight (X kg)
10.0	= X × 10.0
5.0	= X × 5.0
2.5	= X × 2.5

7.5.1.5 Injection Route and Rate

For this trial, NH002 must be injected by an IV bolus method. **Do not administer by intra-arterial injection**.

Slow injection is important for safety (according to prior experience in animal studies). **Sites should always have cardiopulmonary resuscitation personnel and equipment readily available before administration and monitor all subjects for acute reactions**.

NH002 will be injected as an IV bolus, within 30 to 60 seconds, followed by a 10 mL saline flush over 10 seconds.

7.5.1.6 Emergency Event Management

Subjects will be closely monitored by a medical team during the injection and for at least 4 hours (240 minutes) following dosing for the presence of symptoms of allergic or hypersensitivity reaction(s). Subjects who receive 4-hour monitoring after the dose on Day 1 will be encouraged to quickly report any symptoms at the time during this period. The necessary rescue material, equipment, and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

7.5.2 Identity of Investigational Products

7.5.2.1 NH002 (perflutren lipid microspheres) Injectable Suspension

Formulation, Dosage and Strength

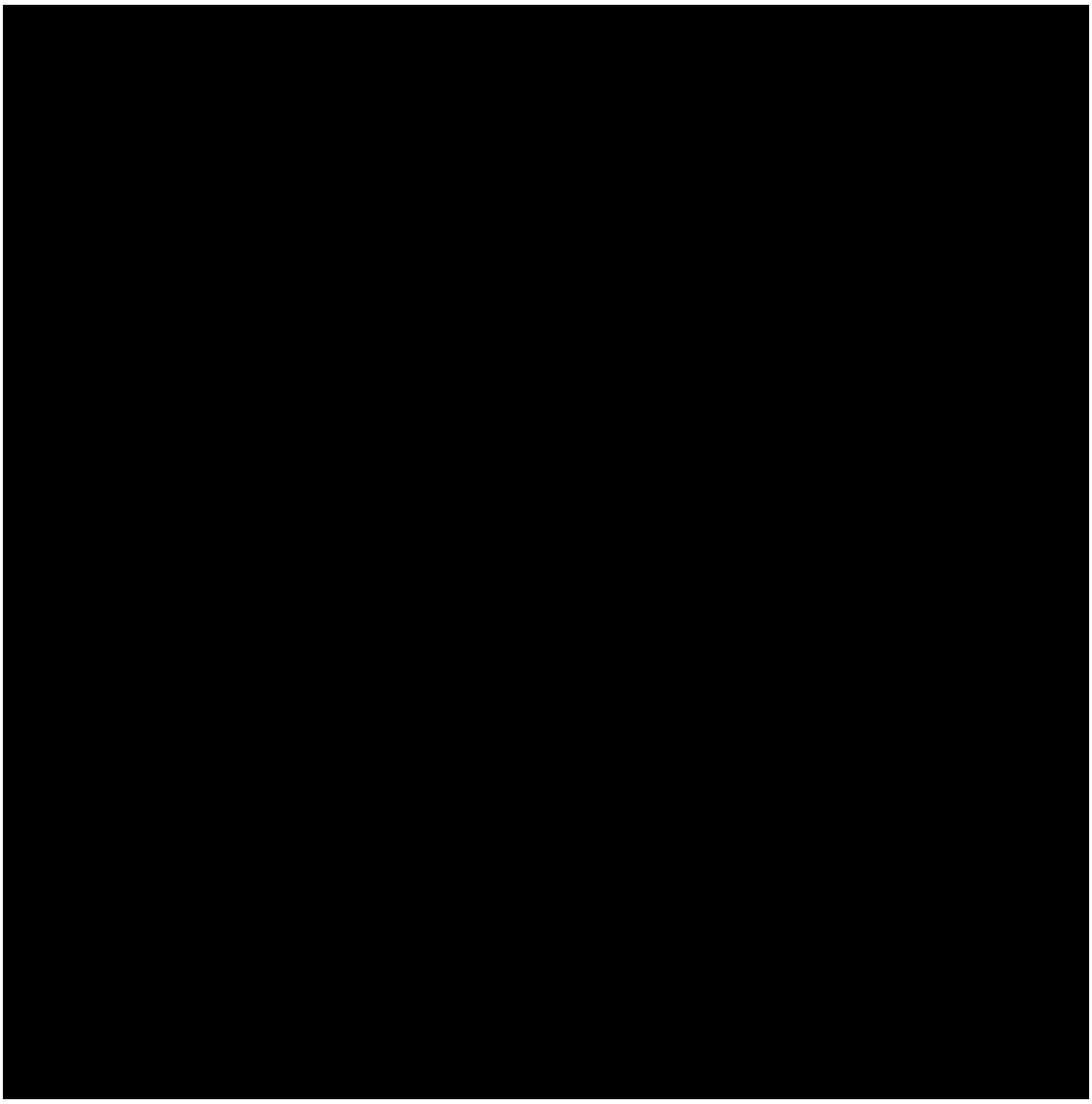
The gas core in NH002 is perflutren. The structure is a gas core surrounded by a lipid shell (Figure 6). NH002 is formulated as a microbubble injectable suspension of perflutren in single-use, 1-mL lipid solution in 2-mL vials with [redacted] C_3F_8 in headspace for IV administration.

Chemical Name

NH002 (perflutren lipid microspheres)

Chemical Structure

Molecular formula: C_3F_8



How Supplied

NH002 is supplied as a single use, 1-mL lipid solution in 2-mL clear glass vial containing clear liquid in packages of 4 single-use vials.

Storage and Handling

Store NH002 between 2-8°C (36°-46°F).

Source

Trust Bio-sonics (TRUST) will supply NH002 (and the associated TRANSMIX™ apparatus). The study agent will be manufactured and imported according to the relevant regulatory requirements.

