Protocol for the Heart Failure Clinical Research Network

Nitrate's **E**ffect on **A**ctivity **T**olerance in **H**eart **F**ailure with **P**reserved **E**jection **F**raction
NEAT-HFpEF

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List of Abbreviations

Abbreviation	Definition
6MWD	6-minute walk distance
6MWT	6-minute walk test
AAU	Arbitrary accelerometry units
AAU_{14}	14-day averaged arbitrary accelerometry units
ACC	American College of Cardiology
AE	Adverse event
AHA	American Heart Association
ARB	Angiotensin receptor blocker
BB	Beta blocker
CC	Coordinating Center
CI	Cardiac index
CO	Cardiac output
COPD	Chronic obstructive pulmonary disease
DCRI	Duke Clinical Research Institute
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EF	Ejection fraction
cGMP	Cyclic guanosine monophosphate
HF	Heart failure
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HFN	Heart Failure Clinical Research Network
HHS	Department of Health and Human Services
HR	Heart rate
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISDN	Isosorbide dinitrate
ISMN	Isosorbide mononitrate
ITT	Intention to treat
KCCQ	Kansas City Cardiomyopathy Questionnaire
LV	Left ventricular
MLHFQ	Minnesota Living with Heart Failure Questionnaire
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NHLBI	National Heart, Lung, and Blood Institute
NYHA	New York Heart Association
PE	Physical examination
QOL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized clinical trial
ROS	Reactive oxygen species
SAE	Serious adverse event
SAR	Suspected adverse reaction
SBP	Systolic blood pressure
SV	Stroke volume
VO_2	Volume of oxygen

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1 EXECUTIVE SUMMARY

Title	**N**itrate's **E**ffect on **A**ctivity **T**olerance in **H**eart **F**ailure with **P**reserved **E**jection **F**raction (NEAT-HFpEF)
Indication	Heart failure with preserved ejection fraction
Location	Approximately 20 clinical centers in the United States
Brief Rationale	Approximately 50% of patients have clinical heart failure with preserved ejection fraction (HFpEF). No therapy has been proven to relieve symptoms or improve outcomes in HFpEF. The physiology of HFpEF is unique with increased systolic and diastolic left ventricular and vascular stiffness, endothelial dysfunction and impaired systolic and diastolic reserve function, which contribute to exercise intolerance. Use of nitrates for symptom relief in patients with HFpEF is endorsed by the American Heart Association and American College of Cardiology guidelines and the Heart Failure Society of America guidelines for heart failure management. However, there are no data to support this recommendation (expert opinion only). Efficacy, tolerance and dose response are undefined. Evidence of equipoise as to efficacy of nitrates for symptom relief in HFpEF comes from a community-based HFpEF cohort and a large randomized clinical trial in HFpEF where only 20-25% of HFpEF patients were on nitrates. Beneficial effects of nitrates in ischemic heart disease or in heart failure with reduced ejection fraction (HFrEF) cannot be assumed to be equivalent in HFpEF. Some studies suggest that nitrates may enhance arterial compliance while other studies suggest that nitrates may induce or worsen endothelial dysfunction, which may prominently contribute to pathophysiology of HFpEF. While nitrate-induced preload and afterload reduction may lower activity-related increases in filling pressures, preload and afterload reduction may result in disproportionate reduction in cardiac output owing to the steep end-systolic pressure volume relationship in HFpEF. Many HFpEF patients are elderly, and typically, nitrates are not well-tolerated in this population. Further, HFpEF patients have autonomic dysfunction including chronotropic incompetence and reduced baroreceptor function which may heighten intolerance to nitrates. Accelerometry-assessed daily activity is an endpoint that provides patient-centric, high density, quantitative data on daily physical activity, which should increase in response to interventions that improve exercise tolerance.
Study Design	A randomized, double-blinded, placebo-controlled crossover study to assess effect of isosorbide mononitrate with dose up-titration on activity

	tolerance as assessed by (hip-worn, tri-axial) accelerometry. Approximately 100 participants will be enrolled in this 2*2 crossover study.
Treatment	Once daily isosorbide mononitrate vs. placebo with dose up-titration (30 to 120 mg/day over 4 weeks).
Primary Objective	To evaluate whether isosorbide mononitrate increases daily activity as assessed by 14-day averaged arbitrary accelerometry units in comparison to placebo.
Secondary Objectives	1.To evaluate whether isosorbide mononitrate improves functional capacity, quality of life and natriuretic peptide levels in comparison to placebo as measured by: • 6-minute walk distance • Borg score during 6-minute walk test • Quality of life (Kansas City Cardiomyopathy Questionnaire score) • N-terminal pro-B-type natriuretic peptide level 2. To evaluate whether isosorbide mononitrate in comparison to placebo improves daily activity as measured by additional accelerometry endpoints: • Hours active per day during maximal dose of study drug • Slope of daily averaged arbitrary accelerometry units during study drug administration • Area under the curve of daily averaged arbitrary accelerometry units during study drug administration 3. To evaluate whether patients prefer isosorbide mononitrate at the end of study.
Primary Endpoint	14-day averaged arbitrary accelerometry units during maximally-tolerated dose of study drug (comparison of weeks 5-6 and 11-12).
Secondary Endpoints	1. Standard HF endpoints: • 6-minute walk distance • Borg score during 6-minute walk test • Quality of life (Kansas City Cardiomyopathy Questionnaire score) • N-terminal pro-B-type natriuretic peptide level 2. Alternate accelerometry endpoints: • Hours active per day during maximal dose of study drug • Slope of daily averaged arbitrary accelerometry units during study drug administration • Area under the curve of daily averaged arbitrary accelerometry units during study drug administration 3. Participant preference for active study drug at end study

Abbreviated Study Flow	**Screen potential HFpEF patients for eligibility criteria and interest** **Week 0: Study visit 1:** • Administer consent form • Perform the following: Review history, physical exam, NT-proBNP, Kansas City Cardiomyopathy Questionnaire, Minnesota Living with Heart Failure Questionnaire and 6-minute walk test • Review accelerometer instructions and dispense devices • Randomization • Dispense phase-1 study drug: o Weeks 1 and 2: No study drug (baseline) o Week 3: 30 mg ISMN or placebo o Week 4: 60 mg ISMN or placebo † o Weeks 5 and 6: 120 mg ISMN or placebo † • Call participant weekly to enhance compliance with study procedures † If side effects develop, stop or return to previously tolerated dose **Week 7: Study visit 2:** • Perform the following: Review interim history, physical exam, NT-proBNP, Kansas City Cardiomyopathy Questionnaire, Minnesota Living with Heart Failure Questionnaire and 6-minute walk test • Accelerometer change out • Dispense phase-2 study drug: o Weeks 7 and 8: No study drug (washout) o Week 9: 30 mg ISMN or placebo† o Week 10: 60 mg ISMN or placebo † o Weeks 11 and 12: 120 mg ISMN or placebo † • Call participant weekly to enhance compliance with study procedures † If side effects develop, stop or return to previously tolerated dose **Week 13: Study visit 3**: • Perform the following: Review interim history, physical exam, NT-proBNP, Kansas City Cardiomyopathy Questionnaire, Minnesota Living with Heart Failure Questionnaire • Return accelerometer • End of study drug (phase out) **Week 15: Phone visit and end of study**

2 OBJECTIVES AND HYPOTHESES

2.1 *Primary Objectives*

To evaluate whether isosorbide mononitrate (ISMN), compared to placebo, increases daily activity as assessed by 14-day averaged arbitrary accelerometry units (AAU_{14}).

The primary hypothesis of the NEAT-HFpEF study is that ISMN, compared to placebo will improve daily activity as assessed by AAU_{14} during the maximally-tolerated dose of study drug (comparison of weeks 5-6 and 11-12).

The significance of this study is that it will provide evidence as to whether nitrate therapy improves symptoms in patients with HFpEF, and thus support or refute guideline recommendations that are based solely on expert opinion.

2.2 *Secondary Objectives*

1. To evaluate whether ISMN, compared to placebo, improves functional capacity, quality of life (QOL) and natriuretic peptide levels as measured by:
    - Six-minute walk distance (6MWD) (higher with ISMN vs. placebo phase)
    - Borg score during 6-minute walk test (6MWT) (lower with ISMN vs. placebo phase)
    - QOL (Kansas City Cardiomyopathy Questionnaire [KCCQ] score) (higher with ISMN vs. placebo phase)
    - NT-proBNP level (lower with ISMN vs. placebo phase)

2. To evaluate whether ISMN, compared to placebo, improves daily activity as measured by additional accelerometry endpoints:
    - Hours active per day during maximally-tolerated dose of study drug (comparison of weeks 5-6 and 11-12)
    - Slope of daily-averaged arbitrary accelerometry units (AAU) during study drug administration (comparison of weeks 3-6 and 9-12)
    - AUC of daily-averaged AAU during study drug administration (comparison of weeks 3-6 and 9-12)

3. To test the hypothesis that patients will prefer the active drug phase of the study.

2.3 *Tertiary Objectives*

1. To determine whether the following subgroups of patients that have heart failure (HF) with preserved ejection fraction (HFpEF) derive differential benefit from ISMN:
    a) Patients treated or not treated with drugs known to ameliorate nitrate tolerance (renin-angiotensin-aldosterone system [RAAS] antagonists, carvedilol, statins, hydralazine).
    b) Patients with baseline N-terminal pro-B-type natriuretic peptide (NT-proBNP) above and below the median.

c) Patients with systolic blood pressure (SBP) above and below the median.
d) Patients with or without known coronary artery disease.

