Protocol for the Heart Failure Clinical Research Network

Inorganic **N**itrite **D**elivery to **I**mprove **E**xercise Capacity in HFpEF
INDIE-HFpEF

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1 List of Abbreviations

Abbreviation	Definition
6MWD	6-Minute Walk Distance
6MWT	6-Minute Walk Test
AAU	Arbitrary Accelerometry Units
ACC	American College of Cardiology
ACE	Angiotensin-Converting Enzyme
AE	Adverse Event
AHA	American Heart Association
ARB	Angiotensin Receptor Blocker
AXM	Accelerometer
BNP	Brain Natriuretic Peptide
BUN	Blood Urea Nitrogen
CC	Coordinating Center
CFR	Code of Federal Regulations
cGMP	Cyclic Guanosine Monophosphate
CPET	Cardiopulmonary Exercise Test
CRF	Case Report Form
DBP	Diastolic Blood Pressure
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DHF	Diastolic heart failure
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EE	Expedited Event
EF	Ejection fraction
ER	Emergency Room
FDA	Food and Drug Administration
GFR	Glomerular Filtration Rate
GTN	Glyceryl Trinitrate
HF	Heart Failure
HFN	Heart Failure Clinical Research Network
HFN	Heart Failure Network
HFpEF	heart failure with preserved ejection fraction
HFrEF	Heart Failure with reduced Ejection Fraction
HFSA	Heart Failure Society of America
ICF	Informed Consent Form

ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ISDN	Isosorbide Dinitrate
ISMN	Isosorbide Mononitrate
ITT	Intention To Treat
IV	Intravenous
IVRS	Interactive Voice Recording System
KCCQ	Kansas City Cardiomyopathy Questionnaire
LV	Left Ventricular
NO	Nitric Oxide
NO_2	Inorganic Nitrite
NO_3	Inorganic Nitrate
NO-CGMP	Nitric Oxide and cGMP transduction pathway
NT-proBNP	Pro-B-type Natriuretic Peptide
NYHA	New York Heart Association
PA	Pulmonary artery
PCI	Percutaneous Coronary Intervention
PCP	Primary Care Physician
RCC	Regional Clinical Center
RCT	Randomized Clinical Trial
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SBP	Systolic Blood Pressure
VO_2	Oxygen consumption

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3 EXECUTIVE SUMMARY

Title	**I**norganic **N**itrite **D**elivery to **I**mprove **E**xercise Capacity in **HFpEF** (INDIE-HFpEF)
Indication	Heart failure with preserved ejection fraction
Location	Approximately 20 clinical centers in the United States
Brief Rationale	Approximately half of Americans with heart failure (HF) have preserved ejection fraction (HFpEF). There is no proven treatment that improves outcome. The pathophysiology is complex and includes left ventricular (LV) systolic and diastolic dysfunction, pulmonary vascular disease, endothelial dysfunction, and peripheral abnormalities. Multiple lines of evidence point to impaired NO-cGMP bioavailability as playing a central role in each of these abnormalities. NO-cGMP levels can be increased using direct NO donors, such as the organic nitrates. The HFN NEAT trial tested whether treatment with organic nitrates (isosorbide mononitrate, ISMN) for 4 weeks could improve daily activity levels as assessed by accelerometry. However, organic nitrates have several shortcomings, including the development of tolerance, greater vulnerability to hypotension in patients with HFpEF, development of 'pseudo-tolerance', where chronic venodilation leads to renal sodium retention, and increases in oxidative stress and endothelial dysfunction. An alternative strategy to restore Nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling is via inorganic nitrite (NO_2). While previously considered to be an inert byproduct of NO metabolism, NO_2 has been shown to be an important in vivo reservoir for NO generation. Notably, reduction of NO_2 to NO is enhanced under hypoxic and acidemic conditions, meaning that the molecule becomes most active at the time of greatest need (e.g., during exercise). Ancillary analyses from the CHAMPION trial have revealed that reduction in pulmonary artery (PA) and PA wedge pressure are associated with marked reduction in HF hospitalizations in HFpEF, emphasizing the importance of filling pressure reduction to improve morbidity in HFpEF. To this end, recent studies have shown that acute NO_2 infusion markedly blunts the increase in PA and PA wedge pressure during exercise in HFpEF, while improving cardiac output reserve. A similar noninvasive study using inorganic nitrate (NO_3) (naturally occurring in beetroot juice) similarly improved aerobic capacity and enhanced cardiac output reserve and systemic vasodilation in HFpEF. Accordingly, INDIE-HFpEF will test the effects of longer term NO_2 therapy on exercise capacity and chronic daily activity levels in HFpEF.

Study Design	A randomized, double-blind, placebo-controlled crossover study to assess the effect of inorganic nitrite (NO_2) on aerobic capacity (peak VO_2) after four weeks of dosing. Approximately 100 participants will be enrolled in this 2*2 crossover study.
Treatment	Inhaled, nebulized inorganic sodium nitrite vs. inhaled, nebulized placebo at a dose of 80 mg (or maximally tolerated dose) administered at a minimum of 4 hours apart, three times per day, during the active part of the day.
Primary Objective and Endpoint	The primary endpoint will be the peak VO_2 after 4 weeks treatment with inorganic nitrite as compared to the peak VO_2 after 4 weeks treatment with placebo as assessed by cardiopulmonary exercise testing (CPET) performed at peak drug levels.
Secondary Objectives and Endpoints	1. To evaluate whether inorganic nitrite improves submaximal activity tolerance chronically. • Average arbitrary accelerometer units (AAU) during at least 14 days and up to 21 days of the maximally tolerated dose of study drug (from 28 days post Study Visit 1 until Study Visit 2 and from 28 days post Study Visit 2 until Study Visit 3). 2. To evaluate whether inorganic nitrite improves quality of life. • Quality of Life (Kansas City Cardiomyopathy Questionnaire score, KCCQ) • NYHA class • Participant preference for study phase (active vs. placebo) 3. To evaluate whether inorganic nitrite improves chronic filling pressures as assessed by echocardiography and natriuretic peptide levels. • E/e' ratio on echocardiography • Left atrial volume index on echocardiography • Pulmonary artery systolic pressure on echocardiography • N-terminal pro-B-type natriuretic peptide level 4. To evaluate whether inorganic nitrite improves ventilatory efficiency or submaximal exercise capacity at peak drug levels. • V_E/VCO_2 slope • VO_2 at ventilatory threshold 5. To evaluate safety and tolerability of inhaled, nebulized sodium inorganic nitrite. • Headache • Lightheadedness/orthostasis • Other AEs
Abbreviated Study Flow	Screen potential HFpEF patients for eligibility criteria and interest

	Study Visit 1 • Initiate consent process and obtain written informed consent. • Confirm with the participant that HF symptoms are the primary limitation to activity. If so, they proceed to CPET screening. If not, they are considered a screen fail. • Obtain baseline bloods *- CBC, complete chemistry panel, biomarkers, biorepository and genetics (if agreed to participate) . • Obtain CPET to verify patient eligibility peak VO2 ≤ 75% predicted and RER ≥ 1.0 (within 3 days prior to randomization) and establish baseline value. • Qualifying patients perform additional baseline studies: history, assess NYHA class, physical exam, ECG, and KCCQ. • Open label, single-dose run-in where patient receives maximal dose (80 mg) inhaled inorganic nitrite. Patients who do not tolerate the run-in are considered screen failures. • Randomize qualifying patients. • Dispense phase-1 study drug, nebulizers and accelerometers • Participants take no study drug for two weeks (baseline). • Participants take 46 mg study drug at a minimum of 4 hours apart, 3 times a day, during active part of the day for 7 days. • Participants take 80 mg study drug at a minimum of 4 hours apart, 3 times a day, during active part of the day until returning for study visit 2 (at least 42 days but up to 49 days post-baseline visit). • If side effects develop, participants can down-titrate to the previous dose. • Participants are called frequently to reinforce study procedures and assess compliance. Study Visit 2 (42-49 Days Post Study Visit 1) • Participant holds study drug on day of visit. • Review history, assess NYHA class, perform physical exam and KCCQ. • Obtain blood draws ** - CBC, complete chemistry panel, biomarkers, biorepository (if agreed to participate). • Obtain limited echocardiogram **. • Perform CPET with Study Drug administered immediately before starting the CPET (primary endpoint). • Change out accelerometer and dispense phase-2 study drug. • Participants take no study drug for two weeks (washout). • Participants take 46 mg study drug at a minimum of 4 hours apart, 3 times a day, during active part of the day for 7 days. • Participants take 80 mg study drug at a minimum of 4 hours apart, 3 times a day, during active part of the day until returning for study visit 3 (at least 42 but up to 49 days after study visit 2). • If side effects develop Participants can down-titrate to the previously tolerated dose. • Participants are called frequently to reinforce study procedures and assess compliance.

	Study Visit 3 (42-49 Days Post Study Visit 2) • Participant holds study drug on day of visit. • Review history, assess NYHA class, perform physical exam and KCCQ • Obtain blood draws** - CBC, complete chemistry panel, biomarkers, biorepository (if agreed to participate). • Obtain limited echocardiogram**. • Perform CPET with Study Drug administered immediately before starting the CPET (primary endpoint). • Return accelerometer and phase-2 study drug. • End of study drug (phase out). Phone Visit and End of Study (14 Days Post Study Visit 3) • A final phone visit is conducted to assess for adverse events. **Visit 1: baseline blood draw needs to be completed prior to the CPET (if this is not feasible, then they cannot be obtained for at least 3 hours post the CPET and prior to the run-in test dose).* ***Visit 2 and Visit 3: blood draws and limited echo need to be obtained prior to study drug administration (if this is not feasible, then it cannot be obtained for at least 3 hours post study drug administration)*

4 OBJECTIVES AND HYPOTHESES

4.1 Primary Objectives

To evaluate whether inhaled, nebulized inorganic sodium nitrite, as compared to placebo, improves maximal exercise capacity as assessed by cardiopulmonary exercise testing performed at peak drug levels.

The primary hypothesis of the INDIE-HFpEF study is that inorganic nitrite, compared to placebo, will improve exercise capacity (peak oxygen consumption, VO2).

The significance of this study is that it will provide evidence as to whether inorganic nitrite therapy improves aerobic capacity in patients with HFpEF. This would then provide rationale to pursue a larger phase 3 trial of inorganic nitrate/nitrite therapy in HFpEF.

