A Proof of Concept Study to Determine the Efficacy of EntrestoTM in HFpEF Based on Circulating Neprilysin Levels: The Circulating NEP and NEPi (CNEPi) Study

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Principal Investigator: Dr. Naveen L. Pereira
200 First Street SW
Rochester, MN 55905
[redacted]
mayoclinic.org

Funding Sponsor: National Institute of Aging
Building 31, Room 5A52
31 Center Drive MSC 2486
Bethesda, MD 20892

Study Product: Entresto™

Protocol Number: (IRBe): IRB # 18-000044

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List of Abbreviations

ACEIs	Angiotensin converting enzyme inhibitors
AE	Adverse Event/Adverse Experience
ANP	Atrial natriuretic peptide
ARB	Angiotensin receptor blocker
BNP	Brain natriuretic peptide
CCaTS	Center for Clinical and Translational Science
CFR	Code of Federal Regulations
cGMP	Cyclic guanosine monophosphate
CHF	Congestive Heart Failure
CNP	c-type natriuretic peptide
CRF	Case Report Form
CRTU	Clinical Research and Trials Unit
DSMB	Data and Safety Monitoring Board
EF	Ejection Fraction
ES	Executive Secretary
FDA	Food and Drug Administration
GFR	Glomerular Filtration Rate
GCP	Good Clinical Practice
HF	Heart Failure
HFpEF	Heart Failure with Preserved Ejection Fraction
HFrEF	Heart Failure with Reduced Ejection Fraction
HIPAA	Health Insurance Portability and Accountability Act
HR	Hazard ratio
IB	Investigator's Brochure
IRB	Institutional Review Board
IV	Intravenous
LVEF	Left ventricular ejection fraction
NEP	Neprilysin
NEPi	Neprilysin inhibitor
NP	Natriuretic peptide
NPRC	Natriuretic peptide clearance receptor
NYHA	New York Heart Association

Study Summary

Title	A Proof of Concept Study to Determine the Efficacy of Entresto™ in heart failure with preserved ejection fraction (HFpEF) Based on Circulating Neprilysin Levels: The Circulating NEP and NEPi (CNEPi) Study
Running Title	CNEPi
Protocol Number	IRB # 18-000044
Phase	Phase 4
Methodology	Proof of Concept Study
Overall Study Duration	21 months
Subject Participation Duration	5 weeks
Single or Multi-Site	Mayo Clinic Sites
Objectives	Determine biomarker (plasma NT-proANP, NT-proBNP, NT-proCNP, cGMP) responses to Entresto™ in patients with HFpEF who have high and low serum NEP levels
Number of Subjects	40
Diagnosis and Main Inclusion Criteria	Subjects with NYHA 2-4 HFpEF
Study Product, Dose, Route, Regimen	Entresto™ 49/51 mg (sacubitril/valsartan) orally twice-daily
Duration of Administration	Up to 5 weeks
Reference therapy	NA
Statistical Methodology	Paired t-test comparing the change from baseline to up to 5 weeks after administration of Entresto™

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Heart failure with preserved ejection fraction (HFpEF) can result from abnormalities in the process of active relaxation and passive stiffness of the heart[1]. The natriuretic peptides (NPs) play an important role in cyclic guanosine monophosphate (cGMP) mediated inhibition of cardiomyocyte growth, smooth muscle and fibroblast proliferation and collagen deposition that can affect passive stiffness[2-4]. Further, NPs can attenuate angiotensin-II, endothelin-1 and arginine-vasopressin induced increased proliferation of vascular smooth muscle cells[5]. HFpEF is characterized by elevated intra-cardiac pressures[6] and the natriuretic and diuretic properties of NPs play a pivotal role in maintaining normal volume homeostasis[7, 8]. Natriuretic peptides also reduce peripheral vascular resistance and blood pressure[9]. The NPs additionally inhibit the effects of the renin-angiotensin-aldosterone and sympathetic nervous system[10-13] the maladaptive activation of which results in the clinical syndrome of HF. Neprilysin (NEP) mediates the anti-fibrotic, anti-proliferative, myocardial relaxation, vasodilator and diuretic properties of NPs by maintaining NP homeostasis. NPs are degraded and cleared by NEP and the NP clearance receptor (NPRC) respectively. NEP inhibition (NEPi) therefore results in significant increases in plasma BNP, urinary BNP and urinary sodium, increases in cardiac output and reduction in intra-cardiac filling pressures[14].