2. To determine whether ISMN improves symptoms of HF as determined by the quotient of Borg Score and 6MWD during the 6MWT.
3. To evaluate whether ISMN improves QOL as assessed by the Minnesota Living with HF Questionnaire (MLHFQ).
4. To evaluate whether ISMN increases plasma levels of cyclic guanosine monophosphate (cGMP).
5. To determine whether increasing doses of ISMN are associated with increasing AAU
6. To determine the relationship between accelerometry assessed activity and standard measures of heart failure severity (NYHA class, 6MWD, KCCQ score and NT-proBNP levels) at baseline
7. To determine the relationship between changes in accelerometry assessed activity and changes in standard measures of heart failure severity (NYHA class, 6MWD, KCCQ score and NT-proBNP levels) over the different study periods.

3 BACKGROUND AND SIGNIFICANCE

Symptom relief in HFpEF is a critical unmet need: While age and sex-specific HF incidence is not increasing,[1] overall HF survival has improved and the number of persons over age 65 is rapidly increasing. Thus, the absolute number of patients with HF will continue to increase. Currently, half of all patients with HF have a preserved ejection fraction (HFpEF).[2-4] The proportion of HF patients with preserved ejection fraction (EF) is increasing.[2] Resource use associated with HF is high in both the inpatient and outpatient settings, regardless of EF.[5]

While improvement in mortality and morbidity in HFpEF remains an important goal, equally important is the need to improve symptoms. Persistent and progressive impairment in exercise tolerance and dyspnea are well documented in HFpEF and these symptoms limit QOL.[6] While both the American College of Cardiology and the American Heart Association (ACC/AHA) and the Heart Failure Society of America HF guidelines suggest that nitrate therapy may improve symptoms in HFpEF, there are no data to support this recommendation and there remains significant concern over efficacy and tolerance of nitrates in HFpEF as outlined below. The ACC/AHA guidelines lack any class I, evidence-level A recommendations for pharmacological therapy in HFpEF. Thus, evidence based therapy for symptom relief in HFpEF is a critical unmet need and in response, the primary objective of NEAT-HFpEF is to determine whether nitrates improve activity tolerance in HFpEF.

Therapies with proven benefit in HF with reduced ejection fraction (HFrEF) have failed to improve outcomes in HFpEF: The cardiovascular system responds to a wide variety of insults (e.g. myocardial disease, ischemia, valve or pericardial disease) in a finite number of ways, both hemodynamically (elevated filling pressures, depressed output) and symptomatically (dyspnea, fatigue, chest pain). However, these similarities in clinical expression do not indicate that the underlying mechanisms of disease are the same, nor that response to treatment will be similar.

While survival for patients with HFrEF has improved over the past two decades, there has been no improvement in HFpEF survival.[2] RAAS antagonists, beta blockers (BB) and digoxin have all been proven to have benefit in HFrEF.[7] Three large trials of RAAS antagonists in HFpEF failed to show an impact on outcomes, individually or when data was pooled.[8] A recent trial of enalapril in elderly patients with HFpEF reported no improvement in exercise capacity, aortic distensibility or neurohormonal profile compared with placebo.[9] Observational data has failed to demonstrate reduced risk of mortality or hospitalization in association with discharge angiotensin-converting enzyme inhibitor (ACEI)/angiotensin reception blocker (ARB) use in HFpEF, in striking contrast to reductions in events observed in HFrEF.[10] An ancillary analysis of the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) showed that chlorthalidone reduced incidence of both HFpEF and HFrEF compared with amlodipine and doxazosin; yet lisinopril reduced incidence of HFrEF, but not HFpEF.[11]

The efficacy of BB use in HFpEF remains unresolved.[10] Observational studies demonstrated no reduction in morbidity and mortality with discharge BB use in short term or long term follow up in HFpEF, in contrast to HFrEF where significant reductions in maladaptive remodeling, HF hospitalizations and mortality are observed with BB in both registry[10,1210, 12] and trial data[7,10,12]. Ancillary analysis from the Study of the Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in Seniors With Heart Failure (SENIORS) suggested the benefits of the BB nebivolol were also observed in the patients with EF>35%,[13] though few patients in the trial had EF >50%. A recent observational study noted that women with HFpEF discharged on BB had higher 6-month rehospitalization rates compared with those not prescribed BB.[14] The effects of BB on cardiomyocytes appear to differ in HFpEF and HFrEF, with higher resting tension observed in HFpEF patients treated with BB, but no adverse BB effect on myocyte stiffness in HFrEF.[15]

In an ancillary analysis of patients with HFpEF in the Digitalis Investigation Group (DIG) trial, digoxin did lower HF hospitalization;[16] however, this benefit was overcome by an equivalent increase in coronary syndrome hospitalizations.[17] Other therapies with proven benefit in HFrEF, such as aldosterone antagonists or devices, are investigated less frequently in HFpEF. Revascularization for triple vessel disease among patients with reduced EF is associated with improved survival.[18] The role of revascularization is less well-studied in HFpEF, though a case series found that episodes of pulmonary edema tend to recur despite revascularization in HFpEF.[19]

These studies underscore that therapies proven beneficial in HFrEF may not be efficacious in patients with HFpEF owing to the unique pathophysiology present in HFpEF as outlined below.

Selection of appropriate endpoints and study design for the smaller studies feasible in the Heart Failure Clinical Research Network (HFN) is challenging, particularly in HFpEF: The HFN aspires to enhance productivity in the second cycle of funding by conducting several small, but informative trials in an expeditious and fiscally responsible manner. There are only nine regional clinical centers in the HFN and the recruitment potential is limited. Appropriate endpoints are needed to provide “proof of concept” in phase II studies of novel therapies in HF

or for studies designed to provide an evidence base for current guideline recommendations based on expert opinion. These challenges are particularly acute in HFpEF. For example, the trial, Evaluating the Effectiveness of Sildenafil at Improving Health Outcomes and Exercise Ability in People With Diastolic Heart Failure (RELAX) began in the first cycle of the HFN, and stipulated rigorous entry criteria with a primary endpoint of peak volume of oxygen (VO_2) as assessed by cardiopulmonary exercise testing. The trial was successfully completed and enrolled its target of 216 patients, but required 3.5 years of recruitment. The HFN Investigators consider HFpEF a high priority and have carefully considered the barriers encountered in RELAX when designing NEAT-HFpEF. Barriers to timely completion of a trial in HFpEF have been addressed with a novel endpoint, carefully considered entry criteria and a crossover study design uniquely suited to address the primary hypothesis while limiting study size.

Trials using an endpoint based on clinical outcomes (death, HF events) require large numbers of patients and are beyond the scope of the HFN. While three-tiered hierarchical composite endpoints (time-to-death, time-to-hospitalization and a third-tier surrogate assessment) are used in the HFN, power in these studies is primarily dependent on the "third tier", which to date has used QOL scores or changes in biomarkers (NT-proBNP). QOL questionnaires such as the KCCQ have been used successfully in large RCTs, but depend on memory, are subjective, low density, and semi-quantitative, and are subject to high variability over time. For interventions that may improve volume status or systolic or diastolic function, a strong rationale exists for using changes in BNP or NT-proBNP as an indicator of improved clinical status and arguably, a surrogate for clinical outcomes. However, brain natriuretic peptide levels are lower in HFpEF than HFrEF and indeed, often below "HF thresholds" and this reality may limit utility of this biomarker in many HFpEF patients.[20] Further, brain natriuretic peptide levels are both variable over time and low density as they are typically assessed only a few times over the course of a trial. Finally, biomarkers are not inherently relevant to patients and patient centric endpoints are more ideally suited to assess the effect of a therapy on symptom relief. As recently reviewed in the RELAX design paper,[21] cardiopulmonary exercise testing has many advantages over 6MWT as an endpoint in HFpEF. However, it presents operational challenges for some sites in the HFN, requires careful attention to standardization and calibration of devices and techniques, excludes some functional but frailer patients, is low density and assessed only a few times, is subject to training effects and encouragement bias, and would require multiple repetitions to assess dose response. Thus, each of the standard HF endpoints pose limitations in small clinical trials and a novel, highly quantitative, high density and patient-centric endpoint is needed.