4.2 Secondary Objectives

1. To evaluate whether inorganic nitrite, compared to placebo, improves submaximal exercise tolerance chronically throughout the course of the day, as well as at trough when plasma nitrite levels have dropped toward normal as assessed by:
    - Chronic activity by averaged arbitrary accelerometry units (AAU_{14}) during the final weeks of study phases (at least two and up to three weeks of peak dose of study drug/placebo)

2. To evaluate whether inorganic nitrite in comparison to placebo improves quality of life as assessed by:
    - KCCQ score
    - NYHA class
    - Patient preference for study phase (active vs. placebo)

3. To test whether inorganic nitrite improves chronic filling pressures as assessed by echocardiography and blood natriuretic peptide levels
    - E/e' ratio on echocardiography
    - Left atrial volume index on echocardiography
    - Pulmonary artery systolic pressure on echocardiography
    - NT-proBNP levels

4. To evaluate whether inorganic nitrite improves other measures of cardiopulmonary function
    - V_E/VCO_2 slope (ventilatory efficiency)
    - VO_2 at ventilatory threshold (submaximal exercise capacity)

5. To evaluate the safety and tolerability of inhaled, nebulized inorganic nitrite at 80 or 46 mg
    - Headache
    - Lightheadedness/orthostasis
    - Other adverse events

4.3 Tertiary Objectives

1. Pre-specified subgroup analyses will include examination of the primary endpoint in patients according to baseline characteristics:
    - NT-proBNP >400pg/ml vs. ≤400 pg/ml
    - Systolic blood pressure above and below median value
    - PASP>60 mmHg vs <60 mmHg
    - Age above and below median value
    - Women vs. men
    - Estimated glomerular filtration rate by MDRD equation above and below median value
    - Subjects on active drug at study visit vs not on study drug at study visit
    - Subjects in atrial fibrillation/flutter vs not
    - Diabetic vs. non-diabetic patients
    - Patients with or without symptoms of exertional chest pain

2. Novel accelerometry endpoints (guided by HFN NEAT ancillary paper analysis)

3. Plasma nitrosothiols, cystatin-C, cGMP levels

5 BACKGROUND AND SIGNIFICANCE

Therapy for HFpEF is a major unmet need: Heart failure (HF) is the leading cause of hospitalization among older Americans and a major public health problem. The direct and indirect costs of HF in 2010 were estimated at $31 billion; this is expected to increase to $70 billion by 2030, when nearly 8 million Americans (1 in 33) will have HF.[1] One-half of patients with HF have preserved ejection fraction (HFpEF), and the prevalence of HFpEF is growing relative to HF with reduced ejection fraction (HFrEF) by approximately 10% per decade.[2] These secular trends, coupled with the current aging of the U.S. population and increasing prevalence of obesity and metabolic syndrome ensure that HFpEF will be the dominant form of HF by 2020.[3] Importantly, no treatment has been proven to reduce mortality in HFpEF, and few interventions have been observed to improve symptoms or quality of life (QOL). Thus, identification of effective treatments for HFpEF is an enormous unmet public health need.

Targeting hemodynamic mechanisms of exercise intolerance in HFpEF: People with HFpEF suffer from dyspnea and fatigue with activity, limiting exercise tolerance and promoting sedentary lifestyle. Exercise capacity in HFpEF is limited by both “central” and “peripheral” factors. Central (cardiac) abnormalities include pathologic increases in filling pressures caused by diastolic dysfunction, exercise-induced pulmonary hypertension, and blunted increases in cardiac output (CO) with exertion.[4-13] Peripheral abnormalities include changes in skeletal muscle capacity and endothelial dysfunction.[14-17]

The fact that cardiac pressures are often normal at rest but elevated with stress has created a major barrier to treatment in HFpEF,[10] because interventions that reduce filling pressures during exercise also reduce resting pressures, increasing the vulnerability to hypotension in people with HFpEF.[18] It has recently been pointed out that HFpEF is a heterogenous syndrome and

that this may explain the failure of previous trials testing therapies that are efficacious in HFrEF. However, elevation in left ventricular filling pressures and pulmonary artery pressures during exercise is a universal finding in HFpEF that contributes to symptoms and thus represents a viable target. Other abnormalities including limited cardiac output reserve or peripheral limitations may apply to some patients but not others.

Improving hemodynamics by enhancing nitric oxide signaling in HFpEF: Nitric oxide-cyclic guanosine monophosphate (NO-cGMP) dependent, flow-mediated vasodilation is impaired in people with HFpEF.[9] Intriguingly, the magnitude of impaired vasodilation is directly correlated with severity of dyspnea and fatigue during submaximal exercise, as well as the degree of reduction in exercise capacity. Cardiomyocyte studies from human tissue have shown that NO-cGMP deficiency in HFpEF is coupled to increases in diastolic ventricular stiffness.[19] These observations have led Paulus and Tschope to recently propose that concentric ventricular remodeling, fibrosis and increased cardiomyocyte stiffness in HFpEF are all downstream consequences of impaired NO-cGMP signaling, making this a key pathway to target in trials.[20]

Ongoing trials in HFpEF have or are targeting NO-cGMP through organic NO donors (isosorbide mononitrate, HFN NEAT trial), decreased degradation of natriuretic peptides (LCZ 696, PARAGON trial) or direct stimulation of cGMP production (guanylate cyclase activator, SOCRATES-preserved). Inorganic nitrate-nitrite represents a novel candidate molecule to preferentially restore NO-cGMP signaling during exercise stress, since reduction of inorganic nitrite to NO is enhanced in the setting of tissue hypoxia and acidosis, as develop during exercise.

Exercise capacity is a clinically-meaningful, patient-centric endpoint in HF trials: Exercise intolerance is the ultimate symptomatic manifestation of HFpEF and can be objectively quantified by measurement of peak VO_2.[21] Cardiopulmonary exercise testing (CPET), unlike other measures such as 6-minute walk distance (6 MWD), permits direct assessment of the organ system limiting gas exchange. This is crucial in people with HFpEF who are older-aged and often display pulmonary, mechanical, or orthopedic limitations to exercise that might contribute to low 6 MWD. CPET also allows for assessment of the adequacy of exercise effort (respiratory exchange ratio, RER) along with other relevant gas exchange measurements that reflect HF severity and mirror hemodynamic abnormalities observed (V_E/VCO_2 slope, VO_2 at anaerobic threshold). Importantly, unlike changes in alternative trial end points, such as natriuretic peptide levels or echo parameters, there is intrinsic value to patients associated with improving exercise capacity, particularly if improvement of peak exercise capacity translates to improved functionality and quality of life. As assessment of peak VO2 is more technically challenging in HF trials and remains somewhat controversial, the INDIE study will provide unique information about the concordance of peak exercise capacity with daily activity and submaximal exercise capacity.

Crossover study design: Crossover studies have been widely used in cardiovascular medicine, including trials testing the effects of organic nitrates in patients with coronary artery disease[23-28] or HFpEF, as in the HFN NEAT trial.[29] In a crossover design each subject is exposed to both interventions, which aids recruitment, and the interindividual variability is reduced, allowing for a much smaller sample size. The drawbacks of a crossover design include the potential for a period effect and for carry-over effects.

The two potential sources of bias are minimized in INDIE-HFpEF because the exposure to study drug is relatively short (4 weeks), decreasing the likelihood of chronic remodeling effects, and because the washout period is sufficiently long to minimize risk of carryover effect. As in NEAT-HFpEF, the concept in INDIE-HFpEF is that hemodynamic, cardiac and possibly peripheral benefits induced by inorganic nitrite will improve exercise capacity, as shown in two single-dose acute studies performed in HFpEF (see Preliminary Studies).

6 PRELIMINARY STUDIES

Exercise capacity, quantified by the peak VO_2 attained during exercise, is severely reduced in HFpEF.[4, 9, 13, 14] According to the Fick principle, VO_2 is equal to the product of cardiac output (CO) and arterial venous O_2 content difference (A-V O_2diff). These components have been termed 'central' and 'peripheral' determinants of exercise limitation. Recent studies in HFpEF have identified abnormalities in both CO and A-V O_2diff reserve in patients with HFpEF that variably correlate with reduced peak VO_2 in different patients.[13, 14, 30, 31] However, regardless of the primacy of central vs peripheral mechanisms, elevation left ventricular filling pressures during exercise is universal in patients with HFpEF (Figure 1).

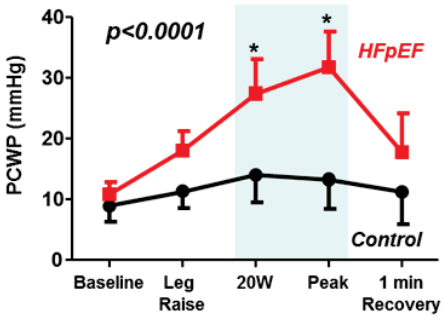


Figure 1: Pulmonary capillary wedge Pressure (PCWP) at rest and exercise.

Among the potential molecular pathways underlying cardiac reserve dysfunction, abnormalities in NO-cGMP-dependent signaling have emerged as important players.[9, 19-21, 32, 33] In the RELAX trial we observed that sildenafil, which increases cGMP by inhibiting its breakdown, failed to improve exercise capacity or QOL in people with HFpEF.[34] This suggests that enhancing NO-cGMP through decreased breakdown is not an effective approach in this population. Alternative strategies include direct provision of NO-cGMP, as with organic NO donors, and this hypothesis was tested in the HFN NEAT trial. However, organic nitrates are limited by tolerance[35], increases in oxidative stress that may cause endothelial dysfunction, pseudo-tolerance, and greater risk of hypotension in people with HFpEF owing to the steep end-systolic pressure volume relationship (ESPVR) observed in this HF phenotype (Figure 2).[18]

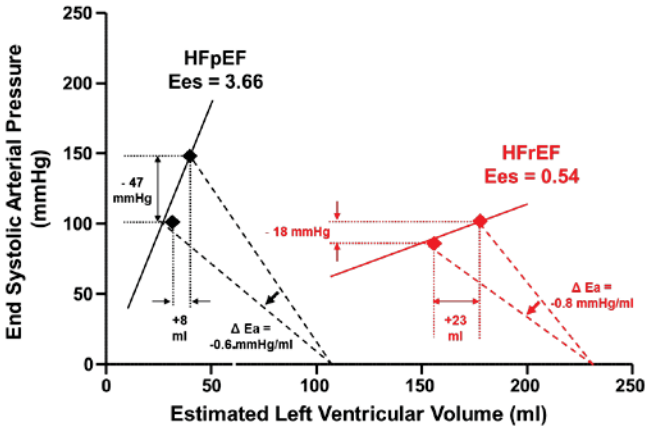


Figure 2: Changes in blood pressure with afterload reduction are amplified in HFpEF (black) compared to HFrEF (red) owing to the steeper ESPVR.

A key factor in HFpEF that complicates treatment is that the hemodynamic perturbations causing morbidity (e.g., high filling pressures) are sometimes mild at rest or present only during stress—being absent at rest (Figure 1).[10, 13] To most effectively treat these derangements, the ideal therapy would become more effective during stress, without untoward effects on resting cardiovascular homeostasis.

Inorganic nitrates represent an attractive alternative approach to treat NO-cGMP deficiency in HFpEF.[36-38] These molecules were previously considered as inert byproducts of endogenous NO metabolism, but recent studies have shown that inorganic nitrite functions as an alternative in vivo source of NO to the classical oxygen-dependent L-arginine-NO-synthase pathway (Figure 3). In the body, dietary nitrate is absorbed and then concentrated in the salivary glands. Oral commensal bacteria then reduce nitrate to nitrite which is absorbed and can then be reduced to NO.

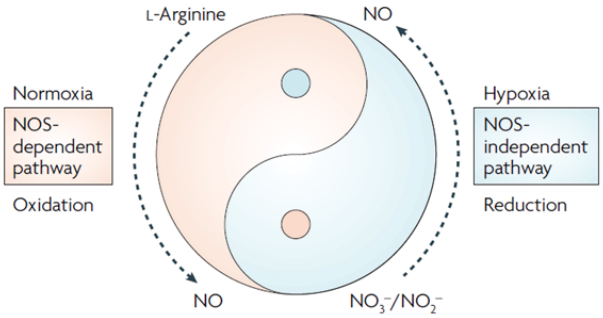


Figure 3: Two parallel sources of endogenous nitric oxide (NO) in mammals.

In contrast to direct NO donors such as organic nitrates, there is no tolerance with inorganic nitrate/nitrite[37] and importantly, reduction of inorganic nitrite to NO is enhanced under conditions of physiologic duress, such as tissue hypoxia and acidosis,[36-38] meaning that inorganic nitrite becomes most effective to unload the heart precisely at the time when it would be most needed in patients with HFpEF, without causing hypotension at rest when filling pressures, pulmonary artery pressures, and CO are normal (Figure 1).