1.2 Preliminary Data

Entresto™, a crystalline complex of a NEPi (sacubitril) and an angiotensin receptor blocker (ARB, valsartan), was recently approved to treat NYHA Class 2-4 patients with HFrEF after the PARADIGM-HF trial was stopped early due to an overwhelming benefit of the drug in reducing all-cause mortality (hazard ratio [HR]: 0.84; p<0.001), CV mortality (HR 0.80; p<0.001), hospitalization for HF (HR 0.79; p<0.001) and in addition reduced the symptoms and physical limitations of HF by 21% (p=0.001)[15]. Entresto™ due to its additional NEPi activity could be a promising new therapy for HFpEF. Entresto™ when administered to patients with HFpEF in a phase 2 study resulted in significantly reduced NT-proBNP levels, a surrogate marker of LV wall stress and NEP activity, as compared to being treated with valsartan alone[16]. Entresto™ therefore is being tested as treatment for HFpEF in a Phase 3 trial, PARAGON-HF, evaluating its efficacy in reducing CV death and HF hospitalizations as compared to valsartan.

NEP is a cell membrane bound metalloendopeptidase and can be released from the cell surface, resulting in the formation of a circulating peptide that maintains its catalytic activity[17, 18]. In recent clinical studies, circulating NEP levels were significantly associated with the composite endpoint of cardiovascular death or HF hospitalization (HR 1.17; p=0.001) and cardiovascular death (HR: 1.19; p=0.002) in patients with HF[19]. The PARAGON-HF trial includes all-comers with HFpEF; the final data collection will be completed May 2019. It is being increasingly recognized that HFpEF is a disease entity that has considerable phenotypic heterogeneity[20, 21]. Therefore, targeting all-comers who suffer from a heterogeneous disease process using a single treatment intervention may not result in desirable outcomes as has been evident with multiple prior negative HFpEF trials[22, 23]. The importance of biomarker based clinical trials that deliver the right drug to the right patient is being increasingly recognized as a useful strategy to limit sample size and decrease costs by enriching such trials with potential responders[24, 25]. Whether the beneficial effects of Entresto™ therapy are dependent on baseline NEP levels is unknown and is being explored in this study. The **hypothesis** in this proof of concept study is that

subjects with lower NEP levels will not have an equivalent response to Entresto™, a NEPi, as compared to those with higher NEP levels. The **primary endpoint** measured is change in biomarkers with Entresto™ administration that reflect NEP activity and myocardial stress (NT pro-ANP, -BNP, -CNP) and drug action (cGMP). This endpoint has been well validated as a measure of Entresto™ drug response[26].

To establish a median value which will be used to categorize subjects recruited for this pilot study into a low and high NEP group we have measured circulating NEP levels in 242 samples from the **RELAX** and **NEAT-HFpEF** studies. The **RELAX study** was a multicenter, double-blind, placebo-controlled, randomized clinical trial of 216 stable outpatients with HFpEF[27]. **NEAT-HFpEF** was a multicenter, randomized study of 110 outpatients with HFpEF[28]. The median circulating NEP level in these populations was 1.55 ng/ml (Q1=0.49, Q3=24.99). Therefore subjects with circulating NEP levels of ≤1.55 ng/ml will be considered as having low NEP levels and those with values >1.55 ng/ml will fall into the high NEP level group.

1.3 Dose Rationale and Risk/Benefits

The effect of administering Entresto™ to subjects with “high” or “low” NEP levels will be studied. Favorable response to Entresto™ therapy in these subjects will be assessed by evaluating a change in biomarkers that reflect NEP activity, myocardial wall stress and clinical outcomes (NT-proANP, NT-proBNP, NT-proCNP)[29] and reflect efficacy of drug action (cGMP)[26]. Entresto is FDA approved for use in HFrEF and will be administered as recommended in the drug labeling information as an off label use in HFpEF as 49/51 mg twice-daily.

2 Study Objectives

Primary Objective: To determine biomarker (plasma NT-proANP, NT-proBNP, NT-proCNP and cGMP) responses to Entresto™ in patients with HFpEF who have high and low serum NEP levels.

3 Study Design

3.1 General Design

Study design: This is a proof of concept single arm study that will be performed in the Clinical Research and Trials Unit (CRTU) at the Center for Clinical and Translational Science (CCaTS), Mayo Clinic. 40 subjects with HFpEF will be assigned to Entresto 49/51 mg (sacubitril/valsartan) twice-daily for a total duration of up to 5 weeks of treatment.