Trust Bio-sonics (TRUST) Address: Room D105, No. 2, Sec. 2, Shengyi Road
Zhubei City, Hsinchu County 30261
Taiwan (R.O.C.)
Telephone: +886-3-6684965

7.5.3 Packaging and Labeling

7.5.3.1 NH002

[redacted]

All packaging and labeling operations will be performed according to Good Manufacturing Practice for Medicinal Products and the relevant regulatory requirements. NH002 sample label is provided in Figure 7.

Figure 7: NH002 Sample Packaging Label

[redacted]

7.5.4 Selection of Doses in the Study

Refer also to Section 5 (Introduction).

7.5.5 Selection and Timing of Dose for Each Subject

On the day of dosing, each subject will receive one injection (of NH002), as per Section 7.5.1.

Up to 36 eligible subjects will be assigned with *sequential allocation* to 1 of 3 doses of NH002 (2.5 µl/kg, 5.0 µl/kg, or 10.0 µl/kg IV bolus).

No dose adjustments will be permitted, per protocol. There are no restrictions on the time of day of dosing. There are also no restrictions in relation of dosing to meals.

7.5.6 Prior and Concomitant Therapy

There is no specific known evidence of contraindications between the ingredients of NH002 and other prior and concomitant therapy. Concomitant medications and therapies will be recorded beginning at the time of screening, as well as during and after research treatment.

7.5.6.1 Prohibited Medication/Therapy

Subjects must not have received an investigational compound within 30 days before enrolling in the study. Subjects must also not have received any contrast agent either intravascularly or orally within 48 hours before NH002 administration. Concomitant medications and procedures will be recorded beginning at the time of screening, as well as during and after research treatment.

Subjects with known right-to-left, bidirectional, or transient right-to-left cardiac shunt(s) are **excluded** from this study, but it is important to note the following: When administering NH002 to subjects with a cardiac shunt, the microspheres can bypass filtering by the lung and enter the arterial circulation. Subjects with shunts should be assessed for embolic phenomena following NH002 administration.

7.5.6.2 Rescue Medication

Slow injection is important for safety (according to prior experience in animal studies). Sites should always have cardiopulmonary resuscitation personnel and equipment readily available before administration and monitor all subjects for acute reactions.

7.5.7 Dosing Compliance

On the day of scheduled dosing (ie, Day 1) dosing compliance will be assessed by the administering clinician(s). At a minimum, date, time, duration, and dose should be recorded in the treatment record(s) to confirm that each dose of NH002 was administered as per protocol.

8 TIMING OF STUDY PROCEDURES

Before performing any study-specific procedure or assessment, written informed consent and authorization for use of protected health information (HIPAA) [21] in the US, must be obtained in accordance with all application policies, regulations, and laws. The planned study assessments are in Section 7.2.

8.1 Pre-dosing

8.1.1 Visit 1: Screening

The following assessment(s) must be collected/performed ***within 28 days prior*** (unless specified) to Day 1. Clinical and laboratory evaluations performed as part of routine standard of care do not need to be repeated if performed within the appropriate window.

- Eligibility: Assess against the inclusion and exclusion criteria
- Medical history: Relevant history including description of all prior therapies, and signs or symptoms
- Vital signs: Measurements include body temperature, blood pressure (BP), heart rate (HR), and respiratory rate (RR)
- Demography
- Height (cm)
- Weight (kg)

- Standard 12-lead ECG
- Physical exam (PE): A complete PE will 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, extremities, and neurological exam
- A urine or serum (beta-human chorionic gonadotropin [β-HCG]) pregnancy test is required for WoCBP; it is not required for postmenopausal or surgically sterilized women.
- Concomitant medications and procedures: Concomitant medications and procedures will be recorded beginning at the time of screening.

8.2 Dosing Period

Collection of concomitant medications and AEs as defined in Section 9.1.2 should occur throughout the study.

For imaging guidelines, refer to Section 7.1.1.

8.2.1 Visit 2: Day 1, Dosing

8.2.1.1 Before Dosing

The following procedures will be performed ***before*** administration of the NH002 contrast injection on Day 1:

- Eligibility: Assess against the inclusion and exclusion criteria
- Medical history, including baseline signs and symptoms should be documented and continually reassessed throughout the study
- VS: Measurements include BP, body temperature, HR, and RR
- Weight (kg)
- Standard 12-lead ECG
- Focused PE: A focused PE will include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments.
- Both a urine and a serum (β-HCG) pregnancy test are required for WoCBP before dosing on Day 1. (Note that at screening, only a urine or serum test is necessary; before dosing on Day 1, both tests are necessary.)
- Complete blood count (CBC) to include hematocrit (HCT), hemoglobin (Hgb), white blood cell (WBC) count with differential, absolute neutrophil count (ANC), and platelet count (PLT)
- Coagulation studies: Thrombin time (TT) and activated partial thromboplastin time (aPTT)
- Metabolic panel: Metabolic panel evaluations to include sodium, potassium, chloride, calcium, magnesium, phosphorus, glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, alkaline phosphatase (ALP), ALT, AST, total bilirubin, and bicarbonate
- Cardiac troponin I
- Pulse oximetry: SpO_2
- Pretreatment AEs: Baseline events, findings, and conditions before NH002 initiation should be documented and continually reassessed throughout the study.

- Concomitant medications and procedures: Concomitant medications and procedures will continue to be recorded.

8.2.1.2 5 Minutes Before Dosing

The following procedures will be performed approximately ***5 minutes before*** dosing:

- Imaging should proceed as specified in Section 7.1.1.
- Modified 12-lead ECG monitoring should be conducted, from 5 minutes before dosing to 10 minutes after dosing. See Section 9.1.2.2.7.
- Pulse oximetry: SpO_2 should be measured and recorded, from 5 minutes before dosing to 10 minutes after dosing.
- VS: BP, body temperature, HR, and RR should be measured and recorded, from 5 minutes before dosing to 10 minutes after dosing.
- Record any AEs that may occur and any changes in concomitant medication.

