The Effect of Vericiguat on Peripheral Vascular Function, Patient Health Status and Inflammation in Patients with Heart Failure with Reduced Ejection Fraction

NCT05420012

Document Type: Study Protocol and Statistical Analysis Plan

Document Date: 5/29/2025

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Protocol Summary

IRB Approval Date of Current Version:	5/29/2025
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Background and Introduction

The incidence of heart failure (HF) continues to increase, along with its associated morbidity, mortality, and cost. Novel therapeutic options have been proposed to address the needs of especially the patients who remain symptomatic despite optimal medical therapy. A number of factors lead to ongoing symptoms in patients with chronic heart failure (HF), including persistent abnormalities in myocardial function, neurohormonal dysregulation, and of the peripheral vascular system.

The Phase 3 VICTORIA trial examined the efficacy of Vericiguat, a novel oral soluble guanylate cyclase (sGC) stimulator in patients with HF and reduced ejection fraction (HFrEF). Vericiguat enhances the cyclic guanosine monophosphate (GMP) pathway by directly stimulating soluble guanylate cyclase independent of nitric oxide (NO). The VICTORIA study showed that patients who received Vericiguat 2.5 mg once daily up-titrated to 10 mg daily had a lower incidence of the primary endpoint of cardiovascular death or first HF hospitalization compared to placebo.

Determining the exact mechanism, or the respective contribution of different mechanisms, through which Vericiguat improves outcomes in HFrEF may allow for better tailoring of its use to individual patients. The preliminary results of an echocardiography sub-study indicate that there was no significant difference in the change of left ventricular ejection fraction (LVEF) between baseline and study end among patients assigned to the active drug vs placebo. We hypothesize that the beneficial effects of Vericiguat in HF may not be linked to improvement in myocardial contractility, but rather to the effects of cGC stimulation on the peripheral vasculature. This was not directly tested in VICTORIA.

Studies from our group and others have collectively identified a marked reduction in vascular function, as determined by flow-mediated vasodilation (FMD) testing, in patients with HFrEF despite optimized pharmacotherapy, indicative of a pervasive, disease-related reduction in endothelial health. Endothelial dysfunction is characterized by NO dysregulation, inflammation, and oxidative stress. These factors impair the capacity of the vascular endothelium to perform its numerous functions including regulation of vascular tone and inflammatory processes. Importantly, endothelial dysfunction is also associated with reduced quality of life and decreased physical capacity in patients with HFrEF. These studies suggest that the consequences of vascular dysfunction are far-reaching and support the concept that interventions targeting the peripheral vasculature to induce systemic effects could prove beneficial in cardiovascular disease. This is particularly relevant given the known relationship between endothelial dysfunction and mortality risk in patients with HFrEF. Improvement in peripheral vascular function in patients with HFrEF would in turn lead to improved physical capacity and health-related quality of life (hrQOL).

Preclinical studies provide evidence of cGC stimulation favorably affecting peripheral vascular function. In a rat model of HF, treatment with Ataciguat normalized endothelial function, improved sensitivity to NO, and reduced platelet activation. However, the impact of Vericiguat on vascular health has not been evaluated in human HF. A recent study also examined the effect of Vericiguat on inflammation and oxidative stress in HF. After 12 weeks of Vericiguat therapy, high sensitivity CRP (hsCRP) decreased significantly, and the probability of hsCRP value being ≤3.0 mg/L at the end of the study was higher in patients treated with Vericiguat compared to placebo. Although the impact of Vericiguat on upstream, pro-inflammatory cytokines such as IL-1β and IL-18 have not been determined, there is strong evidence supporting elevation of these biomarkers that reflect NRLP3 inflammasome activation in patients with HFrEF. Given the recent success in clinical trials targeting the inflammasome in heart failure and recent evidence for the efficacy of sGC stimulation to mitigate NLRP3 inflammasome activity in other organ systems, there is strong rationale for the expectation that Vericiguat may favorably impact both upstream (IL-1β, IL-18, and IL-6) and downstream (hsCRP) proinflammatory biomarkers. Importantly, an inverse correlation between biomarkers of inflammation and endothelial function has been observed in other patient groups, supporting the concept that Vericiguat treatment may result in greater improvements in vascular function in those individuals who experience the largest reductions in vascular inflammation.

Summary.

This background provides clear rationale in support of the concept that direct stimulation of sGC could be a particularly effective approach to increase cGMP in conditions of increased inflammation/oxidative stress, endothelial dysfunction and reduced NO bioavailability. Thus, the aim of the proposed study is to examine the effect of Vericiguat on peripheral vascular function, inflammatory status, and patient health status. We also aim to identify patients who are particularly likely to benefit from Vericiguat treatment and predict that that these patients will be defined by baseline peripheral vascular dysfunction and high inflammatory state.

Purpose and Objectives

This is a randomized, placebo-controlled, parallel-group, single-center, double-blind trial of vericiguat in subjects with heart failure with reduced ejection fraction (HFrEF) to be conducted in conformance with Good Clinical Practice (GCP).

Purpose

The purpose of this study is to determine the effect of Vericiguat in patients with HFrEF.

This study will:

1) Evaluate the impact of Vericiguat on Peripheral Vascular Function;

2) Evaluate the impact of Vericiguat on Patients' Health Status;

3) Evaluate the impact of Vericiguat on Inflammation;

4) publish and disseminate results.