In addition, inorganic nitrite has beneficial effects on skeletal muscle bioenergetics[39] and mitochondrial respiration, improving the O_2 cost of work during submaximal exercise.[40-42] Recently inorganic nitrite has been shown to improve conduit artery compliance and reduce pulsatile aortic load.[43] Thus, inorganic nitrite represents an ideal candidate molecule to target both the central and peripheral limitations to exercise in HFpEF.

Zamani et al. recently performed a double-blind crossover trial (n=17) showing that a single dose of inorganic nitrate (12.9 mmol) administered prior to exercise improves peak VO_2 while enhancing CO reserve and systemic vasodilation in patients with HFpEF (Figure 4).[44] Nitrate also decreased arterial wave reflections and late systolic pressure augmentation. In another study enrolling participants without HF, Omar et al. observed that inorganic nitrite vasodilates

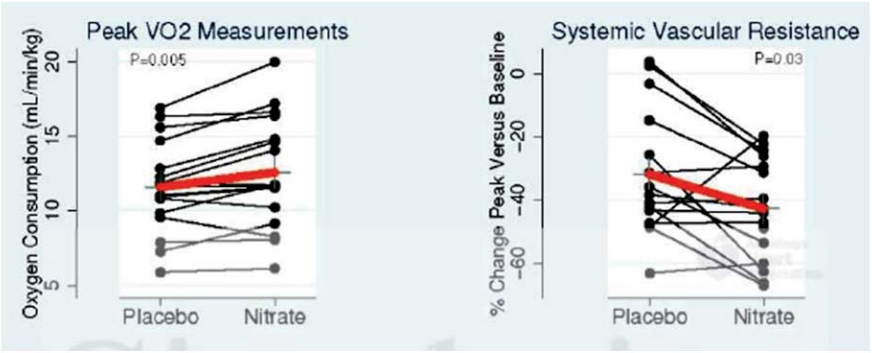


Figure 4: Compared to placebo, nitrate treatment significantly improved peak VO2 and led to greater reductions in systemic vascular resistance in HFpEF patients.

conduit arteries, an effect coupled to significant reductions in central blood pressure, which is typically elevated in HFpEF patients.[43]

Recently, Borlaug et al. completed a randomized, double blind, placebo controlled trial testing the effects of acute intravenous sodium nitrite on exercise hemodynamics and ventricular reserve during submaximal exercise in patients with HFpEF (n=28).[45]

Subjects underwent resting and exercise invasive hemodynamic assessment and were then treated with either intravenous sodium nitrite (250 µg/kg/min * 5 min) or saline. After 15 minutes, repeat rest and exercise hemodynamic assessment was performed at the same workload. After inorganic nitrite infusion, plasma levels increased to 8.39±1.88 µM.[45]

Compared to placebo, NO_2-treated subjects displayed markedly attenuated increases in LV filling pressures during exercise, with a lower slope of the PA pressure-flow relationship, improved exercise CO reserve, and enhanced CO/VO_2 slope (Figure 5).[45]

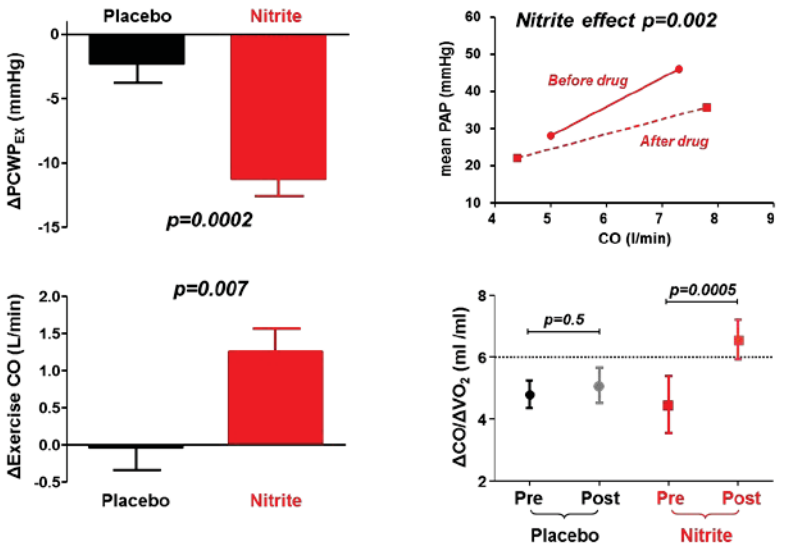


Figure 5: Compared to placebo (black), nitrite infusion (red) reduced exercise pulmonary capillary wedge pressure (PCWP), reduced the slope of the pulmonary artery (PA) pressure-flow relationship, and increased cardiac output (CO) reserve while improving the slope of increase in CO relative to VO_2.

Enhanced CO reserve in inorganic nitrite treated subjects was entirely due to improved stroke volume reserve, as there was no effect on exercise heart rate.[45] Greater increases left ventricular stroke work with exercise were noted with inorganic nitrite, indicating an improvement in myocardial performance, independent of effects on cardiac loading.

More recently, Borlaug et al. have performed an open label pilot study testing the hemodynamic effects of inhaled nitrite (n=4, 80 mg dose) at rest and during exercise, using the same protocol as the intravenous nitrite study above.[45] Inhaled nitrite therapy produced similar increases in plasma nitrite levels as the intravenous formulation (Figure 6A), with similar reductions in PCWP at rest and during exercise as was observed in the intravenous study (Figure 6B).

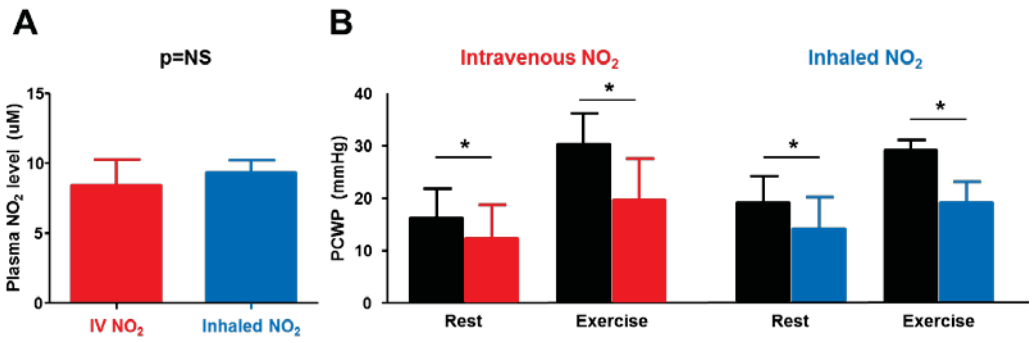


Figure 6: [A] Plasma nitrite (NO_2) levels are similar after intravenous (50 mcg/kg/min, red) and inhaled (90 mg, blue) doses. [B] Reductions in rest and exercise pulmonary capillary wedge pressure (PCWP) with IV/inhaled nitrite (red/blue) are of similar magnitude compared to respective values prior to study drug (black). *$p<0.0001$*

In the HFpEF exercise studies of both Zamani et al.[44] and Borlaug et al.[45] there was improved vasodilation (reduction in systemic vascular resistance) with exercise but no significant decrease in blood pressure with nitrate/nitrite, in contrast to marked reductions in arterial pressure previously noted with organic nitrates in HFpEF.[18] Nitrite infusion achieving plasma levels 30% higher than what will be tested in INDIE-HF had no effect on arterial blood pressure in a study of primates (95±3 vs 98±2 mmHg, p=0.8).[37]

Inhaled nitrite has been administered at a dose of 90 mg three times daily in healthy volunteers, with no hypotension and no orthostatic intolerance.[46] In an unpublished, open label study, 29 patients with Group 1 pulmonary hypertension were treated with inhaled nitrite for 16 weeks, with open label extension out to 57 weeks.[47] There were no withdrawals and no symptomatic hypotensive episodes. In this trial, systolic blood pressure was unchanged during treatment, from 117 (95% CI: 108, 126) mmHg at study entry to 114 (107, 121) mmHg at study completion (p=NS). Diastolic pressure similarly remained unchanged from 74 (68, 80) mmHg to 73 (67, 78) mmHg at study completion (p=NS).

7 BASIC STUDY DESIGN

The INDIE-HFpEF study is a randomized, double-blind, placebo-controlled crossover study to assess the effect of inorganic nitrite on peak exercise capacity (peak VO_2) as assessed by cardiopulmonary exercise testing (CPET).

7.1 Screening / Baseline

Patients with a diagnosis of HFpEF are screened for entry criteria. Willing participants meeting entry criteria will be consented and questioned to confirm that HF symptoms are the primary limitation to activity. If the participant does not meet this criteria, they will be considered screen failures and will not continue.

All consented participants continuing to meet the screening criteria will undergo baseline blood draws to include complete blood count (CBC), complete chemistry panel (Sodium, Potassium, Chloride, Carbon Dioxide, BUN, Creatinine, Glucose, AL, AST, Alkaline Phosphatase & Total Bilirubin), HFN biomarkers (cystatin C, NT-proBNP, cGMP and nitrosothiols), HFN biorepository and genetics samples (if agreed to participate). Afterwards, the participant will undergo a baseline HFN study-specific CPET to confirm Peak VO_2 ≤75% with peak respiratory exchange ratio ≥ 1.0. If the participant does not meet the CPET criteria, they will be considered screen failures and will not continue.

For participants that meet the above criteria, the remaining baseline studies will be performed: history and physical exam, NYHA class assessment, ECG and KCCQ.

Note: The baseline bloods are to be done prior to the CPET as the biomarker values can be affected from the exertion of the CPET. In the event this is not feasible, the blood draws will need to be obtained at least 3 hours post CPET but before the run-in test dose.

All participants will then undergo a single-dose, open label run-in to test study drug tolerance. Just prior to this test dose, a seated and standing BP must be obtained. If the seated SBP is <115 mmHg or the standing SBP is <90 mmHg the patient is considered a screen failure and should not receive the test dose.

Participants that pass the SBP criteria will receive one full dose (80 mg) of nebulized sodium nitrite with monitoring for 60 minutes following dosage for adverse effects or symptomatic hypotension. Systemic blood pressures will be monitored (seated and 3 minutes after standing) every 15 minutes for 60 minutes. Participants developing hypotension (SBP < 90mmHg seated OR standing), lightheadedness, or who otherwise do not tolerate the single-dose run-in will be considered run-in failures and will not be randomized.

7.2 Randomization

Eligible patients successfully completing baseline and run-in activities will then be randomized using procedures determined by the Coordinating Center (CC) to one of 2 treatment groups (placebo first with crossover to inorganic nitrite or inorganic nitrite first with crossover to placebo). A permuted block randomization method stratified by site will be used to ensure relatively equal distribution of participants to each arm within each clinical site.

Following randomization, participants will receive training in use of the nebulizer device and the accelerometry belt. Participants will be required to demonstrate capability to administer study drug using the nebulizer device on their own prior to dismissal from Study Visit 1.

7.3 Study Intervention Phase

Phase 1—Begins after Study Visit 1 which is considered day 0 of Phase 1 as follows:

- Participants will wear the accelerometer devices daily but take no study drug for 14 days (washout period).
- Study staff will call participants 4-7 days post-baseline visit to reinforce study procedures.
- Study staff will call participants 11-14 days post-baseline visit to reinforce study procedures.
- On day 15 post-baseline visit, participants will begin study drug at 46 mg, at minimum of 4 hours apart for 3 doses per day during the active portion of the participant's day. Participants will be encouraged to call study staff promptly to report any adverse reactions.
- Within 1-2 days of study drug initiation, study staff will call participants to discuss tolerability and potential issues with delivery and assess for adverse events.
    - In the case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue study drug.
    - For other intolerable side effects, study drug is discontinued but participants continue other study procedures.
- Study staff will call participants 18-21 days post-baseline visit to reinforce study procedures.
- On day 22 post-baseline visit, participants will increase study drug dose to 80 mg, at a minimum of 4 hours apart for 3 doses per day during the active portion of the

participant's day. Participants will be encouraged to call study staff promptly to report any adverse reactions.