3.2 Primary Study Endpoints

The primary endpoint measured is change in biomarkers with Entresto™ administration that reflect NEP activity and myocardial stress (NT pro-ANP, -BNP, -CNP) and drug action (cGMP). This endpoint has been well validated as a measure of Entresto™ drug response.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

1. Age ≥ 50 years
2. LVEF ≥ 45% assessed by echocardiography, nuclear scan, MRI or left ventriculogram within the past 24 months

3. Current New York Heart Association (NYHA) class 2-4 symptoms of heart failure (HF)
4. Stable medical therapy for 30 days as defined by:
    a. No addition or removal of ACE, ARB, beta-blockers, calcium channel blockers (CCBs) or aldosterone antagonists
    b. No change in dosage of ACE, ARBs, beta-blockers, CCBs or aldosterone antagonists of more than 100%
5. Elevated NT-proBNP (>300 pg/ml) or BNP (>200 pg/ml)
6. One of the following within the last 24 months
    a. Previous hospitalization for HF with radiographic evidence of pulmonary congestion (pulmonary venous hypertension, vascular congestion, interstitial edema, pleural effusion) ***or***
    b. Catheterization documented elevated filling pressures at rest (LVEDP≥15 or PCWP≥20) or with exercise (PCWP≥25) ***or***
    c. Echo evidence of diastolic dysfunction / elevated filling pressures (***at least two***)
        i. E/A > 1.5 + decrease in E/A of > 0.5 with valsalva
        ii. Deceleration time ≤ 140 ms
        iii. Pulmonary vein velocity in systole < diastole (PVs<PVd) (sinus rhythm)
        iv. E/e'≥15
        v. Left atrial enlargement (≥ moderate)
        vi. Pulmonary artery systolic pressure > 40 mmHg
        vii. Evidence of left ventricular hypertrophy
            1. LV mass/BSA ≥ 96 (♀) or ≥ 116 (♂) g/m^2
            2. Relative wall thickness ≥ 0.43 (♂ or ♀) [(IVS+PW)/LVEDD]
            3. Posterior wall thickness ≥ 0.9 (♀) or 1.0 (♂) cm

4.2 Exclusion Criteria

1. History of hypersensitivity or allergy to ACE inhibitors (ACEIs), ARBs, or NEP inhibitors
2. Known history of angioedema to ACE inhibitors (ACEIs) or ARBs
3. Previous LVEF < 40% at any time
4. Systolic blood pressure < 100 mmHg or > 180 mmHg
5. Current acute decompensated HF (exacerbation of chronic HF manifested by signs and symptoms that may require intravenous therapy)
6. Unstable angina, myocardial infarction, stroke, transient ischemic attack, or cardiovascular surgery or urgent percutaneous coronary intervention (PCI) within 3 months of screening or elective PCI within 30 days of entry
7. Significant valvular stenosis or regurgitation (greater than moderate in severity), hypertrophic, restrictive or obstructive cardiomyopathy including amyloidosis, constrictive pericarditis, primary pulmonary hypertension, or biopsy proven active myocarditis
8. Severe congenital heart disease
9. History of heart transplant or with LV assist device
10. Evidence of severe hepatic disease as determined by any one of the following: history of hepatic encephalopathy, history of esophageal varices, or history of porto-caval shunt.
11. Glomerular filtration rate < 20 ml/min/1.73 m2 on most recent clinical laboratories*
12. Serum potassium of > 5.5 mEq/dL on most recent clinical laboratories*
13. Concomitant use of aliskiren in patients with diabetes
14. Currently receiving an investigational drug
15. Inability to comply with planned study procedures

16. Female subject who is pregnant or breastfeeding
* Performed within 90 days of enrollment

4.3 Subject Recruitment, Enrollment and Screening

Subjects meeting the inclusion criteria of the study will be recruited at the Mayo Clinic from the Cardiovascular Diseases Clinic, Community Internal Medicine, General Internal Medicine, Mayo Clinic Cardiovascular Disease database, and past participants of HFpEF studies. The Mayo Clinic Life Science System (MCLSS) will aid recruitment for all the protocols. The MCLSS [redacted] allows identification of patients in real time during an episode of care no matter where (inpatient or outpatient) at Mayo the patients are being seen. [redacted] The study coordinator will query the system daily for potential candidates, review the integrated electronic medical records and if appropriate, contact the patient's physician to discuss the study and the patient's participation. Patients will then be contacted for personal interview in person, by phone or letter. Informed consent will be obtained at the screening visit during which blood will be drawn for safety laboratories and NEP levels. Subjects will be assigned to a low or high NEP level group based on the levels and it is anticipated that up to 100 subjects will undergo a screening visit to ultimately enroll 40 subjects for the study which will be carried out during Visits 1 and 2. Subjects will receive a reimbursement of $100 for participation in each visit (Visits 1 and 2) of the study.