Daily patient activity as assessed by accelerometry is an appealing endpoint for testing interventions designed to enhance activity tolerance: Implanted devices (pacemakers and defibrillators) or externally worn accelerometer devices provide highly quantitative, high density, patient centric data which have been used to characterize activity and to assess the impact of interventions on activity levels in patients with chronic obstructive pulmonary disease (COPD), obesity or arthritis.[22-32] As outlined below, accelerometry data have been shown to reflect changes in clinical status in HF and to correlate with traditional measures of disease severity such as peak VO_2, 6MWD, KCCQ score and prognosis as assessed by the Seattle Heart

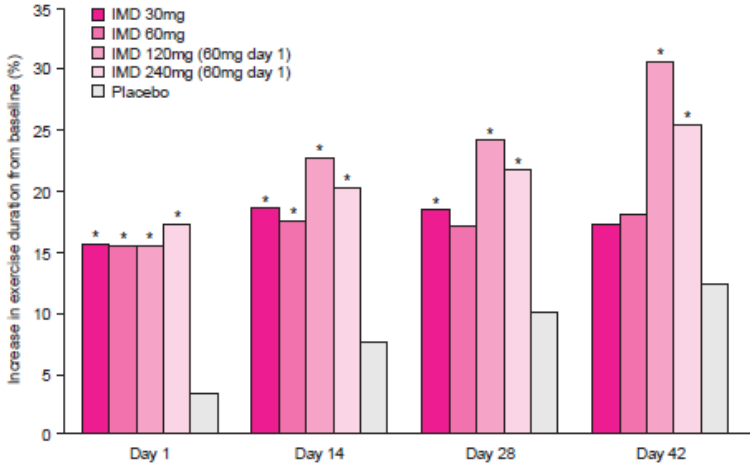
Failure Model.[22,33-43] As such, accelerometry is well-suited to address the primary objective of NEAT-HFpEF: to assess the impact of nitrates on the daily activity tolerance of patients with HFpEF.

Crossover study design: Crossover studies have been widely used in cardiovascular medicine and particularly in studies which have established the effect of nitrates on symptom relief in coronary artery disease.[44-49] There are two major advantages to crossover studies. First, every participant is exposed to all of the alternative interventions, thus reducing the overall sample size. Secondly, by comparing the effects of the interventions against the smaller within individual variations, the model is much more powerful. However, crossover studies are prone to two major areas of bias, period and carry over effects. The treatments are given at different times (periods), and the effect of time may contaminate the result. It is also possible that carry over (residual) effects of the earlier treatment may affect observations related to current treatment, so a "wash out" period is important.

The two potential sources of bias in a crossover design are minimized in NEAT-HFpEF as the total treatment time is relatively brief, thus minimizing the period bias. The carryover issue is minimized in NEAT-HFpEF by the brief treatment period, which will minimize any remodeling effects and the use of a washout period. The concept tested in NEAT-HFpEF is that the hemodynamic effects of nitrates will provide acute symptom relief and the study is not designed to test the potential for chronic remodeling effects. In coronary artery disease, nitrates have rapid onset of hemodynamic effects with improvement in exercise tolerance after 1 dose (Figure 1). Nitrates also have a rapid offset of action as even after 6 weeks of therapy, no improvement in exercise tolerance (as compared to placebo) was seen 24 hours after a dose.[50]

Figure 1. Parallel group study of dose response to ISMN administered once daily in patients with angina.

Exercise performance was assessed at multiple time points after initiation of therapy. These data demonstrate a lack of tolerance at high doses (120-240 mg). Significance at lower doses was lost at day 42 due to gradual improvement in the placebo treated group.[50]



4 PRELIMINARY STUDIES

Benefits of nitrates are well established in HFrEF. Studies of nitrates in HFrEF have shown improvements in exercise time and/or peak VO2 which were sustained with chronic therapy tested up to 3 months.[51-53] The improved exercise tolerance in HFrEF is linked to improvements

in left ventricular (LV) filling pressures and systemic vascular resistance with increases in cardiac output, reduction in severity of mitral regurgitation and myocardial ischemia.[54,55] While the effects of nitrates on hemodynamics and exercise performance are well established in HFrEF no studies have characterized their effects in HFpEF.

Unique pathophysiology in HFpEF may limit symptomatic relief with nitrates. Schwartzenberg et al. compared hemodynamics at rest and during infusion of sodium nitroprusside, a venous and arterial vasodilator in a large cohort of well-characterized patients with HF and preserved (HFpEF). In the left-sided circulation, greater pulsatile arterial loading was evident in HFpEF with greater LV-arterial mismatch in HFrEF, as previously reported.[56-58] The differences in ventricular-arterial properties in HFpEF and HFrEF were associated with fundamental differences in the response to nitroprusside. LV filling pressures were similarly elevated in patients with HFrEF and HFpEF at baseline and dropped to similar extent with nitroprusside in both forms of HF. However, patients with HFpEF had more exaggerated drops in blood pressure and less enhancement in stroke volume (SV) and cardiac output (CO) as compared to HFrEF (Figure 2) suggesting greater vulnerability to venodilator effects and excessive drop in preload. Indeed, 35% of HFpEF patients experienced a *drop* in stroke volume and CO with nitroprusside.

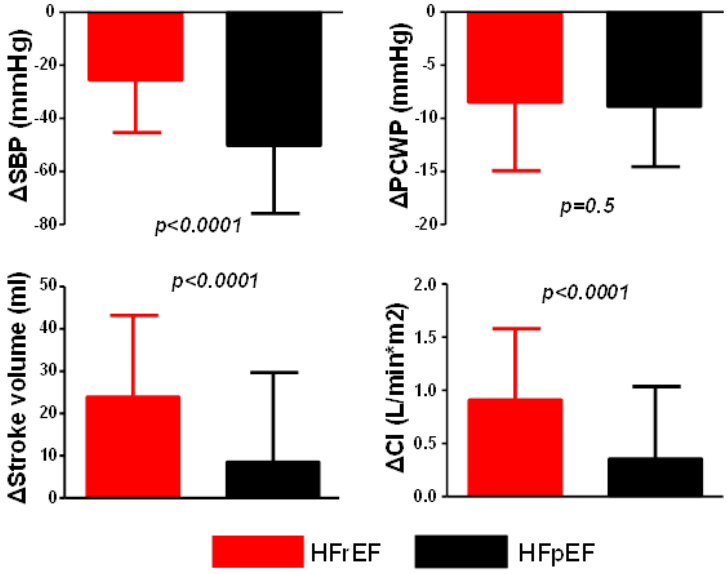


Figure 2. Peripheral and Central Hemodynamic Changes with Nitroprusside in HFrEF and HFpEF.[56]

Administration of nitroprusside is associated with similar decreases in filling pressure in HFrEF and HFpEF. However, SBP drops more in HFpEF while SV and cardiac index (CI) increase less.

These findings emphasize important mechanistic differences stemming from differences in LV systolic and diastolic elastance in HFpEF versus HFrEF (Figure 3), and raise questions regarding the empiric use of vasodilator-based therapies in patients with HFpEF.

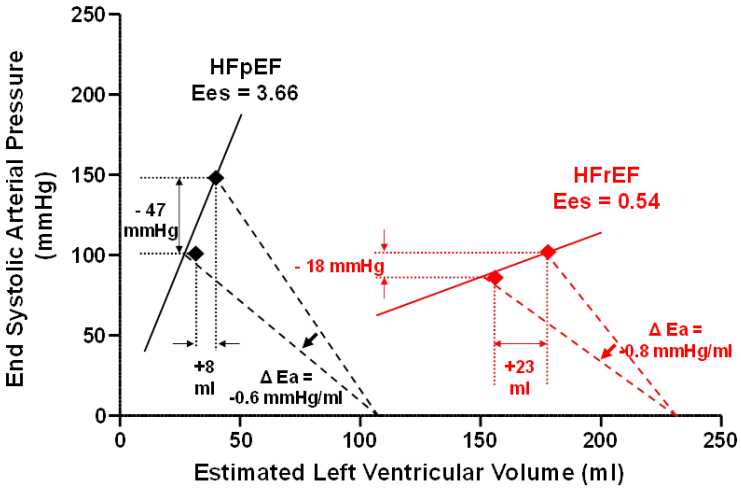


Figure 3. Unique pathophysiology in HFpEF versus HFrEF underlies different hemodynamic response to nitroprusside.[56]

In this study of patients with HF and preserved or reduced EF, systolic elastance (Ees) was greater in HFpEF than HFrEF as expected. Thus, while nitroprusside produced equivalent decreases in arterial elastance (Ea), the effect on blood pressure and stroke volume are determined by the differences in Ees inherent to HFpEF and HFrEF, with greater reduction in SBP and smaller increment in stroke volume.