- Within 1-2 days of study drug up-titration, study staff will call participants to discuss tolerability and potential issues with delivery and assess for adverse events.
    - In the case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue study drug.
    - For other intolerable side effects, the dose of study drug is reduced to the previously tolerated dose (46 mg) but participants continue other study procedures.
- Study staff will call participants 25-28 days post-baseline visit to reinforce study procedures.
- Study staff will call participants 32-35 days post-baseline visit to reinforce study procedures.
- Study staff will call participants 3-4 days prior to Study Visit 2 to reinforce study procedures and important details regarding Study Visit 2.
- During each call, participants will be encouraged to be active within the limitations imposed by their HF symptoms.
- Regardless of participant's ability to tolerate study drug or if the participant requires down-titration, study participants will begin Phase 2 as described below.

Phase 2—Begins with Study Visit 2 which is considered day 0 of Phase 2:

Study Visit 2 is performed at least 42 days but up to 49 days post-baseline visit. Participants should continue to wear the accelerometer and maintain study drug treatment up until Study Visit 2 regardless of timing of Study Visit 2.

At Study Visit 2:

- Study staff will confirm that the participant did not take study drug on the day of Study Visit 2 and undergo the following:
    - Conduct interim history, physical exam, NYHA class assessment and KCCQ.
    - Obtain blood draws prior to the CPET (in the event this is cannot be completed prior to study drug administration, then it cannot be obtained until at least 3 hours post study drug administration) :
        - CBC
        - Complete chemistry panel
        - Biomarkers
        - Biorepository (if agreed to participate)
    - Obtain limited echocardiography prior to study drug administration and CPET. (In the event, the echocardiography cannot be completed prior to study drug administration, then it cannot be obtained until at least 3 hours post study drug administration).
- Next, participants will undergo CPET with a dose of Phase 1 study drug administered immediately before the CPET under the supervision of study staff.
- Accelerometers are returned and changed out.
- Phase 1 study drug is returned.
- Phase 2 study drug is dispensed.

After Study Visit 2:

- Participants will wear the accelerometer devices daily but take no study drug for 14 days (washout period).
- Study staff will call participants 4-7 days post-Study Visit 2 to reinforce study procedures.

- Study staff will call participants 11-14 days post-Study Visit 2 to reinforce study procedures.
- On day 15 post-Study Visit 2, participants will begin study drug at 46 mg, at a minimum of 4 hours apart, for 3 doses per day, during the active portion of the participant's day. Participants will be encouraged to call study staff promptly to report any adverse reactions.
- Within 1-2 days of study drug initiation, study staff will call participants to discuss tolerability and potential issues with delivery and assess for adverse events.
    - In the case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue study drug.
    - For other intolerable side effects, study drug is discontinued but participants continue other study procedures.
- Study staff will call participants 18-21 days post-Study Visit 2 to reinforce study procedures.
- On day 22 post-Study Visit 2, participants will increase study drug dose to 80 mg, at a minimum of 4 hours apart for 3 doses per day during the active portion of the participant's day. Participants will be encouraged to call study staff promptly to report any adverse reactions.
- Within 1-2 days of study drug up-titration, study staff will call participants to discuss tolerability and potential issues with delivery and assess for adverse events.
    - In the case of study drug intolerance due to headache, participants will be encouraged to treat with acetaminophen and continue study drug.
    - For other intolerable side effects, the dose of study drug is reduced to the previously tolerated dose (46 mg) but participants continue other study procedures.
- Study staff will call participants 25-28 days post-Study Visit 2 to reinforce study procedures.
- Study staff will call participants 32-35 days post-Study Visit 2 to reinforce study procedures.
- Study staff will call participants 3-4 days prior to Study Visit 3 to reinforce study procedures and important details regarding Study Visit 3.
- During each call, participants will be encouraged to be active within the limitations imposed by their HF symptoms.
- Regardless of participant's ability to tolerate study drug or if the participant requires down-titration, study participants will return for Study Visit 3 as described below.

Completion—Begins with Study Visit 3:

Study Visit 3 is performed at least 42 days but up to 49 days post-Study Visit 2. Participants should continue to wear the accelerometer and maintain study drug treatment up until Study Visit 3 regardless of timing of Study Visit 3.

At Study Visit 3:

- Study staff will confirm that the participant did not take study drug on the day of Study Visit 3 and undergo the following:
    - Conduct interim history, physical exam, NYHA class assessment and KCCQ.

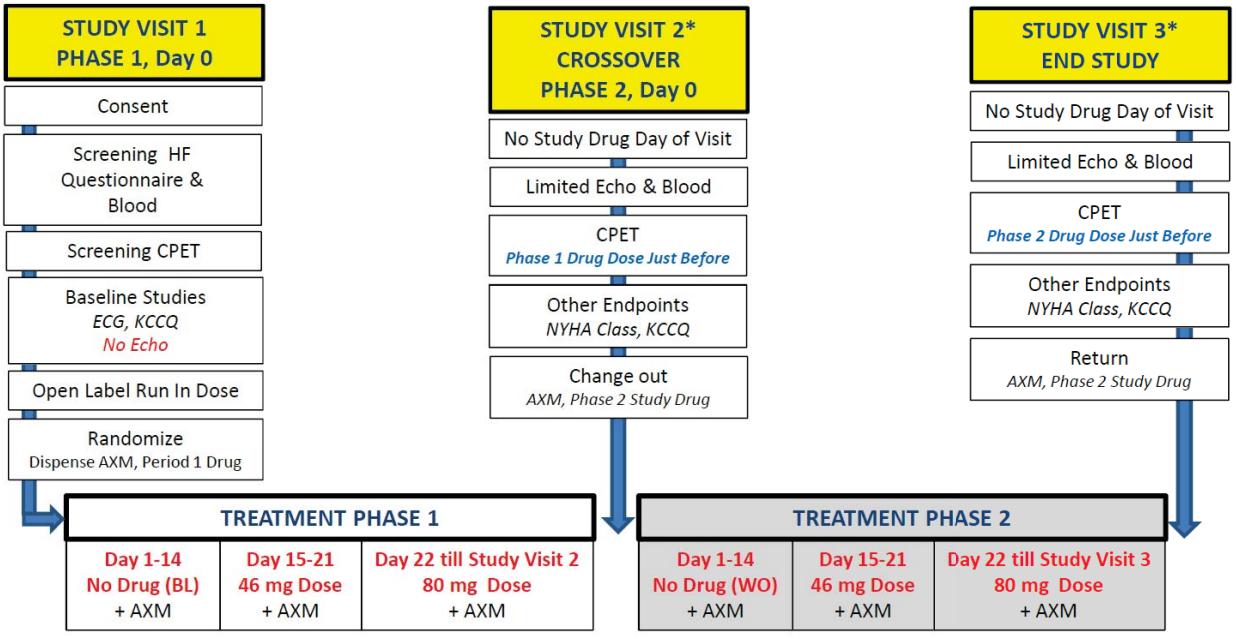
- Obtain blood draws prior to the CPET(in the event this cannot be completed prior to study drug administration, then it cannot be obtained until at least 3 hours post study drug administration) :
  - CBC
  - Complete chemistry panel
  - Biomarkers
  - Biorepository (if agreed to participate)
- Obtain limited echocardiography prior to study drug administration and CPET. (In the event, the echocardiography cannot be completed prior to study drug administration, then it cannot be obtained until at least 3 hours post study drug administration).

- Next, participants will undergo CPET with a dose of Phase 2 study drug administered immediately before the CPET under the supervision of study staff.
- Accelerometers are returned.
- Phase 2 study drug is returned.
- Participants are asked to indicate the study phase during which they felt better (Participant preference secondary endpoint).
- Participants will be reminded of 2 week follow-up phone call.

7.4 Follow-up Phase

A final phone visit is conducted 2 weeks after Study Visit 3 to inquire how they're feeling and to assess AEs and clinical stability.

8 STUDY FLOW DIAGRAM



**Study Visit 2 occurs 42-49 days post Study Visit 1*
**Study Visit 3 occurs 42-49 days post Study Visit 2*
BL = Baseline; WO = Washout; AXM = Accelerometer; CPET, cardiopulmonary exercise test

9 STUDY POPULATION AND ELIGIBILITY CRITERIA

9.1 Study Population

Patients suitable for this protocol are individuals with chronic HF who have normal ejection fraction (LVEF ≥ 50%).

9.2 Inclusion Criteria

1. Age ≥ 40 years
2. Symptoms of dyspnea (NYHA class II-IV) without evidence of a non-cardiac or ischemic explanation for dyspnea
3. EF ≥ 50% as determined on imaging study within 12 months of enrollment with no change in clinical status suggesting potential for deterioration in systolic function
4. One of the following:
    - Previous hospitalization for HF with radiographic evidence (pulmonary venous hypertension, vascular congestion, interstitial edema, pleural effusion) of pulmonary congestion **or**
    - Catheterization documented elevated filling pressures at rest (PCWP ≥15 or LVEDP ≥18) or with exercise (PCWP ≥25) **or**
    - Elevated NT-proBNP (>400 pg/ml) or BNP(>200 pg/ml) **or**
    - Echo evidence of diastolic dysfunction/elevated filling pressures manifest by medial E/e' ratio≥15 and/or left atrial enlargement **and** chronic treatment with a loop diuretic for signs or symptoms of heart failure
5. Heart failure is primary factor limiting activity as indicated by answering # 2 to the following question:

    My ability to be active is <u>most</u> limited by:
    1. Joint, foot, leg, hip or back pain
    2. Shortness of breath and/or fatigue and/or chest pain
    3. Unsteadiness or dizziness
    4. Lifestyle, weather, or I just don't like to be active

6. Peak VO_2 ≤75% predicted with peak respiratory exchange ratio≥1.0 CPET

	Normal Values for Peak VO_2*	Criteria (ml/kg/min) <75% Normal Value
20-29 M	43±7.2	< 32.3
20-29 F	36±6.9	< 27.0
30-39 M	42±7.0	< 31.5
30-39 F	34±6.2	< 25.5
40-49 M	40±7.2	< 30.0
40-49 F	32±6.2	< 24.0
50-59 M	36±7.1	< 27.0
50-59 F	29±5.4	< 21.8
60-69 M	33±7.3	< 24.8

60-69 F	27±4.7	< 20.3
> 70 M	29±7.3	< 21.8
> 70 F	27±5.8	< 20.3

*Fletcher GF et al. *Circulation*. 1995; 91:580-615.