4.4 Early Withdrawal of Subjects

If a subject should stop taking study medication for any reason before completing the 5 weeks of study drug dosing, an attempt should be made to restart the study drug at the lowest starting dose when the subject is stable, per physician discretion. If the treating physician decides to stop study medication and start the patient on an ACEI, then a 36 hour wash-out period is required after the last dose of study drug before starting the ACEI.

4.5 When and How to Withdraw Subjects

If subjects experience an adverse reaction to Entresto™ or if disease progression occurs after consent, the Principal Investigator may consider discontinuing study drug treatment.

4.6 Data Collection and Follow-up for Withdrawn Subjects

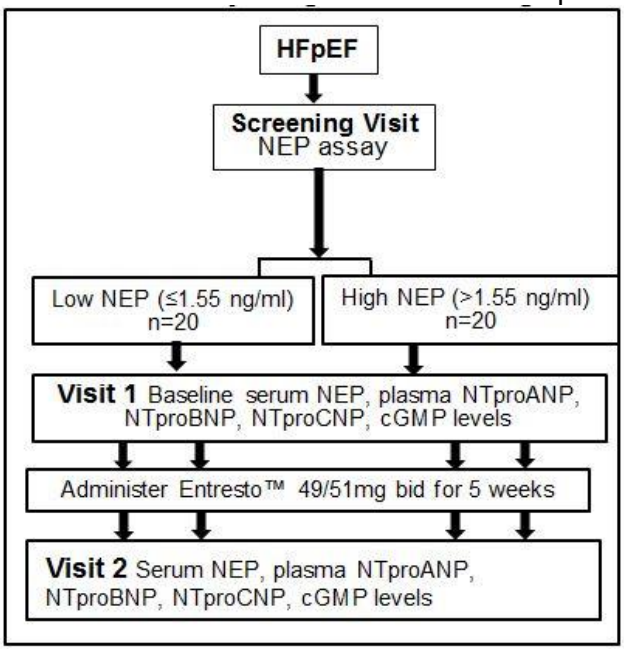
Subjects will complete all study assessments of Study Visit 2 if they have received study drug for at least 2 weeks or more, regardless of whether they have received the full 5 weeks of study drug treatment. The blood collection component of the study assessment will need to occur within 24 hours of drug discontinuation.

5 Study Intervention

5.1 Description

Please see accompanying **Figure**. Study Intervention comprises of eligible HFpEF subjects who have provided informed consent and in whom blood will be collected (screening visit) to measure serum NEP levels and based on these levels will be assigned to a low or high NEP group. These subjects after having blood drawn at Visit 1 for baseline NEP, NT pro-ANP, -BNP,

-CNP and cGMP levels will then receive Entresto™ for up to 5 weeks. They will return in the 5th week for another blood draw to measure NEP, NT pro-ANP, -BNP, -CNP and cGMP levels. At visits 1 and 2 subjects will also provide urine samples for cGMP levels at baseline and post intervention analysis. Blood will also be collected and stored for future transcriptomic, metabolomic and genomic studies.



5.2 Treatment Regimen

40 subjects with HFpEF will be administered their first dose of Entresto™ at Visit 1. Sitting systolic and diastolic BP will be monitored in the subject's dominant arm 3 times with an automatic blood-pressure monitor (Omron, Bannockburn, IL) and an average reading of these 3 measurements will be used. BPs will be checked 60 minutes after first dose is administered at Visit 1 to ensure tolerance of the medication. If tolerated, subjects will receive Entresto™ at a recommended dose of 49/51 mg on an empty stomach twice daily for up to 5 weeks. Patients will be reassessed at Visit 2 in the 5th week.

5.3 Method for Assigning Subjects to Treatment Groups

This single arm study will be performed at in the Clinical Research and Trials Unit (CRTU) at the Center for Clinical and Translational Science (CCaTS), Mayo Clinic. Subjects with circulating NEP levels of ≤1.55 ng/ml will be considered as having low NEP levels and those with values >1.55 ng/ml will fall into the high NEP level group

5.4 Preparation and Administration of Entresto™

- Dispensed by the Research Pharmacy
- Entresto will be taken by mouth by the patient twice daily for up to 5 weeks

5.5 Subject Compliance Monitoring

For this protocol, first dose of Entresto™ is administered in the CRTU by CRTU staff. Subsequent compliance will be monitored by a 2 week phone call made by the study coordinator.