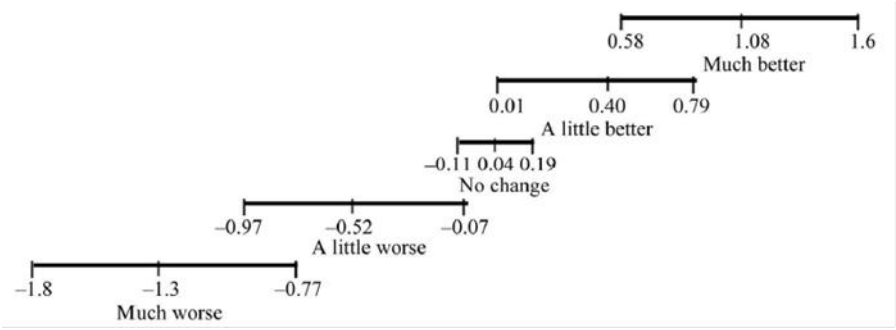
These findings are consistent with the findings of Kitzman et al. in HFpEF where there was no improvement in exercise capacity with enalapril as compared with placebo.[9] These findings are also consistent with the increased incidence of nitrate intolerance in the elderly where excessive hypotension can occur with nitrate use. HFpEF patients are elderly and frequently have autonomic dysfunction including chronotropic incompetence and reduced baroreceptor function, which may heighten intolerance to nitrates.[57,58] While nitroprusside is less veno-specific than ISMN and did reduce filling pressures in HFpEF, these data underscore the potential for excessive hypotension and reduced cardiac output with venous and arterial vasodilators in HFpEF, which may limit symptomatic response to nitrate therapy in HFpEF.

Nitrates may worsen or improve vascular function. Patients with HFpEF have impaired endothelial function and impaired vasodilatory reserve that contributes to impaired ventricular arterial coupling with exercise.[59] It is well established that nitrates are associated with vascular and extravascular changes that limit their hemodynamic effects with long-term administration (tolerance). Systemic effects include neurohumoral activation (sympathetic nervous system, RAAS and vasopressin) and volume expansion, which contribute to “pseudo-tolerance” effects of nitrates. In the vasculature, impaired nitrate biotransformation, increased reactive oxygen species (ROS) production, impaired ROS scavenger function, soluble guanylyl cyclase desensitization, increased production of endothelin, increased sensitivity to other endogenous vasoconstrictors and increases in cGMP-phosphodiesterase activity all may contribute to nitrate tolerance.[55] Anti-oxidants, ACEI/ARB, some beta-blockers, hydralazine and statins have been shown to attenuate nitrate tolerance. Differences in the degree and potential mechanism of tolerance exist between different nitrate preparations. As recently reviewed, there is growing evidence from animal and human studies that nitrates can produce endothelial dysfunction, likely related to ROS activation and local endothelin activation.[55] While once daily ISMN was demonstrated to be devoid of tolerance as evidenced by efficacy in improving exercise time in coronary artery disease patients after 6 weeks of therapy (Figure 1 above)[50,60], a recent study of once daily ISMN dosing in normal humans demonstrated ISMN-induced endothelial dysfunction[61] while animal studies with twice daily oral or continuous ISMN infusion have demonstrated beneficial or deleterious effects on vascular structure and function, respectively.[62,63] No studies have investigated the effects of nitrates on endothelial function in HFpEF and the clinical implications of nitrate-induced ROS activation in HF remain unclear.

Alternatively, improvements in arterial compliance with decreased reflected wave have been described with nitrates and may improve afterload and LV-arterial coupling and theoretically, reduce load induced diastolic dysfunction in HFpEF.[64-67]

Accelerometry data reflect disease severity, intervention effect and change in clinical status in HF. Studies in HF (predominately HFrEF) have shown that accelerometry data correlate with New York Heart Association (NYHA) functional class, 6MWD, peak VO_2, and mortality risk as estimated by the Seattle Heart Failure Model.[22,33-43] A study in *elderly* HF patients confirmed correlation of accelerometer measured activity with NYHA functional class and peak VO_2[40] and three studies have shown that accelerometer-assessed activity increases after cardiac resynchronization therapy in HFrEF.[33,34,39] providing evidence that accelerometer-measured activity can reflect therapeutic response. Accelerometer-measured activity changes in parallel with changes in clinical status as assessed by a Global Rating of Change Scale (GRS) in patients with HFrEF (Figure 4).[43]

Figure 4. Accelerometry data (change in hours active per day) tracks changes in clinical status as assessed by a Global Rating of Change Scale (GRS) in patients with HFrEF[43].



Other studies have examined device-based scores (including, but not limited to accelerometry data) to predict hospitalizations.[68-71] While the role of device-based monitoring continues to be defined, accelerometer-assessed activity declined prior to hospitalizations and may have contributed to declines in heart rate variability. We have measured accelerometer assessed activity (repeated measurement over two weeks at baseline, 3 months and 6 months without any intervention) in 49 elderly sedentary volunteers and shown excellent reproducibility (see power calculations; section 15.5). Accelerometry was used as an endpoint in a COPD trial[25] and studies have addressed analytical issues and compliance with externally worn accelerometry devices in clinical trials in COPD.[24,26,27,40] Compliance was excellent in these studies, and to enhance compliance in NEAT-HFpEF, participants will affix two accelerometry devices (providing variability data) to elastic, clasp-closed belts to enhance ease of wear and reduce chance of loss (Figure 5). For additional compliance, participants will be called weekly throughout the study, participant-specific strategies (e.g., sign posting, bathing-time accelerometer placement and alarms) will be discussed, and a patient-specific strategy formulated. These studies suggest that accelerometer-assessed activity has the potential to reflect response to nitrates in HFpEF.

Accelerometers are affixed to an elastic, clasp-closed belt.

Figure 5. The NEAT-HFpEF accelerometer device.

5 BASIC STUDY DESIGN

The NEAT-HFpEF study is a randomized, double-blinded, placebo-controlled crossover study to assess the effect of extended-release ISMN with forced dose up-titration on activity tolerance as assessed by accelerometry.

5.1 *Study Design*

5.1.1 Screening Phase

Patients with a HFpEF diagnosis are screened for basic entry criteria, including those designed to ensure that HF symptoms are the primary limitation to activity (inclusion criteria # 8) and ability and willingness to wear the accelerometer belt (inclusion criteria # 9 and 10). Willing participants meeting entry criteria will be consented.

5.1.2 Randomization

All eligible participants will undergo baseline studies (history and physical exam, phlebotomy for complete blood count (CBC), basic chemistry panel, HFN biomarkers (including cGMP and NT-proBNP), and HFN biorepository and genetics samples (if agreed to participate), transthoracic echocardiogram (local read), ECG, 6MWT, KCCQ, and MLHFQ). Participants will receive training in accelerometer use.

Participants will then be randomized using procedures determined by the Coordinating Center (CC) to one of 2 treatment groups (placebo first with crossover to ISMN or ISMN 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.

5.1.3 Study Intervention Phase

Phase 1—begins with study visit 1:

- Study drug will be dispensed after consent, completion of baseline studies and randomization. Participants will be instructed to take no study drug for 2 weeks.
- After the 2-week study-drug-free period, participants will be instructed to take 30 mg (1 tablet) of study drug (ISMN or placebo) every morning for one week, 60 mg (2 tablets) of study drug (ISMN or placebo) every morning for one week, and then 120 mg (4 tablets) of study drug (ISMN or placebo) every morning for two weeks.

- At each up-titration (or earlier if new symptoms develop), study staff will discuss tolerability with participants and determine safety of up-titration.
    - If the study drug has been tolerated, participants progress to the next dose.
    - In the case of study drug intolerance, study drug dose is reduced to the previously-tolerated dose.
- Regardless of the participant's ability to tolerate the maximum dose or discontinuation of study drug in Phase 1, study participants will begin Phase 2 as described below.
- Participants will be called weekly to encourage compliance with study-drug regimen and accelerometer use.
- Participants will be encouraged to be active within the limitations imposed by their HF symptoms.

Phase 2—begins with study visit 2:

After the baseline and 4 week up-titration period (6 weeks after the first study visit), participants will return for study visit 2.