7. No chronic nitrate therapy or not using intermittent sublingual nitroglycerin (requirement for >1 SL nitroglycerin per week) within last 7 days
8. No daily use of phosphodiesterase 5 inhibitors or soluble guanylyl cyclase activators and willing to withhold prn use of phosphodiesterase 5 inhibitors for duration of study
9. Ambulatory (not wheelchair / scooter dependent)
10. Body size allows wearing of the accelerometer belt as confirmed by ability to comfortably fasten the test belt provided for the screening process (belt designed to fit persons with BMI 20-40 kg/m^2 but belt may fit some persons outside this range)
11. Willingness to wear the accelerometer belt for the duration of the trial
12. Willingness to provide informed consent

9.3 Exclusion Criteria

1. Recent (< 1 month) hospitalization for heart failure
2. Ongoing requirement for PDE5 inhibitor, organic nitrate or soluble guanylyl cyclase activators
3. Hemoglobin (Hgb) < 8.0 g/dl within 90 days prior to randomization
4. GFR < 20 ml/min/1.73 m^2 within 90 days prior to randomization
5. Systolic blood pressure < 115 mmHg seated or < 90 mmHg standing just prior to test dose
6. Resting HR > 110 just prior to test dose
7. Previous adverse reaction to the study drug which necessitated withdrawal of therapy
8. Significant chronic obstructive pulmonary disease thought to contribute to dyspnea
9. Ischemia thought to contribute to dyspnea
10. Documentation of previous EF < 45%
11. Acute coronary syndrome within 3 months defined by electrocardiographic (ECG) changes and biomarkers of myocardial necrosis (e.g., troponin) in an appropriate clinical setting (chest discomfort or anginal equivalent)
12. PCI, coronary artery bypass grafting, or new biventricular pacing within past 3 months
13. Primary hypertrophic cardiomyopathy
14. Infiltrative cardiomyopathy (amyloid)
15. Constrictive pericarditis or tamponade
16. Active myocarditis
17. Complex congenital heart disease
18. Active collagen vascular disease
19. More than mild aortic or mitral stenosis
20. Intrinsic (prolapse, rheumatic) valve disease with moderate to severe or severe mitral, tricuspid or aortic regurgitation
21. Acute or chronic severe liver disease as evidenced by any of the following: encephalopathy, variceal bleeding, INR > 1.7 in the absence of anticoagulation treatment
22. Terminal illness (other than HF) with expected survival of less than 1 year

23. Regularly (> 1x per week) swims or does water aerobics
24. Enrollment or planned enrollment in another therapeutic clinical trial in next 3 months.
25. Inability to comply with planned study procedures
26. Pregnancy or breastfeeding mothers

10 TREATMENT INTERVENTIONS

10.1 Intervention

Inhaled, nebulized placebo or inhaled nebulized sodium nitrite starting at 46 mg and titrated up to 80 mg 3 times daily, at a minimum of 4 hours apart, with the first dose starting at the beginning of the active part of the day (for example, 8:00, 12:00, and 16:00). The Participant should disperse the doses by at least 4 hours and deliver them over their normal active day time. The doses do not need to be given at the same time each day if the Participant's active period varies by day, for example if the Participant arises later on the weekends.

Figure 7: The Phillips I-neb AAD nebulizer.

The drug or matching placebo is produced supplied in one ml squeezable plastic ampules. The ampules are produced in snap-apart groups of 5 vials place in a nitrogen filled pouch. Participants are instructed to open and utilize one pouch at a time. The medication is stable at room temperature. The Participant is instructed to twist off the top of a vial and squeeze the content into the medication chamber of the nebulizer. The metered, PURPLE latch medication chamber administers the lower dose of 46 mg. The WHITE latch medication chamber administers a full 80 mg dose of medication. Either chamber can be used with the nebulizer allowing two different doses to be delivered during the study protocol. Each Participant will be provided with a PURPLE and a WHITE study drug guide for use in the appropriate time in the protocol.

Inhaled, nebulized sodium nitrite or placebo will be administered utilizing the Phillips I-neb AAD nebulizer over 10-15 minutes for each dose. The I-neb is a small, battery-powered, lightweight, and virtually silent drug delivery device designed to deliver a precise, reproducible dose of drug. (Figure 7) Throughout the treatment, the I-neb provides continuous feedback to the Participant through a liquid crystal display, and upon successful delivery of the treatment, the Participant receives audible and tactile feedback.

The device has a Participant logging system that automatically records adherence and compliance with protocol-specified treatments. The device log will be reviewed by the research coordinator with study participants verbally to optimize adherence.

The time to maximal concentration of NO_2 in plasma is within 5 minutes, immediately after the end of the inhalation period (~15 minutes after start of inhalation). Maximal concentration after nebulization of an 80mg dose is 10-12 micromol/L, which is 2-3-fold higher than what is

achieved with oral NO_2 administration (PK data on file). The mean (SD) terminal half-life of nitrite in plasma in phase 1 testing was 0.6 (0.2) hours.[46]

Permitted dose adjustment: If significant headache occurs, participants will be encouraged to treat headaches with acetaminophen. If participants cannot tolerate the 46 mg dose, they will discontinue study drug but continue with all study procedures and visits. If Participants cannot tolerate the 80 mg dose, they will return to the previously tolerated 46 mg dose and continue all study procedures and visits. If a dose is missed, the 4 hour minimum regimen should be resumed as prescribed with the next scheduled dose. An extra dose or increase in dose should not be taken to make up for the missed dose.

If a participant has a temporary discontinuation for reasons other than intolerance, (e.g., such as hospitalization, lost device, etc), the investigator can restart at the last tolerated dose. If the last tolerated dose was 80mg, and depending on duration of interruption as well as any potential safety concerns, the Investigator will have the flexibility to restart at the reduced dose of 46 mg and up titrate to the 80 mg at their discretion. However, participants that down titrate due to intolerance should not be reintroduced to the higher dose.

Drug interactions: Potential excess nitric oxide mediated effects (e.g., hypotension, headache) might be observed with co-administration of organic nitrates or phosphodiesterase-5 inhibitors or soluble guanylyl cyclase activators. Therefore, co-administration of nitrates, any phosphodiesterase-5 inhibitor formulation or soluble guanylyl cyclase activators is strictly contraindicated due to the risk of hypotension. There are no other known drug interactions and study drug absorption is not affected by food or administration of other inhalers (inhaled steroids or bronchodilators).

10.2 Drug Dispensing

Drug dispensing will be managed by the CC who will obtain and store supplies from the contracted drug supply vendor. At the first study visit, participants will receive a sufficient supply of inorganic nitrite or placebo ampules to permit three doses a day until the second study visit, realizing that participants may not be able to return at the exact 42 day window due to scheduling conflicts (range 42-49 days). To account for potential damage or loss of ampules after opening the pouches containing multiple ampules and to account for unavoidable (for example adverse weather, family illness, etc) further delays in returning for study visits beyond the 42 day (+ up to 7 days) timeline, additional study drug doses will be supplied). Similarly, participants will receive enough inorganic nitrite or placebo ampules at the second study visit to last until the third (final) study visit as above.

Participants will be instructed to take the medication as required by the protocol, and compliance will be assessed at each visit by download of the automatic Participant logging system contained within the nebulizer device and by phone contact (as described in the protocol) between visits. Participants will be instructed to bring the nebulizer and unused drug supplies at each visit.

10.3 Storage, accountability and destruction

Trial products (both unused and in-use) should not be exposed to moisture but can be stored at room temperature. The plastic ampules come in a nitrogen filled foil pouch in snap-apart groups of 5 vials place. Participants are instructed to open one pouch at a time and utilize each ampule before opening another pouch.

10.4 Drug accountability

Participants are instructed to return all used, partly used and unused trial product at each study visit.

Returned trial product(s) (used, partly used or unused) must be stored separately from the non-allocated trial product(s) until drug accountability has been reconciled. The investigators will keep track of all received, used, partly used and unused trial products.

10.5 Destruction

Used and unused investigational product (study drug/nebulizer) can be destroyed at the site according to accepted pharmacy practice, local and national guidelines, using the site's destruction procedure. A copy of the current investigational product (IP) destruction SOP should be maintained in the pharmacy section of the Regulatory Binder and available for review in case of an audit.

Study IP must not be destroyed until the CRA has completed IP accountability and approved and accounted for the study IP to be destroyed. Study IP destruction should be documented in the within the Participant Specific IP Accountability Log (or equivalent tracking system).

10.6 Randomization, Stratification and Blinding

Randomization will occur at the first study visit. Randomization to active drug/placebo during the first phase of the crossover study (1:1 allocation ratio) is stratified by site. Blinding is ensured by preparation of identically appearing placebo and active drug ampules. Participants will be randomized using procedures determined by the CC to one of 2 sequences. A permuted block randomization method stratified by site will be used to ensure relatively equal distribution of participants to each sequences within each clinical site.

10.7 Unblinding

The investigative sites will be given access to the treatment code for their Participants for emergency un-blinding ONLY by calling the CC. Given the safety profile of inorganic nitrite it is anticipated that there should be no need to un-blind the study drug for any reason. Any suspected study drug-related events should be treated as though the Participant received active therapy. Nevertheless, in the rare event of necessary un-blinding, the CC medical monitor must be contacted to discuss a given case.

Randomization data are kept strictly confidential, accessible only to authorized persons, until the time of un-blinding.

10.8 Concomitant Medication

Participants should be treated with standard HFpEF strategies (diuretics for congestion, blood pressure control and heart rate control if Participant is in atrial fibrillation) as per recommended guidelines[48]. Participants should be on stable medications and with adequate blood pressure control prior to entry as outlined in the entry criteria. Further adjustment of diuretics or blood pressure medications during the study period is discouraged and should only be performed according to new and clinically compelling worsening of clinical status. As above, therapy with organic nitrates, phosphodiesterase-5 inhibitors or soluble guanylyl cyclase activators is contra-indicated during the study period.

10.9 Side Effect Risk Reduction Plan

Confirmation of tolerance of the 80 mg dose during the run-in phase, the use of acetaminophen for headache and the ability to down-titrate study drug dose (from the 80 mg dose to the 46 mg dose) if needed are expected to enhance tolerability.

11 RECRUITMENT AND SCREENING PROCEDURES

11.1 Common Recruitment Procedures

All participants admitted to the participating Heart Failure Clinical Research Network (HFN) centers with signs and symptoms suggestive of HFpEF will be screened by a member of the study staff. Patients meeting eligibility criteria will be approached regarding participation in this study.

11.2 Estimated Enrollment Period

This study will enroll approximately 100 participants at approximately 20 clinical centers in the U.S. The anticipated enrollment period is approximately 18 months.

11.3 Informed Consent Procedures

11.3.1 Informed Consent

HFN center clinicians will explain to eligible patients the purpose of the study, study interventions and evaluations, and the potential risks and benefits of participation, and will answer any questions. If a patient agrees to participate in the INDIE-HFpEF study, they will review and sign the site specific IRB approved informed consent form (ICF).

11.3.2 Confidentiality and HIPAA Requirements

All information collected on study participants will be stored in a confidential manner using the procedures in place at each participating center. Only approved study personnel will have access to data collected as part of the study. Consented study participants will be identified by a participant ID number on all study documents. Data will be transmitted to the CC in a secure manner, and stored securely at the CC using standard Duke Clinical Research Institute (DCRI) operating procedures.

11.3.3 Protections of Human Subjects

Protections for human subjects of research are required under Department of Health and Human Services (HHS) regulations at 21 CFR parts 50, 56, and 312.

11.3.4 Summary of the Risks and Benefits

Blood draws: The risks of drawing blood include bleeding at the puncture site, bruising and pain. These occur in a very small portion of the population.

Hypotension related: A potential adverse effect of inorganic nitrite is related to venous and arterial vasodilatation inducing lightheadedness, presyncope or syncope and headache. In prior human studies nitrite-mediated blood pressure reduction typically decreases over time. Elderly Participants may be at increased risk of such reactions. Syncope could be associated with trauma.

Cough: Cough and/or throat irritation may develop after the nebulizer therapy and can be managed symptomatically if needed (e.g., anti-tussives, lozenges). In prior human studies these symptoms have not been limiting.

Headache: Headache is a common and dose-related adverse effect associated with nitrate therapy. Both the severity and incidence of this effect appear to lessen with continued administration. Initiating therapy at a low dose and titrating up slowly is recommended to reduce the incidence of headache. Aspirin and acetaminophen have been successful in treating this headache.