8.2.1.3 Dosing

After all procedures have been performed, NH002 will be administered. Each subject will receive an assignment to 1 of the 3 NH002 dose levels (2.5 µl/kg, 5.0 µl/kg, or 10.0 µl/kg IV bolus). See Section 7.5.1 for details. The following procedures will be performed after the contrast injection:

- Imaging should proceed as specified in Section 7.1.1.
- Modified 12-lead ECG monitoring to continue until completion of imaging (ie, to 10-minutes after dosing). Any clinically significant ECG changes indicative of arrhythmia or S-T segment change identified after dosing from the modified 12-lead ECG monitoring will be recorded in a timely manner, and a standard 12-lead ECG will be conducted subsequently (when the first recorded ECG change occurs). The imaging will be temporarily stopped for the conditionally triggered standard 12-lead ECG examination. The results of the modified 12-lead ECG will also be included into the subject's study file as reference. See Section 9.1.2.2.7.
- Pulse oximetry: SpO_2 should also be measured and recorded until completion of imaging (ie, 10 minutes after dosing).
- VS: BP, body temperature, HR, and RR should be measured and recorded until completion of imaging (ie, 10 minutes after dosing).
- Record any AEs that may occur and any changes in concomitant medication.

8.2.1.4 10 Minutes After Dosing

The following procedures will be performed approximately ***10 minutes after*** the contrast injection:

- VS: Measurements include BP, body temperature, HR, and RR
- Modified 12-lead ECG monitoring to continue until completion of imaging (ie, to 10-minutes post dosing); See Section 9.1.2.2.7.

- Standard 12-lead ECG. Site(s) should make every effort to target the timing to be as close to the end of the modified 12-lead ECG monitoring (10 minutes after dosing) as possible.
- Pulse oximetry: SpO_2
- Imaging should proceed as specified in Section 7.1.1.
- Record any AEs that have occurred since the previous visit and any changes in concomitant medication.

8.2.1.5 30 Minutes After Dosing

The following procedures will be performed approximately ***30 minutes after*** the contrast injection (+5 minutes):

- VS: Measurements include BP, body temperature, HR, and RR
- Standard 12-lead ECG
- Focused PE: A focused PE will include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments.
- Pulse oximetry: SpO_2
- Record any AEs that have occurred since the previous visit and any changes in concomitant medication.

Site(s) should make every effort to target the timing as possible. However, given that these procedures may be performed in different examination rooms in the study sites, a time window of +5 minutes is permitted.

8.2.1.6 60 Minutes After Dosing

The following procedures will be performed approximately ***60 minutes after*** the contrast injection (+5 minutes):

- VS: Measurements include BP, body temperature, HR, and RR
- Standard 12-lead ECG
- Focused PE: A focused PE will include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments.
- CBC to include HCT, Hgb, WBC count with differential, ANC, and PLT
- Coagulation studies: TT and aPTT
- Metabolic panel: Metabolic evaluations include sodium, potassium, chloride, calcium, magnesium, phosphorus, glucose, BUN, creatinine, total protein, albumin, ALP, ALT, AST, total bilirubin, and bicarbonate
- Pulse oximetry: SpO_2
- Record any AEs that have occurred since the previous visit and any changes in concomitant medication.

Site(s) should make every effort to target the timing as possible. However, given that these procedures may be performed in different examination rooms in the study sites, a time window of +5 minutes is permitted.

8.2.1.7 120 Minutes After Dosing

The following procedures will be performed approximately ***120 minutes after*** the contrast injection (+5 minutes):

- VS: Measurements include body temperature, BP, HR, and RR
- Focused PE: A focused PE will include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments.
- CBC to include HCT, Hgb, WBC count with differential, ANC, and PLT
- Coagulation studies: TT and aPTT
- Metabolic panel: Metabolic panel evaluations include sodium, potassium, chloride, calcium, magnesium, phosphorus, glucose, BUN, creatinine, total protein, albumin, ALP, ALT, AST, total bilirubin, and bicarbonate
- Pulse oximetry: SpO_2
- Record any AEs that have occurred since the previous visit and any changes in concomitant medication.

8.2.1.8 240 Minutes After Dosing

The following procedures will be performed approximately ***240 minutes after*** the contrast injection (+5 minutes):

- CBC to include HCT, Hgb, WBC count with differential, ANC, and PLT
- Coagulation studies: TT and aPTT
- Cardiac Troponin I
- Record any AEs that have occurred since the previous visit and any changes in concomitant medication.

8.3 Safety Follow-up Period

8.3.1 Day 4 (+3)

Subjects will be contacted by telephone at least 72 hours after NH002 dosing (ie, Day 4 [+3]) for a follow-up assessment of safety. An additional 3-day window is permitted for this Safety Follow-up, if site(s) have difficulty reaching the subject on Day 4. The following procedures will also be performed during the Safety Follow-up call:

- Record any AEs that have occurred since the previous visit and any changes in concomitant medication. Any AE or SAE should be followed up until resolved.

8.3.2 Day 7 (+3)

Subjects will be contacted by telephone again 7 days after the end of their treatment for another follow-up assessment of safety. An additional 3-day window is permitted for this second Safety Follow-up, if site(s) have difficulty reaching the subject on Day 7. The following procedures will be also performed during the Safety Follow-up call:

- Record any AEs that have occurred since the previous visit and any changes in concomitant medication. Any AE or SAE should be followed up until resolved.

8.4 Duration of Treatment

The duration of treatment is expected to last between 4 to 5 hours during 1 day, barring any unexpected adverse reactions.

9 EFFICACY AND SAFETY VARIABLES

The planned schedule of assessments is in Section 7.2.