- Participants will be instructed to take their study drug the morning of the visit.
- At this visit, participants will return phase 1 study drug, undergo repeat phlebotomy for HFN biomarkers (including cGMP and NT-proBNP), and HFN biorepository samples, 6MWT, KCCQ, and MLHFQ.
- Accelerometers are returned and changed out.
- Phase 2 study drug is dispensed.
- Participants are instructed to take no study drug for 2 weeks.
- After the 2-week period of being study-drug free, participants will be instructed to take 30 mg (1 tablet) of study drug (ISMN or placebo) every morning for one week, 60 mg (2 tablets) of study drug (ISMN or placebo) every morning for one week, and then 120 mg (4 tablets) of study drug (ISMN or placebo) every morning for two weeks.
- At each up-titration (or earlier in the case of new symptoms), study staff will discuss tolerability with participant and determine safety of up-titration.
    - If the study drug has been tolerated, participants progress to the next dose.
    - In the case of potential study drug intolerance, study drug dose is reduced to the previously tolerated dose.
- Participants are called weekly to encourage compliance with study drug regimen and accelerometry use.
- Participants will be encouraged to be active within the limitations imposed by their HF symptoms.

Completion—begins with study visit 3:

After completion of Phase 2, participants will return for study visit 3 (Week 13).

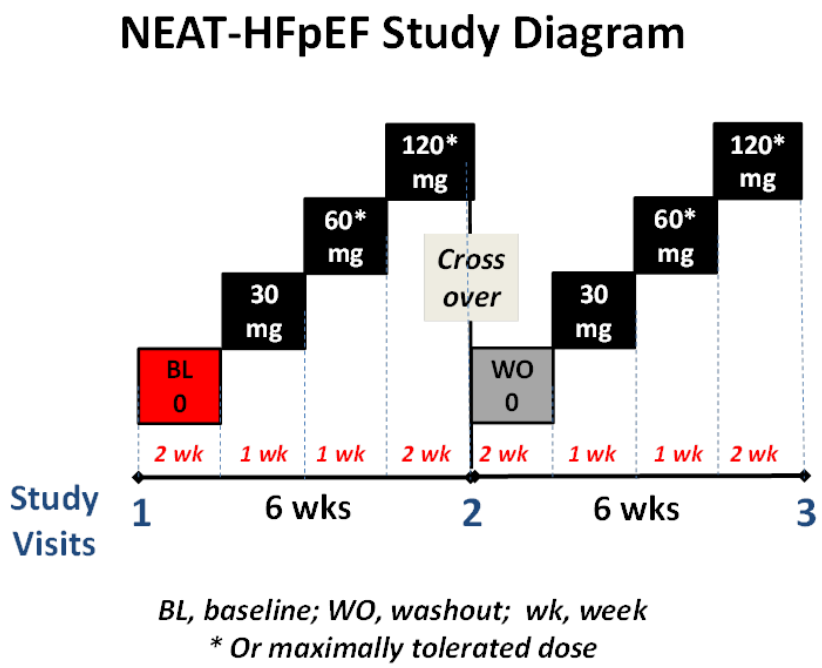
- Participants will be instructed to take their study drug the morning of the visit.
- At this visit, participants will return phase 2 study drug, undergo repeat phlebotomy for HFN biomarkers (including cGMP and NT-proBNP) and HFN biorepository samples, 6MWT, KCCQ, and MLHFQ.
- Accelerometers are returned.

- Participants are asked to indicate the study phase during which they felt better (Patient preference secondary endpoint).

5.1.4 Follow-up Phase

A final phone visit is conducted 2 weeks after study visit 3 to assess clinical stability.

6 STUDY FLOW DIAGRAM



BL, baseline; WO, washout; wk, week
** Or maximally tolerated dose*

7 STUDY POPULATION AND ELIGIBILITY CRITERIA

7.1 *Study Population*

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

7.2 *Inclusion Criteria*

1. Age ≥ 50 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. Stable medical therapy for 30 days as defined by:
    - No addition or removal of ACE, ARB, beta-blockers, calcium channel blockers (CCBs) or aldosterone antagonists
    - No change in dosage of ACE, ARBs, beta-blockers,CCBs or aldosterone antagonists of more than 100%
5. One of the following within the last 12 months
    - Previous hospitalization for HF with radiographic evidence of pulmonary congestion (pulmonary venous hypertension, vascular congestion, interstitial edema, pleural effusion) ***or***
    - Catheterization documented elevated filling pressures at rest (LVEDP≥15 or PCWP≥20) 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 (***at least two***)
        - E/A > 1.5 + decrease in E/A of > 0.5 with valsalva
        - Deceleration time ≤ 140 ms
        - Pulmonary vein velocity in systole < diastole (PVs<PVd) (sinus rhythm)
        - E/e'≥15
        - Left atrial enlargement (≥ moderate)
        - Pulmonary artery systolic pressure > 40 mmHg
        - Evidence of left ventricular hypertrophy
            - LV mass/BSA ≥ 96 (♀) or ≥ 116 (♂) g/m^2
            - Relative wall thickness ≥ 0.43 (♂ or ♀) [(IVS+PW)/LVEDD]
            - Posterior wall thickness ≥ 0.9 (♀) or 1.0 (♂) cm
6. No chronic nitrate therapy or infrequent (≤ 1x week) use of intermittent sublingual nitroglycerin within last 3 months
7. Ambulatory (not wheelchair / scooter / walker / cane dependent)
8. HF is the 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
9. 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)
10. Willingness to wear the accelerometer belt for the duration of the trial
11. Willingness to provide informed consent

7.3 *Exclusion Criteria*

1. Recent (< 3 months) hospitalization for HF
2. Hemoglobin < 8.0 g/dl
3. Glomerular filtration rate < 20 ml/min/1.73 m^2 on most recent clinical laboratories

4. SBP < 110 mmHg or > 180 mmHg at consent
5. Diastolic blood pressure < 40 mmHg or > 100 mmHg at consent
6. Resting HR > 110 bpm at consent
7. Previous adverse reaction to nitrates necessitating withdrawal of therapy
8. Chronic therapy with phosphodiesterase type-5 inhibitors (intermittent use of phosphodiesterase type-5 inhibitors for erectile dysfunction is allowable if the patient is willing to hold for the duration of the trial)
9. Regularly (> 1x per week) swims or does water aerobics
10. Significant COPD thought to contribute to dyspnea
11. Ischemia thought to contribute to dyspnea
12. Documentation of previous EF < 50%
13. Acute coronary syndrome within 3 months defined by electrocardiographic changes and biomarkers of myocardial necrosis (e.g. troponin) in an appropriate clinical setting (chest discomfort or anginal equivalent)
14. Percutaneous coronary intervention, coronary artery bypass grafting or new biventricular pacing within past 3 months
15. Primary hypertrophic cardiomyopathy
16. Infiltrative cardiomyopathy (amyloid)
17. Constrictive pericarditis or tamponade
18. Active myocarditis
19. Complex congenital heart disease
20. Active collagen vascular disease
21. More than mild aortic or mitral stenosis
22. Intrinsic (prolapse, rheumatic) valve disease with moderate to severe or severe mitral, tricuspid or aortic regurgitation
23. 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
24. Terminal illness (other than HF) with expected survival of less than 1 year
25. Enrollment or planned enrollment in another therapeutic clinical trial in the next 3 months.
26. Inability to comply with planned study procedures
27. Pregnant women

8 TREATMENT INTERVENTIONS

8.1 *Intervention*

ISMN or placebo at 30 mg to 120 mg administered orally each morning during the treatment phases as outlined above.

ISMN is the major active metabolite of ISDN and is available as an extended-release preparation.[50] It is an organic nitrate commonly used to prevent angina. It exerts its effect by relaxing vascular smooth muscles resulting in dilatation of peripheral arteries and veins. The study drug should be taken with approximately four ounces of water in the morning. It may be taken with or without food as food does not affect absorption. The capsules should not be split,

chewed or crushed.

Permitted dose adjustment: For all participants, if significant side effects occur during up-titration, return to the previously tolerated dose is permitted. If participants develop severe side effects at the lowest dose (presyncope, syncope or severe headache), study drug is stopped, but the participant continues in the trial. For less severe symptoms (mild lightheadedness or headache), a trial of continued use with symptomatic relief (ie-acetaminophen for headache) is encouraged. The 30 mg dose is a starting dose intended to reduce initial side effects. If a dose is missed, the once-daily 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.

Drug interactions: The only clinically-relevant drug interaction for nitrate administration is administration of phosphodiesterase-5 inhibitors. Co-administration of nitrates and any phosphodiesterase-5 inhibitor formulation is strictly contraindicated due to the risk of excessive hypotension.

8.2 *Drug Dispensing*

Drug dispensing will be managed by the CC in collaboration with the contracted drug supply vendor. At the first study visit, participants will receive a sufficient supply of ISMN or placebo to permit daily dosing until the second study visit. Participants will receive enough ISMN or placebo at the second study visit to last until the third (final) study visit.

Participants will be instructed to take the medication as required by the protocol, and compliance will be assessed at each visit or by phone contact. Participants will be instructed to return unused drug and bottles/packaging at each visit.