This protocol may be hazardous to an unborn child: There are no well-controlled studies of inorganic nitrite to determine whether there are significant risks to a mother or the fetus carried by a mother who is participating in this study. Therefore, female participants must be postmenopausal or have been surgically sterilized or have a serum negative pregnancy test. Women of child bearing potential must agree to use an acceptable method of birth control during the study period.

This study involves administration of an agent (inorganic nitrite) with potential benefits in HFpEF. Thus, during the phase when a participant receives active study drug (inorganic nitrite) rather than placebo, they could potentially experience clinical benefit.

12 BASELINE EVALUATION AND RANDOMIZATION VISIT

A complete schedule of assessments throughout the study is given in Appendix A.

12.1 Screening

Patients will be screened for entry criteria at each site using existing clinical records including their most recent echocardiogram (echo within 12 months) and blood work. Potential participants will be scheduled for Study Visit 1.

12.2 Baseline/Randomization Visit (Visit 1)

Patients who fulfill entry criteria will undergo the consent process and written informed consent will be obtained prior to any study specific procedures. A screening question to assess whether their ability to be active is related to their heart failure symptoms (versus orthopedic, neurologic or behavioral factors) will be completed. If patients indicate that reasons other than heart failure symptoms limit their ability to be active, they will be considered a screen failure and will not continue. Subjects will be assessed for their ability to wear the accelerometer belt confirmed with a "test belt" provided to each center. Willingness to wear the accelerometer belt and to participate in all study procedures is confirmed.

Once confirmed, the participant will undergo baseline blood draws, to include CBC, complete chemistry panel, biomarkers, biorepository and genetics (if agreed to participate), and then an HFN study-specific, baseline CPET will be conducted to ensure patient meets the entry criteria. Patients that do not meet the CPET criteria will be considered a screen failure and will not continue. Note: The baseline bloods are to be obtained prior to the CPET as the biomarker values can be affected from the exertion of the CPET. In the event this is not feasible, the blood draws will need to be obtained 3 hours post CPET and prior to the run-in test dose.

Once confirmed, patients will undergo the remaining baseline studies (below) and undergo single dose unblinded run-in receiving 80 mg nebulized sodium nitrite followed by observation for 60 minutes. Patients that do not tolerate the run-in will not be randomized.

Qualifying patients will be randomized using procedures determined by the CC to one of 2 sequences (inorganic nitrite first or placebo first). Participants will be randomized in a 1:1 allocation ratio. The Participants will be educated in study procedures including wearing of the accelerometer and use of the nebulizer device. Participants will be required to demonstrate independent proficiency with the nebulizer prior to dismissal from Visit 1. Study drug for the first phase of INDIE-HFpEF will be dispensed.

Remaining baseline studies and procedures include:

- Complete Medical History and Medication Review
- Physical Examination and NYHA class assessment
- ECG
- KCCQ

Run-In Test Dose (post completion of baseline studies)

- Verify SBP $\geq$ 115 mmHg seated and $\geq$ 90 mmHg standing and resting HR $\leq$ 110 just prior to test dose (if not, the participant is considered a screen failure and will not receive the test dose).
- Participants passing the SPB and HR criteria receives open label run-in with sodium nitrite (80 mg) given once, with monitoring for AE for 60 minutes following dosage for adverse effects or symptomatic hypotension.
    - Systemic blood pressures (seated and 3 minutes after standing) will be monitored every 15 minutes for 60 minutes.

- Participants developing hypotension (SBP < 90 seated or standing), lightheadedness or who otherwise do not tolerate the single-dose run-in will be considered run-in failures and will not be randomized.

Randomization

- Participants that pass the run-in test, will be randomized to study drug assignment.
- Following randomization, study staff will dispense the accelerometer, nebulizer device and nebulizer supplies and educate participant on how to use the nebulizer device and supplies and on how to wear the accelerometer.
- Compliance plan: A plan for the phone visits will be established with the participant. Participant-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry and study drug administration.
- Dispense Phase 1 study drug—Participant must demonstrate proper administration of both the 46 mg dose and 80 mg dose.

13 FOLLOW-UP EVALUATIONS

13.1 Study Visits

Study Visit 2. Participants will return no sooner than 42 days (but up to 49 days) after the first study visit. Participants requiring extra time beyond the 42 day window must maintain on study drug during this period with no interruption in therapy.

Nebulizer records will be examined by the coordinator to determine compliance with study drug dosing and ensure proper usage. Participants will be instructed to hold the study drug the morning of the visit so that it can be administered in person at the time of the CPET with study staff present. Participants will undergo studies and procedures as below. The Study Visit 2 studies and procedures include:

- Confirm study drug held the day of the visit
- Interim History and Medication Review
- Physical Examination and NYHA class assessment
- KCCQ questionnaire
- Blood draw (prior to CPET) for CBC, Complete Chemistry Panel (Sodium, Potassium, Chloride, Carbon Dioxide, BUN, Creatinine, Glucose, AL, AST, Alkaline Phosphatase & Total Bilirubin) and HFN biomarkers (cystatin C, NT-proBNP, cGMP and nitrosothiols), and biorepository samples (if agreed to participate)
- Limited Echocardiogram prior to study drug administration (in the event this is cannot be completed prior to study drug administration, then it cannot be obtained until at least 3 hours post study drug administration)
- Cardiopulmonary exercise test (with administration of a dose of Phase 1 study drug at the CPET and in the presence of the Study Coordinator immediately before starting the CPET)
- Compliance plan: A plan for the phone visits will be established with the Participant. Participant-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry and study drug administration.
- Change out accelerometer
- Collect Phase 1 study drug and dispense Phase 2 study drug

Study Visit 3. Participants will return no sooner than 42 days (but up to 49 days) after Study Visit 2. Participants requiring extra time beyond the 42 day window must maintain on study drug during this period with no interruption in therapy.

Nebulizer records will be examined by the coordinator to determine compliance with study drug dosing. Participants will be instructed to hold the study drug the morning of the visit so that it can be administered in person at the time of the CPET with study staff present. Participants will undergo studies and procedures as below.

The Study Visit 3 studies and procedures include:

- Confirm study drug held the day of the visit
- Interim History and Medication Review
- Physical Examination and NYHA class assessment
- KCCQ questionnaire
- Blood draws prior to study drug administration (in the event this is not feasible, then it cannot be obtained for at least 3 hours post study drug administration): CBC, Complete Chemistry Panel and HFN biomarkers (cystatin C, NT-proBNP, cGMP and nitrosothiols), and biorepository samples (if agreed to participate)
- Limited Echocardiogram prior to study drug administration (in the event this is not feasible, then it cannot be obtained for at least 3 hours post study drug administration)
- Cardiopulmonary exercise test (with administration of a dose of Phase 2 study drug at the CPET and in the presence of the Study Coordinator immediately before starting the CPET)
- Return accelerometer
- Return Phase 2 study drug
- Participants are asked to indicate the study phase during which they felt better (Patient preference secondary endpoint)

Final study phone visit: A final phone visit is conducted 2 weeks after Study visit 3 to assess clinical stability and any adverse events.

13.2 Phone and Other Media Follow-up

General procedures: At Study Visit 1, participants and study staff will define optimal times and phone numbers for the protocol specified phone contacts to encourage compliance with study procedures.

During the follow-up phone visits, the participant will receive:

- Reminder of appropriate study drug dose for the stage of the protocol.
- Reminder regarding appropriate use of nebulizer device, dose specific study drug guide and compliance
- Encouragement of compliance with accelerometry use
- Encouragement of activity within the limits of their HF symptoms
- Confirm plans for future study visits
- Confirm need to bring study drug and accelerometers to future study visits
- Reminder to hold study drug on days of Study Visit 2 and 3.

Participants are also called 14 days (+ 5 days) after Study Visit 3 to assess clinical stability and any adverse events.

14 OUTCOME DETERMINATIONS

14.1 Primary Endpoint

The primary endpoint will be the peak VO_2 performed at peak drug levels after 4 weeks treatment with inorganic nitrite as compared to the peak VO_2 after 4 weeks treatment with placebo.

14.2 Secondary Endpoints

1. Standard HF endpoints:
    - Average arbitrary accelerometer units (AAU) during at least 14 days and up to 21 days of the maximally tolerated dose of study drug (from 28 days post Study Visit 1 until Study Visit 2 and from 28 days post Study Visit 2 until Study Visit 3).
    - Medial E/e' ratio at echocardiography
    - Left atrial volume index at echocardiography
    - Pulmonary artery systolic pressure at echocardiography
    - KCCQ
    - NT-proBNP
    - NYHA class
    - Other CPET endpoints including V_E/VCO_2 slope, VO_2 at VAT
    - Participant preference for study phase (active vs. placebo)
    - Safety and tolerability

14.3 Tertiary Endpoints

1. Pre-specified subgroup analyses will include examination of the primary endpoint in patients according to baseline characteristics:
    - NT-proBNP >400 pg/ml vs. ≤400 pg/ml
    - Systolic blood pressure above and below median value.
    - PASP>60 mmHg vs <60 mmHg
    - Age above and below median value.
    - Women vs. men
    - Estimated glomerular filtration rate by MDRD equation above and below median value
    - Subjects on active drug at study visit vs not on study drug at study visit
    - Subjects in atrial fibrillation/flutter vs not
    - Diabetic vs. non-diabetic patients
    - Patients with or without symptoms of exertional chest pain
2. Novel accelerometry endpoints (guided by HFN NEAT ancillary paper analysis)
3. Plasma nitrosothiols, cystatin-C, cGMP

15 METHODS TO PROMOTE ADHERENCE

15.1 Adherence to Study Procedures

Protocol training and adherence will be a major focus of the investigator training. Based on our experience in prior studies, identifying and correcting non-adherence is best accomplished in a stepped approach. The site will be responsible for managing participant compliance with study drug administration, accelerometer utilization and visit compliance. The CC will provide data quality reports for review and will follow-up to reemphasize the importance of adherence as needed. Routine data quality reports, will be provided to the sites and significant non-adherence issues will be discussed with the Executive Committee.

16 PARTICIPANT SAFETY AND ADVERSE EVENTS

16.1 Institutional Review Boards

All HFN sites will submit the study protocol, consent form, and other study documents to their IRB for approval. The approval letter for each clinical center will be reviewed by the CC. Any amendments to the protocol, other than minor administrative changes, must be approved by each IRB before they are implemented.

16.2 Definitions

16.2.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in a participant, whether or not considered drug or biologic related. An AE can therefore be any undesirable sign, symptom or medical condition occurring after starting study drug, even if the event is not considered to be related to the pharmaceutical product. Study drug includes the drug under evaluation, and any reference or placebo drug given during any phase of the trial. Headache, dizziness and lightheadedness are AEs that will be specifically questioned about during phone calls and study visits.

16.2.2 Suspected Adverse Reaction

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the drug caused the event. “Reasonable possibility” suggests there is a causal relationship between the drug and the adverse event. “Suspected adverse reaction” implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

16.2.3 Serious Adverse Events (SAE)

An adverse event or suspected adverse reaction is considered serious if the investigator or sponsor believes any of the following outcomes occurred:

- Death

- Life-threatening AE: Places the participant at immediate risk of death at the time of the event as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Inpatient hospitalization or prolongation of hospitalization.
- Congenital anomaly or birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition above.

This determination is based on the opinion of either the investigator or sponsor (e.g., if either believes it is serious, it must be considered serious).