5.6 Prior and Concomitant Therapy

Subjects should be treated with standard HF strategies (diuretics for congestion, blood pressure control and heart rate control if subject is in atrial fibrillation) as per recommended guidelines. Subjects may not take ARBs, ACE inhibitors and direct renin inhibitors during the study. If subjects are on ACEi or ARBs, these drugs will be withheld 36 hours before the initiation of treatment with Entresto™ at Visit 1. All other medications will be allowed.

5.7 Masking/Blinding of Study Drug

There is no masking or blinding of study drug since this is an open label study.

5.8 Receiving, Storage, Dispensing and Return

Will be managed by Research Pharmacy.

5.9 Receipt of Drug Supplies

Will be managed by Research Pharmacy

5.10 Storage

Entresto™ will be kept in a secure, limited-access storage area by Research Pharmacy.

5.11 Dispensing of Entresto™

Entresto™ reconciliation will be performed by the Research Pharmacy per their standard procedures.

5.12 Return or Destruction of Entresto™

Patients will be asked to destroy any unused Entresto™ tablets.

6 Study Procedures

6.1 Screening Visit

Possible study participants will meet with study coordinator to review consent form. Medical history and current medications will be reviewed. Blood will be drawn for baseline serum NEP levels, safety laboratory tests (potassium, creatinine) and pregnancy test if necessary at the CRTU. Dependent on the results of the NEP levels which will be available within 48 hours, subjects will be assigned to a high or low NEP group. If subjects are found to be eligible and are on ACEi or ARBs, subjects will be instructed to withhold these drugs 36 hours before the initiation of treatment with Entresto™ at Visit 1 and throughout the duration of the study while subjects are on Entresto™.

6.2 Visit 1

Visit 1 will occur within 30 days of the Screening Visit. Qualified subjects will be evaluated as outpatients at the CRTU. Interim history, a review of medications, NYHA class assessment and a brief physical exam will be performed by a qualified study team member. Blood will be drawn for serum NEP levels, NT-proANP, NT-proBNP, NT-proCNP, cGMP and a pregnancy test will be performed, if necessary; urine sample will also be collected for cGMP levels. An additional 55 mL blood sample will be collected from subjects, for future transcriptomic, metabolomic and genomic analysis, who provide consent to allow their blood to be stored in the BAP laboratory for future studies. Subjects will be given the option to opt in or out of this component of the study. Following this, subjects will receive their first dose of Entresto™. BPs will be monitored at baseline prior to

administration of Entresto™ and 60 minutes after this first dose to ensure tolerance of the medication. Sitting systolic and diastolic BP will be monitored in the subject's dominant arm 3 times with an automatic blood-pressure monitor (Omron, Bannockburn, IL) and an average reading of these 3 measurements will be used. Before dismissal, subjects will be provided with Entresto™ tablets for the duration of the study. Study coordinator will follow-up with the subject by telephone on day 14 ± 5 post-visit 1 to monitor compliance and to assess tolerance.

6.3 Visit 2

Upon completion of 4 weeks of drug intervention, during the 5th week of therapy subjects will be admitted as outpatients to the CRTU. Subjects will be asked to take their last dose of Entresto™ prior to their clinical visit. Interim history, a review of medications, NYHA class assessment and a brief physical exam will be performed by a qualified study team member. Baseline sitting systolic and diastolic BP will be monitored in the subject's dominant arm 3 times with an automatic blood-pressure monitor (Omron, Bannockburn, IL) and an average reading of these 3 measurements will be used. Blood will be drawn for serum NEP levels, NT-proANP, NT-proBNP, NT-proCNP and cGMP; urine sample will also be collected for cGMP levels. An additional 55 mL blood sample will also be collected from subjects, for future transcriptomic, metabolomic and genomic analysis, who provide consent to allow their blood to be stored in the BAP laboratory for future studies as in Visit 1.

7 Potential Risks and Adequacy of Protection against Risks

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.

7.1 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.