8.3 *Drug storage*

Study drug is to be stored at 25°C (77°F) with excursions permitted to 15–30°C (59–86°F). Excessive moisture should be avoided.

8.4 *Drug accountability*

Participants are instructed to return all used, partly used and unused trial product (study drug, bottling/packaging) at each study visit. Returned trial product(s) (used, partly used or unused including empty packaging material) 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.

8.5 *Drug Destruction*

Unused study drug can be destroyed at the site according to accepted pharmacy practice, and local and national guidelines, using the site's destruction procedure. A copy of the drug destruction procedure should be maintained in the pharmacy section of the Regulatory Binder. Study drug destruction should be documented in the comments section of the Subject Specific

Drug Accountability Log.

8.6 *Randomization, Stratification and Blinding*

Randomization will occur at the first study visit. Randomization to active drug or 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. Participants will be randomized using procedures determined by the CC to one of 2 treatment sequences. A permuted block randomization method stratified by site will be used to ensure relatively equal distribution of participants to each sequence within each clinical site. Blinding to treatment groups will be preserved by the use of matching placebo tablets.

8.7 *Unblinding*

Given the safety profile of ISMN, 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.

Unblinding should be a very rare occurrence. The potential physiologic actions of the therapy are well characterized. Hypotension or headache should be addressed as described above. The investigative sites will be given access to the treatment code for their participants for emergency unblinding ONLY by calling the CC. In the rare event of necessary unblinding, the CC Medical Monitor must be contacted to discuss the case.

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

8.8 *Concomitant Medication*

Patients with HFpEF should be treated with standard HFpEF strategies (diuretics for congestion, blood pressure control and heart rate control if patient is in atrial fibrillation) as per recommended guidelines.[7] Patients should be on stable medications and have adequate or optimal 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, clinically compelling worsening of clinical status. As above, therapy with phosphodiesterase-5 inhibitors is contraindicated during the study period.

8.8.1 Side Effect Risk Reduction Plan

The gradual up-titration of study drug, use of acetaminophen for headache, and ability to return to a previously-tolerated dose may enhance tolerability.

9 RECRUITMENT AND SCREENING PROCEDURES

9.1 *Common Recruitment Procedures*

All patients admitted to the participating HFN centers with signs and symptoms suggestive of HFpEF will be screened by a Study Coordinator. Patients meeting eligibility criteria will be approached regarding participation in this study.

9.2 *Estimated Enrollment Period*

This study will enroll approximately 100 participants at approximately 20 clinical centers in the U.S. The anticipated enrollment period is approximately18 months.

9.3 *Informed Consent Procedures*

9.3.1 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 NEAT-HFpEF study, they will review and sign the site-specific Internal Review Board (IRB) approved informed consent form (ICF).

9.3.2 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. 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.

9.3.3 Protections of Human Subjects

Protections for human subjects of research are required under Department of Health and Human Services (HHS) regulations at 45 CFR 46. Subpart A of the HHS regulations constitutes the Federal Policy (Common Rule) for the Protection of Human Subjects, which has been adopted by an additional 16 Executive Branch Departments and Agencies.

Each institution engaged in (non-exempt) HHS-supported human subjects research must provide a written Assurance of Compliance, satisfactory to the Office for Protection from Research Risks, that it will comply with the HHS human subjects regulations—45 CFR 46.103(a).

9.3.4 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: Potential adverse effects of ISMN are related to venous and arterial vasodilatation

include lightheadedness, presyncope or syncope and headache. Such adverse reactions are dose-related and typically decrease over time with incidence of ≤ 5%. Elderly patients may be at increased risk of such reactions.

Headache: Nitrate headache is a common and dose-related adverse effect associated with ISMN 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. In controlled North American clinical studies, the incidence of headache increased from 38% to 51% to 57% as daily doses of ISMN advanced from 60 milligrams to 240 milligrams. Between 5% and 8% of patients discontinued therapy because of headache.

Participating in the research study may be hazardous to an unborn child: There are no well-controlled studies of ISMN 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 negative serum pregnancy test.

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

10 BASELINE EVALUATION AND RANDOMIZATION VISIT

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

10.1 *Screening*

Patients will be screened for entry criteria at each site using existing clinical records including their most recent echocardiogram (imaging study within 12 months of enrollment) and blood work. Discussion of the trial by direct contact or phone is encouraged. Potential participants will be scheduled for study visit 1.

10.2 *Baseline and Randomization Visit (Visit 1)*

Patients will be interviewed and their records reviewed to determine if they meet all entry criteria. Ability to wear the accelerometer belt will be confirmed with a “test belt” provided to each center. Willingness to wear the accelerometer belt and to participate in all study procedures is confirmed. After providing informed consent and signing the ICF, all subjects who fulfill all the inclusion criteria and none of the exclusion criteria will undergo the baseline studies (below) and will then be randomized using procedures determined by the CC to one of 2 treatment groups (ISMN first or placebo first). Participants will be randomized in a 1:1 allocation ratio. The patients will be educated in study procedures including wearing of the accelerometer. Study drug for the first phase of NEAT-HFpEF will be dispensed.

The baseline studies and procedures include:

- Complete Medical History and Physical Examination
- NYHA class assessment
- Medication review
- Blood draw for CBC, basic chemistry panel, HFN biomarkers (including cGMP and NT-proBNP), and biorepository and genetics samples (if consented)
- Transthoracic Echocardiogram (local read)
- ECG
- 6-minute walk test
- KCCQ
- MLHFQ
- Compliance plan: A plan for the weekly phone visits will be established with the patient. Participant-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry.
- Dispense accelerometer and educate participant on how to wear device
- Dispense study drug

11 FOLLOW-UP EVALUATIONS

11.1 *Study Visits*

Study Visit 2. Participants will return no sooner than 6 weeks (but up to 8 weeks) after the first study visit where the Phase 1 study drug and accelerometers will be returned and exchanged for Phase 2 study drug and accelerometers. Participants will be instructed to take their study drug the morning of the visit. Patients will continue to take the maximally tolerated dose of drug until the second study visit. Compliance with study procedures during Phase 1 will be assessed. Participants will undergo studies and procedures as below. The Study Visit 2 studies and procedures include:

- Interim History and Physical Examination
- NYHA class assessment
- Medication review
- Blood draw for HFN biomarkers (including cGMP and NT-proBNP), and biorepository samples (if consented)
- 6-minute walk test
- KCCQ
- MLHFQ
- Compliance plan: A plan for the weekly phone visits will be established with the patient. Patient-specific strategies such as sign posting, bathing-time accelerometer placement, and alarms may be used to enhance compliance with accelerometry.
- Change out accelerometer
- Collect study and dispense new study drug

Study Visit 3. Participants will return no sooner than 6 weeks (but up to 8 weeks) after the second study visit where the Phase 2 study drug and accelerometers will be returned. Participants will be instructed to take their study drug the morning of the visit. Compliance with study procedures during Phase 2 will be assessed. Patient will undergo studies and procedures as below. The Study Visit 3 studies and procedures include:

- Interim History and Physical Examination
- NYHA class assessment
- Medication review
- Blood draw for HFN biomarkers (including cGMP and NT-proBNP) and biorepository samples (if consented)
- 6-minute walk test
- KCCQ
- MLHFQ
- Discontinuation of study drug
- Collect accelerometer

Final study phone visit: A final phone visit is conducted 2 weeks after Study visit 3.

11.2 *Phone and other media follow-up*

General procedures: At Study Visit 1, participants and study staff will define optimal times and phone number for weekly phone contact to encourage compliance with study procedures. During the weekly phone visits, the participant will receive:

- Reminder regarding appropriate study drug dose
- Encouragement of compliance with accelerometry use
- Encouragement of activity within the limits of their HF symptoms

Participants are also called 14 days ± 5 days after Study Visit 3 for adverse event status.

12 OUTCOME DETERMINATIONS

12.1 *Primary Endpoint*

The primary endpoint is the AAU_{14} during maximally tolerated dose of ISMN vs. placebo.

Patients will receive an accelerometer belt, which includes two accelerometers affixed to a clasp-closed elastic belt designed to fit persons with BMI ≈ 20-40 kg/m^2. The belt will be tailored by the site study coordinators to provide a comfortable fit over or under clothing as outlined in the physical activity measurement core laboratory manual of operations. Participants will wear the belt continuously throughout the study including while sleeping. The device will be removed only for bathing or swimming. The accelerometers continuously record arbitrary accelerometer units (AAU) and store cumulative AAU in 15-minute bins providing 96 data points over each day. The devices can record data for 56 days providing an ample window for data collection over each 42-day study phase with up to 14 days extra recording available to accommodate any

delay in return for study visit 2 or 3. If additional delays are encountered, the data collected remains stored providing the ability to assess the primary endpoint.