16.2.4 Laboratory Test Abnormalities

For laboratory test abnormalities that meet the definition of an SAE, that required the participant to have the investigational product discontinued or interrupted or required the participant to receive specific corrective therapy, the clinical diagnosis rather than the laboratory term will be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

16.2.5 Assessment of Causal Relationship

A medically-qualified investigator must assess the relationship of any AE to the use of study drug, based on available information, using the following guidelines:

- **Not related:** There is not a reasonable causal relationship to the investigational product and the adverse event.
- **Unlikely related:** No temporal association or the cause of the event has been identified, or the drug or biologic cannot be implicated.
- **Possibly related:** There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
- **Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

16.2.6 Assessment of Adverse Event Severity

The determination of adverse event severity rests on medical judgment of a medically-qualified Investigator. The severity of AEs will be graded using the following definitions:

- **Mild:** Awareness of sign, symptom, or event, but easily tolerated;
- **Moderate:** Discomfort enough to cause interference with usual activity and may warrant intervention;
- **Severe:** Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.

16.2.7 Expectedness

The expectedness of an AE or SAR shall be determined according to the most current investigator's brochure. Any AE that is not identified in nature, severity, or specificity in the

current investigator's brochure is considered unexpected. Events that are mentioned in the investigator's brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but not specifically mentioned as occurring with the particular drug under investigation are considered unexpected.

16.3 Anticipated Disease Related Events

The following AEs are anticipated, disease-related events in patients with HF with preserved ejection fraction (HFpEF).

- **Arrhythmias:** This refers to both atrial and ventricular arrhythmias.
- **Acute coronary syndrome:** This refers to unstable angina, non ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial (STEMI).
- **Unplanned hospitalization, ER visit or clinic visit for worsening HF:** This refers to treatment for acute heart failure such as receiving intravenous diuretics.
- **Cerebrovascular event:** This refers to cerebrovascular accidents (stroke) of any cause (hemorrhagic, ischemic, or embolic) and transient ischemic attack (TIA).
- **Venous thromboembolism:** This includes both deep venous thrombosis and pulmonary embolus.
- **Worsening renal function:** This refers to acute kidney injury, typically defined as a rise in creatinine > 0.3 mg/dL over 48 hours, or progressive loss of renal function over time.

Anticipated disease related events will not be captured as AEs/SAEs during the study, but will be entered on the appropriate electronic case report form (eCRF) module ("Events of Interest" page).

16.3.1 Recording and Reporting of Adverse Events

The site Investigator is responsible for monitoring the safety of participants enrolled into the study at the study sites. Events significant enough to necessitate modification of study drug dosing will be captured on the AE eCRF.

All AEs/SAEs, except for those anticipated AEs listed above, occurring from signed informed consent to 2 weeks post visit 3 will be captured on the AE/SAE eCRF. Patients that have screen failed will be followed for AEs/SAEs until two weeks post last study drug dose. Unless exempted as described above**,** all AEs/SAEs, whether or not deemed drug-related or expected, must be reported by the investigator or qualified designee within 24 hours of first becoming aware of the event. For this study, all cause deaths will be reported on the SAE eCRF, as well as the Death eCRF page. The investigator or qualified designee will enter the required information regarding the AE/SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to DCRI Safety Surveillance. If the eCRF system is temporarily unavailable, the event, including the investigator-determined causality to study drug should be reported via the back-up paper SAE form to DCRI Safety Surveillance. Upon return of the availability of the electronic data capture (EDC) system, the SAE information must be entered into the eCRF.

Follow-up

When additional relevant information becomes available, the Investigator will record follow-up information according to the same process used for reporting the initial event as described above. The Investigator will follow all reportable events until resolution, stabilization or the event is otherwise explained.

DCRI Safety Surveillance will follow all SAEs until resolution, stabilization, until otherwise explained or until the participant completes the final follow-up, whichever occurs first. Investigators are also responsible for promptly reporting AEs to their reviewing IRB/EC in accordance with local requirements. The DSMB will review detailed safety data approximately every 6 months throughout the study.

16.3.2 Suspected Unexpected Serious Adverse Reaction

AEs that meet the criteria of serious, related to study drug, and unexpected per investigator brochure, qualify for expedited reporting to the regulatory authorities. DCRI will notify the FDA and all participating investigators in a written IND safety report of an SAR that is serious and is unexpected, based on the opinion of the investigator and DCRI safety medical monitor, as soon as possible, but not later than 15 calendar days after the event is confirmed to be a serious, unexpected SAR and qualifies for expedited reporting. DCRI will identify all safety reports previously filed with the IND concerning a similar SAR, and will analyze the significance of the SAR in light of the previous, similar reports. Follow-up reports will be sent to investigators to inform and update them about an important suspected adverse reaction if it significantly affects the care of the participants or conduct of the study.

Day Zero - Day zero (0) is the calendar day that DCRI is first notified of an event. Day zero can also be the date the event qualified for expedited reporting as determined by the DCRI Safety Medical Monitor.

16.3.3 Pregnancy

Pregnancy occurring during a clinical investigation, although not considered a serious adverse event, must be reported to DCRI within the same timelines as a serious adverse event. The pregnancy will be recorded on the appropriate paper pregnancy tracking form. The pregnancy will be followed until final outcome. Any associated AEs or SAEs that occur to the mother or fetus will be recorded in the AE/SAE eCRF, within the EDC system.

17 STATISTICAL CONSIDERATIONS

17.1 Overview

All planned analyses will be prospectively defined for this study and approved by the CC prior to unblinding of data. In addition, exploratory analyses will be performed to help explain and understand findings observed from the planned analyses. Statistical tests with a 2-sided p-value <0.05 will be considered statistically significant, unless otherwise stated. Analyses will be

performed using SAS software (SAS Institute, Inc, Cary, NC). The INDIE-HFpEF statistical analysis plan (SAP) will contain detailed information regarding the data analysis. The SAP will be finalized prior to trial completion and will be approved by the coordinating center statistical team as well as the NHLBI program officer.

17.2 Design and Analytical Criteria

The primary analysis will be conducted on an intention-to-treat (ITT) basis. The ITT population includes all participants who are randomized. The primary endpoint is based on the within-patient comparison of peak VO_2 following 4 weeks treatment with inorganic nitrite as compared to following 4 weeks treatment with placebo. It is anticipated that some data will be missing due to participant drop out or inability to complete both exercise tests.

The primary data analysis of the primary endpoint will involve a mixed model with fixed effect terms for the sequence, period and treatment. When available, the baseline value of the variable will be included in the model. A random effect term will be included to account for the correlated measurements within each participant. The estimated treatment effect will be provided with a 95% confidence interval. Given the short half-life of the drug (40 minutes), the two-week washout period, and the assumed lack of remodeling effects with this duration of treatment, we anticipate no significant carry-over or residual effect from Phase 1 to Phase 2.

Baseline data will be collected to characterize the study population and potentially to serve as a covariate in the analysis of the endpoints and to facilitate subgroup analysis, including tertiary endpoint analyses. As a sensitivity analysis, the data from the first period and second period will be presented and analyzed separately. Exploratory analyses will be conducted using the paired t-test and the t-test using the crossover design structure.

17.3 Analysis of Secondary and Tertiary Endpoints

Analysis of continuous outcomes will be conducted using mixed models as described above. For endpoints such as NT-proBNP, we will consider transformations of the data to obtain more valid 95% confidence intervals. For nominal variables, the number and percentages in each category will be presented. For binary outcomes, Chi-square tests and Fisher's exact test will be used for unadjusted comparisons.

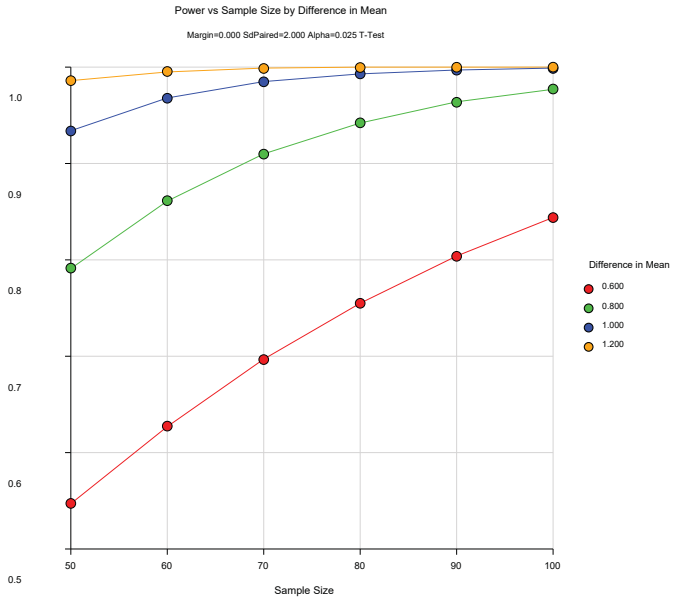
17.4 Analysis of Safety Data and Statistical Monitoring Plan

Interim data analysis for efficacy and futility will not be conducted due to relatively small size, short duration, and crossover structure of this clinical trial. Safety data, summarized at the treatment level, will be assessed approximately every 6 months by the National Heart, Lung, and Blood Institute (NHLBI)-appointed DSMB. The safety analyses will be based on the entire ITT population. Safety will be evaluated by comparing the occurrence of AEs. Based on the RELAX-HFpEF study performed in patients with HFpEF within the HFN, we expect the mortality rate over the 12-week study period to be less than 2% (Data on file at DCRI; RELAX trial analysis).

17.5 Sample Size and Power Calculation

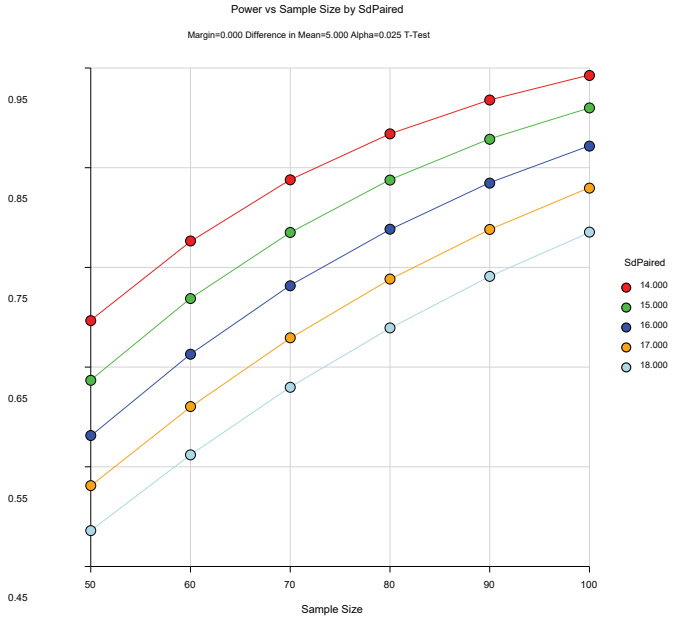
Peak VO_2: From prior HFN studies, the within-patient standard deviation for peak VO_2 in HFpEF is roughly 2 ml/min/kg.[REF 21] A 1.2 ml/min/kg increase in peak VO_2 is considered to be clinically meaningful for a particular patient. Assuming a 2 ml/min/kg standard deviation, a total of 90 participants is adequate to provide 80% power to detect a difference of 0.6 ml/min/kg in peak VO_2.[see Figure 8] The assumed treatment difference allows for not all of the participants to have a clinically meaningful difference. These calculations are based on a 2*2 factorial design and assume a 2-sided 0.05 (or 1-sided 0.025) type I error rate. The total sample size of 100 participants allows for approximately 10% incomplete data due to missed tests, death, and withdrawal of consent. The sample size estimates were calculated using nQuery and PASS software.