Entresto™ (sacubitril/valsartan): The use of Entresto™ compared to the guideline-indicated ACE inhibitor enalapril reduced mortality was shown in the PARADIGM trial to reduce the hazard ratio for cardiovascular death and mortality by 20%. The known risks of Entresto™ overlap with those of valsartan, a drug that has been used in patients with HFpEF. In the PARADIGM trial, use of Entresto™ was associated with a greater incidence of angioedema than the comparator drug enalapril (0.5% vs. 0.2%). The risk of angioedema with Entresto™ compared to enalapril was relatively greater among Black patients (2.4% vs. 0.5%). Patients with a history of angioedema are contraindicated from taking this drug. Trial data indicate adverse reactions occurring ≥5% are hypotension, hyperkalemia, cough, dizziness, and renal failure. Symptomatic hypotension in PARAGON was more common among patients taking Entresto™ than enalapril (18% vs. 12%).

Subjects will be given appropriate instructions to monitor for any untoward effects and to report immediately and seek medical attention if required. Also, a study team member will be following-up with the subjects on a timely basis as described above to monitor compliance and to assess tolerance, Hence, the study has extremely little risk of causing irreversible consequence on the patients.

Plan of intervention in the event of adverse effects to the subjects During the course of the treatment, if the patient does develop angioedema, or signs or symptoms of hypotension, or any

other untoward symptoms the study drug may be discontinued and the clinical care team will manage the subject according to standard policies and procedures.

Other potential risks include:
This protocol may be hazardous to an unborn child. When pregnancy is detected, Entresto™ will be discontinued as soon as possible. Drugs that act directly on the renin-angiotensin system such as Entresto™ can cause injury and death to the developing fetus. Therefore, female participants must be postmenopausal or have been surgically sterilized or have a negative pregnancy test and must agree to use a reliable method of contraception until study completion. Methods of contraception include: birth control pill/implants/injections, intrauterine devices, spermicide, diaphragm or condoms.

Schedule of Events Table

Study Activity	Screen	Visit 1 Day 1	Interim Call Day 14±5	Visit 2 Week 5
Study Agent		X		X
Informed consent	X			
History (NYHA Class)	X	X	X	X
Concurrent meds	X	X	X	X
Physical Exam (Ht, Wt, BSA, VS,		X		X
Blood draw	X[a]	X[b]		X[b]
B-hCG	X[c]	X[c]		
Urine collection		X[d]		X[d]

a: Plasma NEP, serum potassium, creatinine if required

b: Plasma NEP, NT pro-ANP, -BNP, -CNP, and cGMP; Blood for future studies
c: Serum pregnancy test (women of childbearing potential)
d. cGMP levels in urine sample

7 Statistical Plan

7.1 Sample Size Determination

The sample size calculation and statistical analysis have been designed in collaboration with the Division of Biostatistics. This is a proof of concept study; 40 subjects will be recruited, with 20 in each group (Group 1: subjects with HFpEF - low NEP levels and, Group 2: subjects with HFpEF - high NEP levels). It is anticipated that 100 subjects will be screened.

7.2 Statistical Methods

The primary analysis for this study will be a comparison of the changes in values of parameters of interest between the study groups low NEP vs. high NEP following up to 5 weeks of study drug intervention. This analysis will be completed using a paired t-test comparing the change from baseline to 5 weeks after study drug intervention. A secondary analysis will also be performed to evaluate the effects of the varying doses (in case, if the dose of the drug is changed during the course of the study). Analysis of variance methods will be used to compare changes from baseline to 6 hours, and pairwise group comparisons will be made via two-sample t-test if the overall ANOVA F-test is found to be significant. Distributions of changes will be examined and appropriate transformations applied prior to analyses, if necessary. Values will be expressed as the mean $\pm$ SEM. A statistically significant difference will be considered to be present when $p<0.05$.

8 Participant Safety and Adverse Events

8.1 Institutional Review Boards

All 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. Any amendments to the protocol, other than minor administrative changes, must be approved by each IRB before they are implemented.

8.2 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.

8.3 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.

8.4 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).

8.5 Laboratory Test Abnormalities

For laboratory test abnormalities that meet the definition of a serious adverse event (SAE), which required the subject to have the study drug 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).

8.6 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.

8.7 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.

Anticipated Adverse Events and Procedure Effects

The following are anticipated, disease-related events in patients with HF or anticipated events of interest in patients with HFpEF taking Entresto™:

- **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 an increase in eGFR ≥ 20% over 48 hours, or progressive loss of renal function over time.
- **LVAD implantation:** This refers to implantation of a temporary or durable LVAD.
- **Cardiac Transplantation**
- **Hyperkalemia > 6 mEq/L**
- **Acute renal failure with serum creatinine > 2.5 mg/dL**
- **Angioedema**
- **Symptomatic hypotension**

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

8.8 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 CRF, but should be documented in the source documents and followed according to local standard of care.