9.1 Efficacy and Safety Measurements Assessed

9.1.1 Efficacy Variables

9.1.1.1 Echocardiographic Imaging

Efficacy evaluations will be based on blinded central echocardiographic imaging assessments of LVO, duration of useful contrast enhancement, LVEBD, and diagnostic confidence:

- Left ventricular opacification (LVO): The degree of LVO following each injection was graded according to the following four-point rating scale: 0= none (no visible contrast within the LV cavity); 1= faint (weak or trace effect of contrast within the LV cavity); 2= moderate (some areas of the LV cavity fully opacified but without a time when the whole cavity was filled with contrast to the same high density); or 3= complete (homogeneous and high intensity effect).

- Duration of clinically useful contrast enhancement: The time between the disappearance of shadowing effect and the time when moderate or full LV enhancement and contrast enhancement are no longer adequate (useful effect ends).

- Left ventricular endothelial border delineation (LVEBD): To determine the impact of NH002 on EBD, a standard 12-segment model will be used, dividing the LV endocardium of the 4- or 2-chamber apical views into 6 segments, with 2 basal, mid- and apical segments in each view.

9.1.2 Safety Assessments

Each subject must be carefully monitored for the development of any AEs throughout the study from the signing of the ICF to at least 7 days after receipt of the study agent (ie, Day 7 Follow-up call). Safety evaluations will also be assessed and recorded at specified time points: before injection, during injection and imaging (beginning approximately 5 minutes prior to and continuing until 10 minutes after injection), and up to 7 days post-injection.

Safety parameters to be evaluated will include:

- Standard 12-lead ECGs assessed pre-injection, and at 10, 30, and 60 minutes post-injection; during imaging, a standard 12-lead ECG will be conditionally performed at the

earliest time point when any clinically significant ECG changes indicative of arrhythmia or S-T segment change are identified within 10 minutes after injection from the modified 12-lead ECG monitoring; each standard 12-lead ECG assessment will be scored as either normal or abnormal;

- Changes in VS 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; VS measurements will include body temperature, HR, RR, and BP;
- The following parameters will be included in the *complete* PE (at screening): General appearance, skin (turgor, pallor, cyanosis, erythema, lesions), head (including ear, eyes, nose, and throat), neck, lungs and heart (observation, palpation, percussion, auscultation), abdomen, back lymph nodes, extremities, and a neurological exam. A *focused* PE (after screening) will include cardiopulmonary (observation, palpation, percussion, auscultation) and skin (turgor, pallor, cyanosis, erythema, lesions) assessments; each PE assessment will be scored as either normal or abnormal;
- Changes in hematology and metabolic laboratory parameters taken pre-injection and 60, 120, and 240 minutes post-injection;
- TT and aPTT pre-injection and 60, 120, and 240 minutes post-injection;
- Cardiac troponin I pre-injection and 240 minutes (4 hours) post-injection;
- SpO_2 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 assessment of any potential adverse allergic reactions during and post-injection.

9.1.2.1 Overall Study Stopping Rules

The estimated duration of the study is contingent on safety and the number of dose levels evaluated in the dose escalation. See Section 7.5.1.2 for stopping criteria for dosing. The study will be stopped in the event of any new findings that indicate a relevant deterioration of the risk-benefit relationship that would render continuation of the study unjustifiable. Study administration with NH002 will be halted and the DEC will convene to determine if the study should be terminated for any safety concerns. See Section 9.2 for DEC details.

The sponsor may also end the study for administrative reasons.

Should the study be terminated prematurely, the sponsor will provide written notification to all investigators and regulatory authorities specifying the reason(s) for early termination. The investigator must inform the institutional review board (IRB)/independent ethics committees (IEC) promptly and provide the reason(s) for the termination. Previously dosed subjects will be assessed through all planned study safety visits.

9.1.2.2 Adverse Events

Adverse Event Definition

An AE is defined as any untoward medical occurrence in a clinical study subject administered a medicinal product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the indication under investigation that is not recorded elsewhere in the CRF under specific efficacy assessments. Anticipated fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered AEs.

It is the responsibility of the investigator to document all AEs that occur during the study. Any AEs will be elicited by asking the subject a nonleading question, for example, "Have you experienced any new or changed symptoms since we last asked/since your last visit?". All AEs should be reported on the appropriate page of the CRF.

Assessment of Severity

Each AE will be assigned a category by the investigator as follows:

Mild:	An AE that is easily tolerated by the subject, causes minimal discomfort, and does not interfere with everyday activities.
Moderate:	An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed.
Severe:	An AE that prevents normal everyday activities; treatment or other intervention usually needed.

If there is a change in severity of an AE, it must be recorded as a separate event.

Assessment of Causality

Every effort will be made by the investigator to assess the relationship of the AE, if any, to the study agent. Causality should be assessed using the categories presented in the following:

Unrelated:	Clinical event with an incompatible time relationship to study agent administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the study agent.
Unlikely:	Clinical event whose time relationship to study agent administration makes a causal connection improbable, but that could plausibly be explained by underlying disease or other drugs or chemicals.

Possible:	Clinical event with a reasonable time relationship to study agent administration, but that could also be explained by concurrent disease or other drugs or chemicals.
Probable:	Clinical event with a reasonable time relationship to study agent administration, and is unlikely to be attributed to concurrent disease or other drugs or chemicals.
Very Likely/Certain:	Clinical event with plausible time relationship to study agent administration, and that cannot be explained by concurrent disease or other drugs or chemicals.

Action Taken

The investigator will describe the action taken (with regard to the study agent and/or the subject) in the appropriate section of the CRF, as follows:

- None
- Study agent stopped
- Study agent temporarily interrupted
- Concomitant medication
- Other, specify.

Follow-up of Adverse Events

All investigators should follow up subjects with AEs until the event is resolved or until, in the opinion of the investigator, the event is stabilized or determined to be chronic. Details of AE resolution must be documented in the CRF.

Subjects should also be followed up 3 days (+3) and 7 days (+3) after receiving NH002 (ie, Day 4 and Day 7), and any AEs that occur during this time should be reported according to the procedures outlined in this section.