Figure 8. Power curve for the primary endpoint



KCCQ overall summary score: Clinically significant differences have been established for these endpoints. From earlier HFN studies, the within-patient standard deviation for the KCCQ overall summary score has ranged from 14 to 18 points. In the NEAT study, the within-patient standard deviation for the KCCQ overall summary score was approximately 14 points. For the KCCQ, a clinically-significant difference is considered 5 points and a moderately large clinical difference is considered to be 10 points. Assuming a 14 point standard deviation, a total of 70 participants is adequate to provide >80% power to detect a clinically-significant difference of 5 points in the KCCQ overall summary score. Assuming a 16 point standard deviation, a total of 90 participants is adequate to provide >80% power to detect a clinically-significant difference of 5 points in the KCCQ overall summary score. Assuming an 18 point standard deviation, a total of 100 participants (50 per sequence) is adequate to provide approximately 79% power to detect a clinically-significant difference of 5 points in the KCCQ overall summary score. [See Figure 9]

Figure 9. Power curve for the KCCQ overall summary endpoint



18 DATA MANAGEMENT PROCEDURES

18.1 Overview of Data Management

The CC will have primary responsibility for data management, including the development of data collection systems, data monitoring processes, and data storage and back-up. State-of-the-art technology will be used for the management of the network's data.

18.2 Data Security

Access to databases will be controlled centrally by the CC through user passwords linked to appropriate privileges. This protects the data from unauthorized changes and inadvertent loss or damage. Database and web servers will be secured by a firewall and through controlled physical access. Database back-up will be performed daily using standard procedures in place at the CC. All disk drives that provide network services, and all user computers, will be protected using virus-scanning software.

18.3 Publication Policy

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, investigators will not be allowed to perform subset analyses at any point before the conclusion of the study, and any data, other than safety data, cannot be used for publication or reporting outside of this study until the study is completed or discontinued by the DSMB or HFN Steering Committee.

19 STUDY ADMINISTRATION

19.1 Data and Safety Monitoring Board

A DSMB has been appointed by the NHLBI for the HFN, and will function as the DSMB for this trial. This committee consists of a group of highly experienced individuals with extensive pertinent expertise in HF and clinical trials. The DSMB will advise the HFN Steering Committee regarding the continuing safety of current participants and those yet to be recruited, as well as the continuing validity and scientific merit of the trial. Safety data, summarized at the treatment level, will be assessed approximately every 6 months by the DSMB. The safety analyses will be based on the entire ITT population. Safety will be evaluated by comparing the occurrence of AEs and changes in laboratory values of the active arm compared to placebo.

19.2 Coordinating Center

The DCRI will function as the CC for this trial as specified by the National Institute of Health and NHLBI HFN grant.

19.3 Core Laboratories

19.3.1 Cardiopulmonary Exercise Testing Core Laboratory

The Harvard DCC will serve as the core laboratory for interpretation of cardiopulmonary exercise tests.

19.3.2 Biomarker Core Laboratories

The University of Vermont will serve as the core laboratory for measurement of HFN biomarkers. Plasma specimens will be collected at Study Visits 1-3, processed at the clinical centers according to the procedures provided by the core laboratory, and shipped to the core laboratory on dry ice (Refer to Biomarker Core Laboratory Manual of Procedures). Any patients who agree to participate in the HFN genetics biorepository will have samples collected at the clinical sites, stored at the University of Vermont biomarker core laboratory, and subsequently shipped to the HFN Genetic Core Laboratory at the University of Montreal for processing.

19.3.3 Echocardiograph Core Laboratory

A limited echocardiography will be performed at the clinical sites at visits 2 and 3. All echocardiograms will be reviewed at the Mayo Clinic echocardiographic core laboratory using the techniques described in the Echocardiography Manual of Operations for the HFN.

19.3.4 Physical Activity Measurement Core Laboratory

The Mayo Clinic will serve as the core laboratory for the production and distribution of accelerometry devices, and will provide training to the sites in procedures related to accelerometer devices. At the completion of each phase, the devices will be returned to the Physical Activity Measurement Core Laboratory for downloading of accelerometry data, analysis and transmittal of data to the CC (refer to Physical Activity Measurement Core Laboratory Manual of Procedures).

20 REGULATORY ISSUES

20.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol. These procedures are designed to ensure adherence to Good Clinical Practice, as described in the following documents:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).

Participating investigators agree to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

20.2 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to participants must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC). Documentation that the protocol and informed consent have been approved by the IRB/IEC must be given to the Coordinating Center before study initiation. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

20.3 Informed Consent

The investigator or designee must explain to each participant (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each participant must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

The informed consent form(s) must be submitted by the investigator for IRB/IEC approval. The Coordinating Center will supply template informed consent forms, which comply with regulatory requirements, and are appropriate for the study. Any changes to the template consent form suggested by the Investigator must be agreed to by the Coordinating Center before submission to the IRB/IEC, and a copy of the approved version must be provided to the Coordinating Center after IRB/IEC approval.

21 Monitoring

A CC monitor will visit sites during the enrollment period to verify that data collection is being handled properly. In addition, monitors may provide in-service training, review source, and address questions from site investigators and coordinators as needed. Remote monitoring activities will also be conducted to monitor compliance with the study protocol.

22 Protocol Amendments after Trial Commencement

Amended Protocol v.2.0 dated Nov 10, 2016 –Included are the following changes:

- Section 9.2, Inclusion Criteria #7 - "No chronic nitrate therapy or not using intermittent sublingual nitroglycerin (requirement for >1 SL nitroglycerin per week) within last 6 months" was modified to be "within last 7 days". The reduction in the drug use period for a minimum of 1 week offers no risk to patients and will enrich the population of potentially eligible participants.

- Section 9.3, Exclusion Criteria #3 and #4 – modified exclusion timeframe from "30 days" to "90 days" prior to randomization:

    - Hemoglobin (Hgb) < 8.0 g/dl within 90 days prior to randomization
    - GFR < 20 ml/min/1.73 m2 within 90 days prior to randomization

This increased timeframe will still promote the elimination of subjects whose symptoms may be related to anemia and/or kidney function instead of heart failure and will remove an added burden of having to unnecessarily obtain new lab values prior to the run-in test dose.

- Section 7.3, Study Visit 3 procedures, page 22 - states participants will be dosed with their "Phase 1 drug" prior to CPET. This was corrected to state participants will be dosed with their "Phase 2 drug" which is correctly stated on page 22, Section 8 and page 32, Section 13.1.

- Section 13.2, last sentence - modified from "Participants are also called 14 days + 5 days after Study Visit 3 to assess clinical stability and any adverse events" to "Participants are also called 14 days (+ 5 days) after Study Visit 3 to assess clinical stability and any adverse events" to ensure that follow-up is at least 14 days after last dose.

- Appendix A, Schedule of Assessments table

o The visit days listed for Study Visit Window Day 22 for Phase 2 was corrected from "15-21" to "22-28"

o Study Visit Window – "Day 17" modified to "Day 16-17" for both Phase 1 and Phase 2 to be consistent with protocol section 7.3.

o Added a footnote reminder, to Visit 1 for the Test Dose (80 mg Run-in) & BP Observation (60 mins)6 , to emphasize AE collection requirements as noted in section 16.3.1 of the protocol: "^^^Run-in failures must be contacted 2 weeks after dosing for adverse event assessment".

- Section 21 – Updated from "Remote Monitoring" to reflect on-site activities in addition to the remote activities. This section now reads:

"Monitoring

A CC monitor may visit sites during the study to verify that data collection is being handled properly. In addition, monitors may provide in-service training, review source, and address questions from site investigators and coordinators as needed. Remote monitoring activities will also be conducted to monitor compliance with the study protocol."

23 Appendix A – Schedule of Assessments

Study Visit Window	Phase 1											Phase 2												2 Week Follow-up ***
	Study Visit 1 (-3 to 0) Baseline	Day 4-7 (Washout)	Day 11-14 (Washout)	Day 15	Day 16-17	Day 18-21	Day 22	Day 23-24	Day 25-28	Day 32-35	Day 38-41	Study Visit 2*	Day 4-7 (Washout)	Day 11-14 (Washout)	Day 15	Day 16-17	Day 18-21	Day 22	Day 23-24	Day 25-28	Day 32-35	Day 38-41	Study Visit 3**	
Days	0	1-7	8-14	15-21	15-21	15-21	22-28	22-28	22-28	29-35	36-42	42-49	1-7	8-14	15-21	15-21	15-21	22-28	22-28	22-28	29-35	36-42	42-49	14 days Post Visit 3
Study Drug Dose (Nitrite vs Placebo)				46mg	46mg	46mg	80mg	80mg	80mg	80mg	80mg	80mg			46mg	46mg	46mg	80mg	80mg	80mg	80mg	80mg	80mg	
Screening (Inclusion/Exclusion)	X																							
Informed Consent*	X																							
Phone Follow-Up		X	X		X	X		X	X	X	X		X	X		X	X		X	X	X	X		X
Clinical Evaluation																								
Complete Medical History [1]	X																							
Interim Medical History		X	X		X	X		X	X	X	X	X				X	X		X	X	X	X	X	
Physical Exam	X											X											X	
NYHA Class	X											X											X	
Medication Review	X											X											X	
12-Lead ECG	X																							
Systolic BP[2]	X																							
Laboratory Evaluation																								
Serum Pregnancy Test	X																							
CBC	X^^											X[3]											X[3]	
Complete Chemistry Panel[4]	X^^											X[3]											X[3]	
Core-Biomarkers & Repository	X^^											X^[3]											X^[3]	
Imaging Evaluation																								
Limited Echo[3]												X[3]											X[3]	
Exercise Evaluation																								
CPET[5]	X											X											X	
QOL Evaluation																								
KCCQ	X											X											X	
Study Drug and Accelerometer																								
Test Dose (80 mg Run-in) & BP Observation (60 mins)[6]	X^^^																							
Randomization**	X																							
Dispense/Change out Accelerometer and Instruction	X											X												
Dispense Study Drug, Device and Instructions	X											X												
Return Accelerometer (AXM), Study Drug and Device												X											X	
Study Drug Initiation				X											X									
Study Drug Tolerance	X				X	X		X	X	X	X	X				X	X		X	X	X	X	X	
Up-titration (if tolerated)							X											X						
Study Drug / AXM Adherence		AXM only	AXM only		X	X		X	X	X	X	X	AXM only	AXM only		X	X		X	X	X	X	X	
Adverse Events	X	X	X		X	X		X	X	X	X	X	X	X		X	X		X	X	X	X	X	X

*Consented patients will be screened through IXRS
**Only patients who have completed all inclusion/exclusion criteria, baseline studies and tolerated the test dose will be randomized through IXRS
*Visit 2 is expected to be completed 42-49 days post Study Visit 1
**Study Visit 3 is expected to be completed 42-49 days post Study Visit 2
***2 Week Follow-up visit is expected to be completed 14 days post Study Visit 3
^ Biomarkers & Biorepository only (genetics is only required at Visit 1)
^^Baseline bloods must be obtained prior to CPET (in the event that is not feasible, then bloods must be at least 3 hours post CPET but prior to the run-in test dose)

[1]Includes etiology and duration of HF
[2]BP should be obtained just prior to test dose to confirm run-in eligibility
[3]Obtain prior to study drug administration (in the event, this has to be scheduled after , then it must be at least 3 hours post last dose of study drug administered)
[4] Sodium, Potassium, Chloride, Carbon Dioxide, BUN, Creatinine, Glucose, ALT, AST, Alkaline Phosphatase & Total Bilirubin
[5]HFN study-specific CPET: Baseline- within 3 days prior to randomization; Visit 2 & 3 – obtained immediately post study drug administration
[6]BP(seated and standing) obtained every 15 minutes for 60 minutes; patients are not to be randomized if SBP < 90 mmHg
^^^Run-in failures must be contacted 2 weeks after dosing for adverse event assessment.

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