SAEs, except for those anticipated AEs listed above, occurring from *signed informed consent* to Week 5 visit will be captured on the SAE CRF. 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 CRF, which will be distributed to the appropriate sponsor contact.

Information collected on the adverse event worksheet (and entered in the research database):

- Subject’s name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5)
- If any intervention was necessary:

- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution

8.2.7 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. Investigators are also responsible for promptly reporting AEs to their reviewing IRB/EC in accordance with local requirements.

8.2.8 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 overall study PI within the same timeline used for reporting to regulatory authorities.

8.2.9 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 CRF.

8.9 Stopping Rules

The study would be stopped only if advised because of unanticipated adverse events.

8.10 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events. After informal discussions with IRB staff in the midst of the study, given that 1. This was not a Phase 1,2,,3 study or clinical trial providing definitive information about effectiveness or safety of the drug 2. Prior data suggesting that the drug was safe to use leading to FDA approval for its use in heart failure 3. No major clinical endpoints were being evaluated to assess safety and effectiveness 4. No ethical issues were being raised in this proof of concept study regarding efficacy or safety that would lead to premature stopping of the study - a decision was made not to engage a DSMB.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction. Study CRF components will include an enrollment and demographics form; forms for recording relevant history, HF symptoms, physical exam results, laboratory results, 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.

9.4 Data Quality Control

Prior to the start of enrollment, the Investigators and Study Coordinators will be trained on the clinical protocol and data collection procedures. Personnel at the clinical sites will enter the data mandated by the protocol into the CRFs. 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. Follow-up training and training for new study personnel will be conducted as needed.

9.5 Data Security

Access to databases will be controlled centrally 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. All disk drives that provide network services, and all user computers, will be protected using virus-scanning software.

9.6 Publication Policy

Dissemination of preliminary information can adversely affect the objectivity of study data. For this reason, Investigators will be prohibited from 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.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Regulatory Issues

11.1 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.

11.2 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.

11.3 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.

12 Study Administration

12.1 Coordinating Center

Mayo Clinic will function as the CC for this trial.

12.2 Core Laboratories

Mayo Clinic will serve as the core laboratory for measurement of serum NEP and other biomarkers. Plasma specimens will be collected at Study Visits (weeks 0, and 5), 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 subjects who agree to participate in the biorepository will have samples collected at the clinical sites stored at the Mayo Clinic BAP.

13 Study Finances

13.1 Funding Source

This study is financed through a grant from the US National Institute of Aging, National Institutes of Health.

13.2 Subject Stipends or Payments

Participants completing the study will receive $100.00 each for Study Visit 1 and 2 for a total remuneration of $200.

14 Publication Plan

The primary responsibility for publication of the results lies with the principal investigator. The trial will be registered on ClinicalTrials.gov.

15 References

1. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. Circulation. 2002;105(11):1387-93.

2. Huntley BK, Sandberg SM, Noser JA, Cataliotti A, Redfield MM, Matsuda Y, Burnett JC, Jr. BNP-induced activation of cGMP in human cardiac fibroblasts: interactions with fibronectin and natriuretic peptide receptors. J Cell Physiol. 2006;209(3):943-9.

3. Itoh H, Pratt RE, Dzau VJ. Atrial natriuretic polypeptide inhibits hypertrophy of vascular smooth muscle cells. J Clin Invest. 1990;86(5):1690-7.

4. Sangaralingham SJ, Huntley BK, Martin FL, McKie PM, Bellavia D, Ichiki T, Harders GE, Chen HH, Burnett JC, Jr. The aging heart, myocardial fibrosis, and its relationship to circulating C-type natriuretic Peptide. Hypertension. 2011;57(2):201-7. PMCID: 4440480.

5. Hashim S, Li Y, Anand-Srivastava MB. Small cytoplasmic domain peptides of natriuretic peptide receptor-C attenuate cell proliferation through Gialpha protein/MAP kinase/PI3-kinase/AKT pathways. Am J Physiol Heart Circ Physiol. 2006;291(6):H3144-53.

6. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, et al. 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2013;128 CIR.0b013e31829e8776.

7. Marin-Grez M, Fleming JT, Steinhausen M. Atrial natriuretic peptide causes pre-glomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. Nature. 1986;324(6096):473-6.

8. Sonnenberg H, Honrath U, Chong CK, Wilson DR. Atrial natriuretic factor inhibits sodium transport in medullary collecting duct. Am J Physiol. 1986;250(6 Pt 2):F963-6.