Documentation and Reporting of Adverse Events

All AEs should be reported and documented in accordance with the procedures outlined in this section. All AEs occurring during the study must be documented on the relevant CRF pages. The following data should be documented for each AE:

- Description of the symptom event
- Classification of ‘serious’ or ‘not serious’
- Severity
- Date of first occurrence and date of resolution (if applicable)
- Action taken
- Causal relationship
- Outcome of event (unknown, recovered, not yet recovered, recovered with sequelae, death [with date and cause reported])

9.1.2.2.1 Serious Adverse Events

Serious Adverse Event Definition

An SAE is any untoward medical occurrence or effect that, at any dose,

- Results in death.
- Is life-threatening (an AE is life-threatening if the subject was at immediate risk of death from the event as it occurred; ie, it does not include a reaction that might have caused death if it had occurred in a more serious form).
- Requires or prolongs inpatient hospitalization. (Complications occurring during hospitalization are AEs and are SAEs if they cause prolongation of the current hospitalization. Hospitalization for elective treatment of a pre-existing nonworsening condition is not, however, considered an AE. The details of such hospitalizations must be recorded on the medical history or physical examination page of the CRF).
- Results in persistent or significant disability/incapacity. (An AE is incapacitating or disabling if it results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions).
- Results in a congenital anomaly/birth defect.

In addition, medical and scientific judgement is required to decide if prompt notification is required in situations other than those defined for SAEs above. This may include any event that the investigator regards as serious that did not strictly meet the criteria above but may have jeopardized the subject or required intervention to prevent one of the outcomes listed above, or that would suggest any significant hazard, contraindication, side effect, or precaution that may be associated with the use of the investigational product.

Reporting of Serious Adverse Events

Any SAE must be reported by the investigator if it occurs during the clinical study or within 4-7 days of receiving the study agent, whether or not the SAE is considered to be related to the investigational product. An SAE report consists of the SAE form, the AE form, and the concomitant medication form. A copy of these forms must be emailed **within 24 hours** for the attention of the product safety scientist at:

Name: Ting-Yu Huang
Company: Trust Bio-sonics, Inc.
E-mail: [redacted]

The investigator should not wait to receive additional information to document fully the event before notification of a SAE, though additional information may be requested. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the investigator at any

time after cessation of study agent administration and linked by the investigator to this study, should be reported to the study monitor.

The sponsor and/or the appointed representative(s) will promptly notify all relevant investigators and the regulatory authorities of findings that could adversely affect the safety of subjects, impact on the conduct of the study, or alter the IEC/ IRB approval/favorable opinion of the study. In addition, the sponsor and/or the appointed representative(s), will expedite the reporting to all concerned investigators, to the IEC(s)/IRB(s), where required, and to the regulatory authorities of all adverse reactions that are both serious and unexpected.

Details of the procedures to be followed if a pregnancy occurs are also provided in Section 7.4.4.

9.1.2.2.2 Unexpected Adverse Reactions

Unexpected Adverse Reaction Definition

An unexpected adverse reaction is any untoward and unintended response that is related to the administration of the study agent, at any dose that is not consistent with the applicable product information (eg, current version of the Investigator's Brochure for an unauthorized investigational medicinal product or summary of product characteristics for an authorized product).

All suspected unexpected serious adverse reactions (SUSARs) will be the subject of expedited reporting. The sponsor and/or the appointed representative(s) shall ensure that all relevant information about a SUSAR that is fatal or life-threatening is reported to the relevant competent authorities and IEC/IRB within 7 days after knowledge by the sponsor of such a case and that relevant follow-up information is communicated within an additional 8 days. All other SUSARs will be reported to the relevant competent authorities and IEC/IRB within 15 days after knowledge by the sponsor of such a case. All investigators should follow up SUSARs until the event is resolved or until, in the opinion of the investigator, the event is stabilized or determined to be chronic. Poststudy SUSARs that occur after the subject has completed the clinical study must be reported by the investigator to the sponsor.

Warnings and Precautions

Hypersensitivity Reactions: Do not administer NH002 to subjects with known or suspected hypersensitivity to perflutren.

Serious Cardiopulmonary Reactions: Serious cardiopulmonary reactions including fatalities have occurred uncommonly during or shortly following perflutren-containing microsphere administration, typically within 30 minutes of administration. The risk for these reactions may be increased among subjects with unstable cardiopulmonary conditions (acute myocardial infarction, acute coronary artery syndromes, worsening or unstable congestive heart failure, or serious ventricular arrhythmias). Always have cardiopulmonary resuscitation personnel and equipment readily available before NH002 administration and monitor all subjects for acute reactions.

Systemic Embolization: Subjects with known right-to-left, bidirectional, or transient right-to-left cardiac shunt(s) are **excluded** from this study, but it is important to note the following. When administering study agent to subjects with a cardiac shunt, the microspheres can bypass filtering by the lung and enter the arterial circulation. Subjects with shunts should be assessed for embolic phenomena following study agent administration. The study agent is only for IV administration; do not administer by intra-arterial injection(s).

Ventricular Arrhythmia Related to High Mechanical Index: High ultrasound 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. Study agent is not recommended for use at MIs greater than 0.8.

9.1.2.2.3 Clinical Laboratory Evaluation

The hematology and clinical chemistry laboratory analyses will be performed at local laboratories. Reference ranges will be supplied by the local laboratories and used by the investigator to assess the laboratory data for clinical significance and pathological changes.

Methods (including whether samples are taken fed or fasting) and timing for assessing, recording and analyzing each laboratory variable should follow local guidelines, within the confines of the protocol timeframes. The following laboratory safety tests will be performed at screening and treatment visits:

Hematology

Hgb, HCT, WBC count (total and differential), red blood cell count, PLT, mean cell volume, mean cell hemoglobin (MCH), and MCH concentration

Clinical Chemistry

Creatinine, BUN, AST, ALT, ALP, total bilirubin, albumin, total protein, sodium, potassium, chloride, glucose, bicarbonate, calcium, magnesium, and phosphorus

9.1.2.2.4 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).