12.2 *Secondary Endpoints*

1. Functional, QOL and Natriuretic HF endpoints:
   - 6MWD
   - Borg score during 6MWT
   - KCCQ
   - NT-proBNP

2. Alternate accelerometry endpoints:
   - Hours active/day during maximally-tolerated dose of study drug (comparison of weeks 5-6 and 11-12)
   - Slope of daily-averaged AAU during study drug administration (comparison of weeks 3-6 and 9-12)
   - AUC of daily-averaged AAU during study drug administration (comparison of weeks 3-6 and 9-12)

3. Participant preference for study phase

12.3 *Tertiary Endpoints*

1. Pre-specified subgroup analyses will include examination of the primary endpoint in patients:
   - Treated or not treated with agents known to ameliorate nitrate tolerance (RAAS antagonists, carvedilol, statins or hydralazine)
   - NT-proBNP above and below median
   - SBP above and below median
   - Patients with or without known coronary artery disease

2. Quotient of 6MWD and Borg Score during 6MWT

3. MLHFQ to determine if ISMN improves QOL as assessed by MLHFQ

4. Within patient-averaged AAU at 30, 60 and 120 mg of study drug to assess dose response

5. Plasma levels of cyclic guanosine monophosphate (cGMP)

13 METHODS TO PROMOTE ADHERENCE

13.1 *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 CC will contact each site to offer per-participant feedback on adherence; will review episodes of non-adherence and reemphasize the importance of adherence; and will

provide adherence reports to the Executive Committee.

14 PARTICIPANT SAFETY AND ADVERSE EVENTS

14.1 *Institutional Review Boards*

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

14.2 *Definitions*

14.2.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in a subject whether or not considered drug or biologic related. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of a pharmaceutical product or biologic.

14.2.2 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.

14.2.3 Serious Adverse Events

An AE or SAR is considered serious if the Investigator or sponsor believes any of the following outcomes may occur:

- Death
- Life-threatening AE: Places the subject 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 subject 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).

14.2.4 Laboratory Test Abnormalities

For laboratory test abnormalities that meet the definition of a serious adverse event (SAE), which required the subject to have the investigational product discontinued or interrupted, or required the subject to received 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).

14.2.5 Assessment of Causal Relationship and Severity

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.

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.

14.2.6 Expectedness

The expectedness of an AE or SAR shall be determined according to the specified reference document containing safety information (e.g., most current product label). Any AE that is not identified in nature, severity, or specificity in the current study drug reference document(s) is considered unexpected. Events that are mentioned in the product label 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.

14.3 *Anticipated Adverse Events and Procedure Effects*

The following AEs are anticipated, disease-related events in patients with HF with preserved EF (HFpEF):

- Arrhythmias
- Sudden cardiac death
- Acute coronary syndrome
- Unplanned hospitalization, ER visit or clinic visit for worsening HF
- Cerebrovascular event
- Venous thromboembolism
- Lightheadedness, presyncope or syncope
- Worsening renal function

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).

14.3.1 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. For this study, non-serious AEs will not be collected on the safety reporting page of the eCRF, but should be documented in the source documents and followed according to local standard of care. Events significant enough to necessitate modification of study drug dosing will be captured on the appropriate eCRF module ("Study Drug Dosing" page).

SAEs, except for those anticipated AEs listed above, occurring from *signed informed consent* to Week 15 phone visit will be captured on the SAE eCRF. Unless exempted as described above**,** all SAEs, whether or not deemed drug-related or expected, must be reported by the investigator or qualified designee within 1 working day of first becoming aware of the event. The investigator or qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to the appropriate sponsor contact. 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 the appropriate sponsor contact. 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 last subject completes the final follow-up, whichever occurs first. DCRI Safety Surveillance will report all SAEs to the Data Safety Monitoring Board (DSMB) chair monthly.

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.

Suspected Unexpected Serious Adverse Reaction

AEs that meet the criteria of serious, related to study drug, and unexpected for that drug, qualify for expedited reporting to the regulatory authorities. The Site Investigator will assess all SAEs occurring at his/her site and evaluate for "unexpectedness" and relationship to study drug. The Site Investigator is required to complete and submit a voluntary MedWatch Report for the events identified as serious, study drug related and unexpected at: https://www.accessdata.fda.gov/scripts/medwatch/

A copy of this report should be kept at the site and also forwarded to the DCRI within the same timeline used for reporting to regulatory authorities.

Pregnancy

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

15 STATISTICAL CONSIDERATIONS

15.1 *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).

15.2 *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 AAU_{14} during maximally-tolerated dose period of ISMN versus placebo at weeks 5-6 and 11-12. It is anticipated that some data will be missing as the accelerometers will not be used at all times for every participant on every day. Some of this missing data is required as the devices cannot be used during certain activities such as bathing and water activities. We propose to apply the imputation approach of Catellier et.al. to create a pseudo-complete dataset

prior to analysis.[72] Details of this imputation algorithm will be included in the statistical analysis plan. The imputation plan will account for potential differences in activity between weekdays and weekends. Additionally the imputation will account for portions of the day with missing data.

The primary data analysis will involve a mixed model with fixed effect terms for the sequence, period and treatment. A random effect term will be included to account for the correlated measurements within each participant.[48] For the primary analysis, the response variable will use data from the maximally-tolerated dose of study drug period during each treatment (ISMN vs. placebo) phase obtained from weeks 5-6 and weeks 11-12. The estimated treatment effect will be provided with a 95% confidence interval. Given the short half-life of the drug, the two-week washout period, and the assumed lack of remodeling effects, we anticipate no significant carry-over or residual effect from Phase 1 to Phase 2.

The NEAT statistical analysis plan (SAP) will contain detailed information regarding the accelerometer 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.

The anticipated plan for use of the data from the two accelerometers is based on expertise supplied by the Physical Activity Core Laboratory as well as the HFN Data Coordinating Center statisticians and will incorporate the following concepts:

1. If one accelerometer has clearly "failed" for all or part of the study period but the other accelerometer data shows the expected data pattern throughout the study period, the failed accelerometer data will be not be utilized.
2. If both accelerometers are functioning, the NEAT-DHF primary endpoint (14 day averaged arbitrary accelerometer units (AAU_{14})) from both accelerometers will be averaged.
3. The agreement between the AAU_{14} from the two accelerometers at each study period will be characterized and reported.

Baseline data (history, physical, qualifying echo data, medication use, accelerometry data, 6MWD, KCCQ, MLHFQ, NT-proBNP) will be collected to characterize the study population and potentially to serve as a covariate in the analysis of the endpoint and to facilitate subgroup analysis. As a sensitivity analysis, the data from the first period and second period will be presented separately. Presentation of the study results will be based on the criteria described by Mills et.al.[73]

15.3 *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.

15.4 *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 preliminary analysis).

15.5 *Sample Size and Power Calculation*

As the primary endpoint has never been examined in the proposed study population, the justification of the sample size is based on two key secondary endpoints: the overall summary score from the KCCQ and the 6MWT distance.

KCCQ 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 is roughly 17-18 points. For the KCCQ, a clinically-significant difference is considered 5 points and a moderately large clinical difference is considered to be 10 points.[74,75] Assuming a 17 point standard deviation, a total of 94 participants (47 per sequence) 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 104 participants (52 per sequence) is adequate to provide 80% power to detect a clinically-significant difference of 5 points in the KCCQ overall summary score.

6MWT: From prior HFN studies in HFpEF, the within-patient standard deviation for the 6MWT distance is expected to be between 85 and 95 meters. Given the clinically-important difference of 43 meters,[76] a sample size larger than 60 participants (30 per sequence) would be expected to provide greater than 90% power in this 2*2 crossover design. These calculations assume a two-sided Type I error rate of 0.05 and are based on a crossover ANOVA.[48]

We propose a sample size of 100 participants (50 per sequence) which should provide greater than 80% power for 2 key secondary endpoints (KCCQ and 6MWT distance) with an allowance for approximately 5% missing data due to deaths and/or withdrawal of consent. The proposed sample size is sufficient to provide 80% or better power to detect differences larger than 28% of the standard deviation of the difference between active and placebo within participants. The planned sample size of 100 participants is larger than the sample size in previous cross-over studies used to establish the effectiveness of nitrates for HF and angina.[52,77-80]

ACCELEROMETRY DATA: We have measured accelerometer- assessed activity as the cumulative AAU_{14} at baseline, 3 months and 6 months without any intervention in 49 elderly sedentary volunteers. The average of the within-patient standard deviation between the

baseline and 3-month measurements was 337 AAU (mean baseline 4462 AAU, mean 3-month 4496 AAU). If the baseline AAU_{14} and the within-patient variability in HFpEF patients is similar to that observed in healthy elderly sedentary persons, NEAT-HFpEF would have 90% power to detect a difference between the active and placebo phases of 114 AAU (approximately 2.5% of the baseline measurement).