9. Janssen WM, de Jong PE, van der Hem GK, de Zeeuw D. Effect of human atrial natriuretic peptide on blood pressure after sodium depletion in essential hypertension. Br Med J (Clin Res Ed). 1986;293(6543):351-3.

10. Dillingham MA, Anderson RJ. Inhibition of vasopressin action by atrial natriuretic factor. Science. 1986;231(4745):1572-3.

11. Harris PJ, Thomas D, Morgan TO. Atrial natriuretic peptide inhibits angiotensin-stimulated proximal tubular sodium and water reabsorption. Nature. 1987;326(6114):697-8.

12. Steele MK, Gardner DG, Xie PL, Schultz HD. Interactions between ANP and ANG II in regulating blood pressure and sympathetic outflow. Am J Physiol. 1991;260(6 Pt 2):R1145-51.

13. Yang RH, Jin HK, Wyss JM, Chen YF, Oparil S. Pressor effect of blocking atrial natriuretic peptide in nucleus tractus solitarii. Hypertension. 1992;19(2):198-205.

14. Chen HH, Lainchbury JG, Harty GJ, Burnett JC, Jr. Maximizing the natriuretic peptide system in experimental heart failure: subcutaneous brain natriuretic peptide and acute vasopeptidase inhibition. Circulation. 2002;105(8):999-1003.

15. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile MR. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med. 2014;371(11):993-1004.

16. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, Shi V, Bransford T, Takeuchi M, Gong J, Lefkowitz M, Packer M, McMurray JJ. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: a phase 2 double-blind randomised controlled trial. Lancet. 2012;380(9851):1387-95.

17. Komada Y, Peiper S, Tarnowski B, Melvin S, Kamiya H, Sakurai M. Shedding of the common acute lymphoblastic leukemia antigen (CALLA) by lymphoblastoid cell lines. Leuk Res. 1986;10(6):665-70.

18. Malfroy B, Kuang WJ, Seeburg PH, Mason AJ, Schofield PR. Molecular cloning and amino acid sequence of human enkephalinase (neutral endopeptidase). FEBS Lett. 1988;229(1):206-10.

19. P Bayes-Genis A, Barallat J, Galan A, de Antonio M, Domingo M, Zamora E, Urrutia A, Lupon J. Soluble neprilysin is predictive of cardiovascular death and heart failure hospitalization in heart failure patients. J Am Coll Cardiol. 2015;65(7):657-65.

20. Kitzman DW, Upadhya B. Heart failure with preserved ejection fraction: a heterogenous disorder with multifactorial pathophysiology. J Am Coll Cardiol. 2014;63(5):457-9.

21. Shah AM, Solomon SD. Phenotypic and pathophysiological heterogeneity in heart failure with preserved ejection fraction. Eur Heart J. 2012;33(14):1716-7.

22. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, et al. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. JAMA. 2013;309(12):1268-77. PMCID: 3835156.

23. Pitt B, Pfeffer MA, Assmann SF, Boineau R, Anand IS, Claggett B, Clausell N, Desai AS, Diaz R, Fleg JL, Gordeev I, Harty B, Heitner JF, Kenwood CT, Lewis EF, et al. Spironolactone for heart failure with preserved ejection fraction. N Engl J Med. 2014;370(15):1383-92.

24. Ahmad T, Fiuzat M, Pencina MJ, Geller NL, Zannad F, Cleland JG, Snider JV, Blankenberg S, Adams KF, Redberg RF, Kim JB, Mascette A, Mentz RJ, O'Connor CM,

Felker GM, et al. Charting a roadmap for heart failure biomarker studies. JACC Heart Fail. 2014;2(5):477-88.

25. Pereira NL, Sargent DJ, Farkouh ME, Rihal CS. Genotype-based clinical trials in cardiovascular disease. Nat Rev Cardiol. 2015;12(8):475-87.

26. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Belohlavek J, et al. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. Circulation. 2015;131(1):54-61.

27. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, et al. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. JAMA. 2013;309(12):1268-77. PMCID: 3835156.

28. Zakeri R, Levine JA, Koepp GA, Borlaug BA, Chirinos JA, LeWinter M, VanBuren P, Davila-Roman VG, de Las Fuentes L, Khazanie P, Hernandez A, Anstrom K, Redfield MM. Nitrate's effect on activity tolerance in heart failure with preserved ejection fraction trial: rationale and design. Circ Heart Fail. 2015;8(1):221-8. PMCID: 4304404.

29. Felker GM, Hasselblad V, Hernandez AF, O'Connor CM. Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials. Am Heart J. 2009;158(3):422-30