Other laboratory variables collected are:

- Coagulation studies of TT and aPTT
- Cardiac troponin I

9.1.2.2.5 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.

9.1.2.2.6 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.

9.1.2.2.7 Physical Examination

At screening, a complete physical examination will be performed. A focused physical examination will be performed before dosing and during dosing at the following time points for each contrast injection: 30, 60, and 120 minutes post-injection. Any changes will be recorded and assessed. Each PE assessment will be scored as either normal or abnormal.

9.1.2.2.8 Electrocardiogram

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 following parameters will be evaluated: First degree heart block (HB), second degree HB, complete HB, atrial arrhythmia, junctional rhythms, ventricular arrhythmia, LVH (left ventricular hypertrophy), other chamber enlargement, Q-wave infarct, ST-T wave changes; ischemia suspected, nonspecific ST-T wave changes, QTc interval prolongation. Each 12-lead ECG assessment will be scored as either normal or abnormal.

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.

To further enhance the quality of the modified 12-lead ECGs:

1. The investigator should make best efforts to minimize the displacement of the electrodes for the modified 12-lead ECG compared with the standard 12-lead ECG.

2. The repositioning of the ECG leads for echocardiography should only involve the V5-V6 leads, which have been shown to have minimal effect on ECG waveform.[22]

3. To describe the shifted leads, study personnel should make appropriate annotations in the study records/subject file.

Refer to Image Evaluation Guidelines for information on central review procedures.

9.1.3 Appropriateness of Measurements

The efficacy and safety assessments planned for this study are generally recognized as reliable, accurate, and relevant to the diagnostic modality and underlying disease/condition.

9.2 **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 (ie, 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.

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

10 STATISTICAL METHODS

The statistical planning and analysis of the trial will be performed by the biostatistics department of the sponsor or designated contract research organization.

10.1 **Statistical and Analytical Plans**

A statistical analysis plan will be prepared and finalized prior to database lock of the study. The statistical analysis plan will include full details of all planned statistical analyses and the dose selection strategy.

10.2 **Datasets or Populations Analysed**

Safety Set

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

Full Analysis Set

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.

Per Protocol Set

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. Further definition of the per protocol set will be specified in the statistical analysis plan.

10.3 Demographic and Other Baseline Characteristics

Demographic and baseline characteristic data will be summarized for each cohort and overall. 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 impact of any imbalances in demographics or baseline characteristic variables on treatment effect may be investigated in exploratory analyses on select efficacy or safety variables; such analyses will be documented in the statistical analysis plan, if applicable.

10.4 Safety Assessments

All safety assessments, including AEs, PEs, VS, ECGs, and clinical laboratory evaluations, where indicated, will be presented using descriptive statistics for each dose of NH002. Data will be summarized for each dose group and overall.

10.4.1 Safety Variables

- Adverse events recorded before injection, during injection and imaging, and post-injection;
- Any changes in PE findings
- Standard 12-lead ECGs assessed before injection and at 10, 30, and 60 minutes post-injection; an additional standard 12-lead ECG will also be conditionally performed at the earliest time point when any clinically significant ECG changes indicative of arrhythmia or S-T segment change are identified within 10 minutes after injection from the modified 12-lead ECG monitoring;
- Changes in VS assessed before 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;
- Changes in hematology and metabolic laboratory parameters taken before injection and 60, 120, and 240 minutes post-injection;
- SpO_2 assessed before 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.

10.5 Efficacy Variables

10.5.1 Definition of Primary Efficacy Endpoint

There are no primary efficacy endpoints for this study.

10.5.2 Definition of Secondary Efficacy 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).

10.6 Methods of Analysis

The safety and efficacy variables of each dose of NH002 will be presented using descriptive statistics. A series of selection rules based on efficacy and safety will be applied to determine the maximum tolerated dose. If no single NH002 dose shows a clear benefit in safety or efficacy, the lowest dose of NH002 will be selected.

Secondary endpoints will be summarized by descriptive statistics. Graphical presentations of the data will be provided, where appropriate.

10.7 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 nonmissing data available. No imputation process will be used to estimate/substitute missing data.

10.8 **Determination of Sample Size**

Each of the expected 36 completed subjects will receive 1 dose of NH002 (with 12 subjects per NH002 cohort). As this is a dose finding study, a formal sample size calculation was not required. The number of subjects dosed within each cohort is based on safety, with the objective of exposing the lowest possible number of subjects to the investigational agent, while still being able to assess safety.

10.9 **Protocol Deviations**

Major protocol deviations will be defined in the statistical analysis plan and agreed upon during a Data Review Meeting prior to database lock.

11 QUALITY ASSURANCE AND QUALITY CONTROL

11.1 **Audit and Inspection**

Study centers and study documentation may be subject to Quality Assurance audit during the course of the study by the sponsor or its nominated representative. In addition, inspections may be conducted by regulatory authorities at their discretion.

11.2 **Monitoring**

Data for each subject will be recorded on a CRF. Data collection must be completed for each subject who signs an ICF and is administered study agent.

In accordance with current good clinical practice (cGCP) and International Council for Harmonisation (ICH) guidelines, the study monitor will carry out source document verification at regular intervals to ensure that the data collected in the CRF are accurate and reliable.

The investigator must permit the monitor, the IEC/IRB, the sponsor's internal auditors, and representatives from regulatory authorities direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the CRFs.

11.3 **Data Management and Coding**

The sponsor and/or the appointed representative(s) will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant standard operating procedures of the data management and biostatistics departments of the sponsor and/or the appointed representative(s).

Study centers will complete the CRF. Data entered into the CRF must be verifiable against source documents at the study center. Data to be recorded directly on the CRF will be identified and the CRF will be considered the source document. Any changes to the data entered into the data capture system will be recorded in the audit trail (and if electronic, will be FDA CFR 21 Part 11 compliant).