16 DATA MANAGEMENT PROCEDURES

16.1 *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.

eCRF: The CC management team will develop eCRF modules necessary for NEAT-HFpEF. Common fields and data elements will be used across the HFN trials to promote data standardization and facilitate cross-network analyses. Study eCRF components will include an enrollment and demographics form; forms for recording relevant history, HF symptoms, physical exam results, laboratory results, baseline biomarker levels, and other baseline presenting characteristics; follow-up forms for use during regular follow-up visits; forms to track the participant's clinical course over time; and event forms for recording the circumstances and details surrounding the occurrence of a death or hospitalization.

EDC System: The data will be collected in a validated, 21 CFR Part 11-compliant EDC system. The DCRI has an internal team of skilled data managers and programmers that will design and produce a tailored network system that provides operational efficiency and meaningful reporting of metrics.

Data Management Process: The EDC system will be used for data entry and simple reports. All data will be entered into the eCRF by personnel at the clinical sites. Any out-of-range values and missing key variables will be flagged and addressed in real-time at the site during data entry. When a query is generated on a particular variable, a flag is raised in a database field; the system tracks the queries and produces reports of outstanding queries. Queries can also be generated from manual or statistical review of the data forms.

The CC will create reports to identify trends in the data that may require additional clarification and training. These reports will be available to the sites and to the study leadership as we work with the sites to correct negative trends and eliminate future data errors. The CC will perform internal database quality-control checks during the study to identify systematic deviations requiring corrections.

Data Quality Control: A three-step approach to data quality control will be implemented.

1. Training: Prior to the start of enrollment, the Investigators and Study Coordinators will be trained on the clinical protocol and data collection procedures. Recent site surveys indicate that most Coordinators are very familiar with the EDC system, so training is typically targeted to a specific protocol. For Coordinators new to the InForm database, the CC will provide training with hands-on database interaction, demonstration of key EDC system functionality, and practice exercises. Personnel at the clinical sites will enter the data mandated by the protocol into the eCRFs. The data will be extracted from the participant's medical charts and other source documents. All CRFs will be completed according to the current Good Clinical Practice (GCP) guidelines. The CC will conduct follow-up training and training for new study personnel as needed.

2. Monitoring: A CC monitor will visit sites during the enrollment period to ensure that data collection is being handled properly, to provide in-service training, and to address questions from site investigators and coordinators. Additional details will be outlined in the Clinical Monitoring Plan.

3. Managing data: A series of computerized data validation checks will be programmed by the CC to check for missing data, inconsistencies in the data or data that is out of range. After the data have been exported from the EDC system to SAS for statistical summarization and data analysis, further cross-checking of the data will be performed by the CC and queries issued through the EDC system for any discrepancies.

16.2 *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.

16.3 *Publication Policy*

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, Investigators will be prohibited performing subset analyses at any point prior to 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.

17 STUDY ADMINISTRATION

17.1 *Data and Safety Monitoring Board (DSMB)*

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.

17.2 *Coordinating Center*

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

17.3 *Core Laboratories*

17.3.1 Biomarker Core Laboratory

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).

17.3.2 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).

18 REGULATORY ISSUES

18.1 *Ethics and Good Clinical Practice*

This study must be carried out in compliance with the protocol and in accordance with DCRI standard operating procedures. 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).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

By signing the protocol, the investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice to which it conforms.

18.2 *Institutional Review Board/Independent Ethics Committee*

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be provided to the CC before study initiation. The name and occupation of the chairman and the members of the IRB/IEC must be supplied to the CC if this information is released by IRB/IEC. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

18.3 *Informed Consent*

The Investigator or designee must explain to each subject (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 subject 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.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If written consent is not possible, oral consent can be obtained if witnessed by a signed statement from one or more persons not involved in the study, mentioning why the patient was unable to sign the form. No patient can enter the study before his/her informed consent has been obtained. The informed consent forms are part of the protocol, and must be submitted by the investigator with it for IRB/IEC approval. The CC will supply proposed informed consent forms, which comply with regulatory requirements, and are considered appropriate for the study. Any changes to the proposed consent form suggested by the Investigator must be agreed to by the CC before submission to the IRB/IEC, and a copy of the approved version must be provided to the CC after IRB/IEC approval.

19 REMOTE MONITORING

The study will be monitored remotely by representatives of the DCRI or its designee according to the prospective clinical monitoring plan for the following purposes:

- To enable real-time monitoring of compliance with study protocol inclusion and exclusion criteria is enabled via triggers and range checks programmed in the InForm database.
- To assist site personnel who will verify data identified within query reports against source documents through frequent telephone and email contact.

- To verify that written informed consent was obtained before initiation of any screening procedures that are performed solely for the purpose of determining eligibility for the clinical study and/or prior to the participant's randomization to a procedure.

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20 APPENDICES

20.1 *Appendix A. Schedule of Assessments*

	Study Visit 1 (Baseline)						Study Visit 2						Study Visit 3		
(Beginning of) Week No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Study Drug Dose (ISMN versus placebo)	**0 mg**	**0 mg**	**30 mg**	**60 mg**	**120 mg**	**120 mg**	**0 mg**	**0 mg**	**30 mg**	**60 mg**	**120 mg**	**120 mg**			
Visit															
In-person (clinic)	X						X						X		
Phone		X	X	X	X	X		X	X	X	X	X			X
Informed Consent	X														
Randomization	X														
Clinical Evaluation															
Complete Medical History	X														
Interim Medical History							X						X		
Physical Examination	X						X						X		
NYHA Class	X						X						X		
Medication Review	X						X						X		
12-lead ECG	X														
Laboratory Evaluation															
Local - Electrolytes, hematology*	X														
Core - Biomarkers^	X						X						X		
Imaging Evaluation															
Local-Transthoracic Echocardiography	X														
Functional and QOL Evaluation															
6 MWT	X						X						X		
Kansas City Cardiomyopathy Questionnaire (KCCQ)	X						X						X		
Minnesota Living with Heart Failure Questionnaire	X						X						X		
Study Drug and Accelerometer															
Dispense/Change Out Accelerometer	X						X								
Return Accelerometer													X		
Dispense Study Drug	X						X								

*Includes basic chemistry panel (sodium, potassium, chloride, carbon dioxide, BUN, creatinine, glucose) and complete blood count.

^Biomarkers to be determined by HFN Biomarker Committee but to include cGMP and NT-proBNP

20.2 *Appendix B. Kansas City Cardiomyopathy Questionnaire*

The KCCQ is a self-administered, 23-item questionnaire developed to provide a better description of health-related QOL in patients with HF. It quantifies physical limitation, symptoms, QOL, social interference and self-efficacy. The survey requires 4-6 minutes to complete, and is scored by assigning each response an ordinal value, beginning with 1 for the response that implies the lowest level of functioning and summing items within each domain. Scale scores are transformed to a 0 to 100 range by subtracting the lowest possible scale score, dividing by the range of the scale and multiplying by 100. A clinical summary score will be calculated by combining the functional status with the QOL and social limitation domains.

21.3 Appendix C. 6-Minute Walk Test and Borg Scores

Because usual daily activities generally require much less than maximal exertion, the measurement of submaximal exercise capacity may provide information that is complementary to that provided by maximal exercise testing.[18] 6MWT is the most common of the fixed-time tests; it measures the distance walked on level ground in 6 minutes. In this test, the participant is asked to walk along a level corridor as far as he or she can in 6 minutes. The participant can slow down or even stop, may be given a carefully controlled level of encouragement, and is told when 3 and 5 minutes have elapsed. The 6MWT is moderately predictive of maximal oxygen consumption, and independently predicts morbidity and mortality in HF.[19,20] For a complete description of the indications, contraindications, technical aspects, safety issues, and interpretation of the 6MWT, the investigator is referred to the 2002 guidelines published by the American Thoracic Society. At the completion of the 6MWT, the patient will be asked to rank their level of dyspnea and perceived level of exertion using the Borg Score Dyspnea Score (1-10) and Borg perceived level of exertion score (6-20) as outlined in the Manual of Operations.

21.4 Appendix D. New York Heart Association Functional Classification

Class	NYHA Classification
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitations of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

21.5 Appendix E. Minnesota Living with Heart Failure Questionnaire

The MLHFQ is a self-administered, disease-specific measure of health-related QOL that assesses patients' perceptions of the influence of HF on physical, socioeconomic and psychological aspects of life.[81-83] Patients respond to 21 items using a six-point response scale (0-5). The total summary score can range from 0-105 with a lower score reflecting better HF-related QOL. Two sub-scale scores reflect physical (8 items) and emotional (5 items) impairment. This instrument has been extensively validated and widely used to assess treatment effect on clinical status in multiple trials of therapeutic interventions in HF.