Medical coding will use Medical Dictionary for Regulatory Activities for concomitant diseases and AEs and WHODrug for medications.

Missing or inconsistent data will be queried in writing to the investigator for clarification. Subsequent modifications to the database will be documented.

11.4 **Quality Management and Risk Evaluation**

Details are provided in Section 7.3.2.

12 RECORDS AND SUPPLIES

12.1 Drug Accountability

On receipt of the study agent (including rescue medication, if relevant), the investigator (or designee) will conduct an inventory of the supplies and verify that study agent supplies are received intact and in the correct amounts before completing a supplies receipt. The investigator will retain a copy of this receipt at the study center and return the original receipt to the study monitor. The monitor may check the study supplies at each study center at any time during the study.

It is the responsibility of the study monitor to ensure that the investigator (or designee) has correctly documented the amount of the study agent received, dispensed, and returned on the dispensing log that will be provided. A full drug accountability log will be maintained at the study center at all times. The study monitor will arrange collection of unused study agent returned by the subject. The study monitor will also perform an inventory of study agent at the close-out visit to the study center. All discrepancies must be accounted for and documented.

12.2 Financing and Insurance

Financing and insurance of this study will be outlined in a separate agreement between the contract research organization and the sponsor.

13 ETHICS

13.1 Independent Ethics Committee or Institutional Review Board

Before initiation of the study at each study center, the protocol, the ICF, other written material given to the subjects, and any other relevant study documentation will be submitted to the appropriate IEC/IRB. Written approval of the study and all relevant study information must be obtained before the study center can be initiated or the study agent is released to the investigator. Any necessary extensions or renewals of IEC/IRB approval must be obtained for changes to the study such as amendments to the protocol, the ICF or other study documentation. The written approval of the IEC/IRB together with the approved ICF must be filed in the study files.

The investigator will report promptly to the IEC/IRB any new information that may adversely affect the safety of the subjects or the conduct of the study. The investigator will submit written summaries of the study status to the IEC/IRB as required. On completion of the study, the IEC/IRB will be notified that the study has ended.

13.2 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating countries, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

13.3 Ethical Conduct of the Study

The investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, cGCP, ICH guidelines, and the applicable national and local laws and regulatory requirements.

13.4 Informed Consent

The process of obtaining informed consent must be in accordance with applicable regulatory requirement(s) and must adhere to cGCP.

The investigator is responsible for ensuring that no subject undergoes any study related examination or activity before that subject has given written informed consent to participate in the study.

The investigator or designated personnel will inform the subject of the objectives, methods, anticipated benefits and potential risks and inconveniences of the study. The subject should be given every opportunity to ask for clarification of any points s/he does not understand and, if necessary, ask for more information. At the end of the interview, the subject will be given ample time to consider the study. Subjects will be required to sign and date the ICF. After signatures are obtained, the ICF will be kept and archived by the investigator in the investigator's study file. A signed and dated copy of the subject ICF will be provided to the subject or their authorized representative.

It should be emphasized that the subject may refuse to enter the study or to withdraw from the study at any time, without consequences for their further care or penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give or who withdraw written informed consent should not be included or continue in the study.

If new information becomes available that may be relevant to the subject's willingness to continue participation in the study, a new ICF will be approved by the IEC(s)/IRB(s) (and regulatory authorities, if required). The study subjects will be informed about this new information and reconsent will be obtained.

13.5 **Subject Confidentiality**

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the IEC(s)/IRB(s) approving this research, and the United States (US) FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the HIPAA,[21] applicable to national and/or local laws and regulations on personal data protection.

14 REPORTING AND PUBLICATION, INCLUDING ARCHIVING

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study (end of study defined as the date of the last visit of the last subject), all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the sponsor or its representatives. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years since the discontinuation of clinical development of the investigational product. It is the responsibility of the sponsor to inform the study center when these documents no longer need to be retained. The investigator must contact the sponsor before destroying any study related documentation. In addition, all subject medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

The sponsor must review and approve any results of the study or abstracts for professional meetings prepared by the investigator(s). Published data must not compromise the objectives of the study. Data from individual study centers in multicenter studies must not be published separately.

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16 APPENDICES

16.1 **Image Evaluation Guidelines**

Sponsor-recommended equipment/platforms*:

Ultrasound System	[redacted] with contrast LVO mode
Video Recording Instrument	[redacted]
Image Evaluation Software	Windows Media Player/DICOM viewer

**Equipment other than the listed can be used after discussion and approval by the sponsor.*

Equipment technical settings to be used at each site: Site should follow standard institutional guideline(s) for equipment technical settings and subject preparation, positioning, and overall comfort measures.

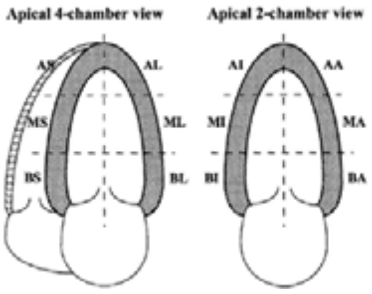
Site qualification process: Local site imaging technicians must be trained and qualified in equipment operation, including the ability to identify faulty or unacceptable images and understand the importance of repeating imaging when appropriate. (In the event of faulty or unacceptable images, an unscheduled visit can be scheduled to repeat all necessary procedures.)

Acquisition quality control monitoring process:
A checklist will be used for quality control. Checklist for each treatment:

- ☐ Subject ID and treatment number were input correctly on ultrasound imaging system and external video recorder
- ☐ Investigator confirmed settings of mechanical index, depth, focal depth, dynamic range, gain, and time gain compensation were optimized for each subject
- ☐ 4-chamber view of pre-injection B mode acquired
- ☐ 2-chamber view of pre-injection B mode acquired
- ☐ 4-chamber view of pre-injection contrast mode acquired
- ☐ 2-chamber view of pre-injection contrast mode acquired
- ☐ Treatment administered
- ☐ 4-chamber view of post-injection contrast mode acquired
- ☐ 2-chamber view of post-injection contrast mode acquired
- ☐ 19 transitions between 2-chamber and 4-chamber view post-injection. Two or less transitions may be skipped when a standard 12-lead ECG is conditionally triggered during the imaging session.
- ☐ A video containing consecutive pre-injection and 10-minute post-injection sessions acquired
- ☐ 4 pre-injection DICOM cineloop sequences acquired
- ☐ 20 post-injection DICOM cineloop sequences acquired (including 10 DICOM cineloop sequences of 4-chamber view and 10 DICOM cineloop sequences of 2-chamber view). Two or less sequences may be skipped when a standard 12-lead ECG is conditionally triggered during the imaging session.)

Data storage and transfer:
USB flash disks or CD(s) labeled with subject ID will be used to store and transfer video files and DICOM files acquired in the study. Each subject's image data will be stored in one USB flash disk or CD(s) and shipped to the sponsor or designated contract research organization for randomized sequencing (for central blinded reviews).

Standards for Image Evaluation:
(a) LVO:
0 = none (no visible contrast within the LV cavity);
1 = faint (weak or trace effect of contrast within the LV cavity);
2 = moderate (some areas of the LV cavity fully opacified but without a time when the whole cavity was filled with contrast to the same high density);
3 = complete (homogeneous and high intensity effect).
(b) LVEBD: The apical 4-chamber and 2-chamber views of the left ventricle were divided into segments by using cardiac maps. A total delineation score (0-24) was obtained by adding the scores (0 = inadequate, ie, border not visible; +1 = sufficient, ie, border barely visible; +2 = good, ie, border clearly visible) from the six individual segments in each of the two views. [redacted]



0 = inadequate, ie, border not visible;
1 = sufficient, ie, border barely visible;
2 = good, ie, border clearly visible.

Image display sequence and randomization.
The sponsor or designated contract research organization will merge the grouped images obtained in the study from each subject and present the merged set to the blinded reader in a random sequence. The whole recorded image data will be used for scoring by the blinded reader(s). The blinded reader(s) should document the time points of the best images used for LVEBD scoring as well as the start and end time points of useful contrast imaging.

Central blinded reader training and qualification:
Central blinded reader(s) have been selected to assess the echocardiographic images for this study because of their background training and qualifications. These selected blinded central reader(s) will also be trained in scoring procedures for LVO and LVEBD using sample images, prior to the central image evaluations.

Timing of image reads and the read process:
Merged/randomized image scoring of blinded central reader(s) will be done after all the images in the study are obtained.

Imaging data lock process:
Each blinded evaluation will be locked in the dataset shortly after it is obtained and before additional types of image evaluations are performed.

If indicated and applicable, consensus image evaluations (or consensus reads) can be performed after the individual readings (if applicable) are completed and locked. (These consensus reads will NOT serve as the primary image evaluation used to demonstrate any preliminary efficacy of NH002.) If used, the consensus reads will also be locked once obtained and before additional types of blinded readings are performed.

Quality control of the image display and blinded interpretation process:
Central image evaluations will occur offsite to ensure that the blinded reader(s) perform their image evaluations independently of other image evaluations. The blinded central reader(s) will not have any knowledge of subject identifiers nor dose level received to obtain an image. The blinded central reader(s) will also evaluate image groups in a randomized sequence.

Imaging data transfer process to the sponsor:
All USB flash disks or CDs with subject data will be returned to the sponsor or designated contract research organization after the completion of study (from both the local sites and the blinded central reader[s]).

Archiving of images and blind image interpretations:
Images and blind image interpretations will be archived in the sponsor or designated contract research organization’s database.

16.2 **Contrast Administration Guidelines for NH002**

NH002 Administration: for further details, see also the current version of the NH002 Investigator's Brochure.

1. Obtain IV access or evaluate for an existing IV line. (May be given through a peripherally inserted central catheter [PICC] or central line if necessary.)
2. NH002 is to [redacted] using the TRANSMIX™ vial [redacted]
3. NH002 is to be activated for [redacted] TRANSMIX activation cycle, and will appear as a milky-white suspension. Do not use this drug unless it has completed a full [redacted] activation cycle in the TRANSMIX™. Do not reactivate the vial if TRANSMIX™ did not complete a full [redacted]; the product should not be used. Do not reactivate a successfully activated NH002 vial.
4. Invert the vial and withdraw the activated milky white suspension using an 18- to 20-gauge syringe needle. Withdraw the material from the middle of the liquid in the inverted vial. Do not inject air into the vial. Use the product immediately after its withdrawal from the vial; do not allow the product to stand in the syringe.
5. Administer activated NH002 into the IV port closest to the IV site.
6. Injections are to be administered slowly, over 30 to 60 seconds, followed by a 10 mL saline flush over 10 seconds.

NOTE: Contact the sponsor or clinical research associate for further instructions. Do not discard the vial(s).

16.3 **Investigator Signature Page**

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

Protocol Number: NH002-LV

Version: 1.0

Confidentiality and cGCP Compliance Statement

I, the undersigned, have reviewed this protocol (and amendments), including appendices, and I will conduct the study as described in compliance with this protocol (and amendments), cGCP, and relevant ICH guidelines.

Once the protocol has been approved by the IEC/IRB, I will not modify this protocol without obtaining prior approval of Trust Bio-sonics, Inc. and of the IEC/IRB. I will submit the protocol amendments and/or any ICF modifications to Trust Bio-sonics, Inc. and IEC/IRB, and approval will be obtained before any amendments are implemented.

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all CRFs, laboratory samples, or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Trust Bio-sonics, Inc., to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

_____ _____

Investigator Signature Date

_____

Printed Name

_____

Institution