Primary objective:

1. To determine the impact of Vericiguat administration on vascular function in patients with HFrEF.

   Hypothesis: Endothelium-dependent vasodilation, as determined by flow-mediated vasodilation (FMD) testing, will be improved following 12-weeks of Vericiguat treatment compared to placebo.

Secondary objectives:

1. To determine the impact of Vericiguat administration on health-related quality of life and physical capacity in patients with HFrEF.

   Hypothesis 1a: Physical capacity, as determined by change in six minute walk test (6MWT) distance, will be better following 12-weeks of Vericiguat treatment compared to placebo.

   Hypothesis 1b: Quality of life, as determined by change of Kansas City Cardiomyopathy Questionnaire overall summary score, will be better following 12-weeks of Vericiguat treatment compared to placebo.

2. To explore the relationship between inflammation and vascular function in response to vericiguat in patients with HFrEF.

   Hypothesis: A positive correlation between changes in FMD and biomarkers of inflammation will be observed following 12-weeks of Vericiguat treatment, such that the greatest improvements in FMD will be observed in those patients who experience the largest decreases in proinflammatory biomarkers (IL-1β, IL-6, IL-18, and hsCRP).

Study Population

Age of Participants: 18+

Sample Size:
At Utah:
All Centers: 30

Inclusion Criteria:

1. History of chronic HF with New York Heart Association (NYHA) Class II-III symptoms. Patients with NYHA 1 (asymptomatic) or NYHA 4 (severe limitations – symptoms even while at rest) symptoms, will not be enrolled.

2. Left ventricular ejection fraction (LVEF) of ≤45% assessed within 12 months prior to randomization by any imaging method.
3. Systemic blood pressure ≥90/60 mmHg.
4. Standard guideline-directed HF therapy.
5. If female of reproductive potential, agrees to avoid becoming pregnant while receiving study drug and for 14 days after the last dose of study drug by complying with abstinence from heterosexual activity or use (or have her partner use) contraception during heterosexual activity.

Exclusion Criteria:

1. Addition of a new disease-modifying HF pharmacotherapy or CRT-D in previous 4 weeks.

2. Current or anticipated use of long-acting nitrates or nitric oxide (NO) donors including isosorbide dinitrate, isosorbide 5-mononitrate, pentaerythritol tetranitrate, nicorandil or transdermal nitroglycerin (NTG) patch, and molsidomine.
3. Current or anticipated use of phosphodiesterase type 5 (PDE5) inhibitors such as vardenafil, tadalafil, and sildenafil.
4. Current use or anticipated use of a soluble guanylate cyclase (sGC) stimulator such as riociguat.
5. Known allergy or sensitivity to any sGC stimulator.
6. Estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m2 or chronic dialysis.
7. Patients who are pregnant or breastfeeding or plan to become pregnant or to breastfeed.

Design

Blinded Trial (Single or Double Blinded)
Placebo Controlled Trial
Randomized Trial

A Randomized Parallel-Group, Placebo-Controlled, Double-Blind, Single - Center Trial of The Effect of Vericiguat on Peripheral Vascular Function, Patient Health Status and Inflammation in Patients with Heart Failure with Reduced Ejection Fraction.

Study Procedures

Recruitment/Participant Identification Process:

Potential participants will be screened while admitted inpatients at the University and VA Hospitals or while attending routine clinic appointments in the Cardiovascular Medicine clinic.

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 study, they will review and sign the IRB approved informed consent form before any study specific procedures are conducted.

Informed Consent:

Description of location(s) where consent will be obtained:

University of Utah Health Sciences Center Veterans Affairs SLC Health Care System (VAMC)

Description of the consent process(es), including the timing of consent:

Potential participants will be screened while inpatients at the University and VA Hospitals or attending routine clinic visit in Cardiovascular medicine clinic. 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 study, they will review and sign the IRB approved informed consent form before any study specific procedures are conducted.

Procedures:

TRIAL PROCEDURES

The Trial Flow Chart (Table 1) summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled timepoints if deemed clinically necessary by the investigator.

Table 1. Trial Flow Chart

Administrative Procedures

Informed Consent

The investigator or qualified designee must obtain documented consent from each potential subject prior to participating in the clinical trial. If there are changes to the subject's status during the trial (e.g., health or age of majority requirements), the investigator or qualified designee must ensure the appropriate consent is in place.

General Informed Consent

Consent must be documented by the subject's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB's approval/favorable opinion in advance of use. The subject should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB requirements, applicable laws and regulations and Sponsor requirements.

Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

Subject Identification Card

All subjects will be given a Subject Identification Card identifying them as participants in a research trial. The card will contain trial site contact information (including direct telephone numbers) to be utilized in the event of an emergency. The investigator or qualified designee will provide the subject with a Subject Identification Card immediately after the subject provides written informed consent. At the time of treatment allocation/randomization,site personnel will add the treatment/randomization number to the Subject Identification Card.

The subject identification card also contains contact information for the emergency unblinding call center so that a health care provider can obtain information about trial medication/vaccination in emergency situations where the investigator is not available.

Medical History

A medical history will be obtained by the investigator or qualified designee.

Prior and Concomitant Medications Review

Prior Medications

The investigator or qualified designee will review prior medication use, and record prior medication taken by the subject 30 days before starting the trial.

Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial as instructed in the Data Entry Guidelines.

Assignment of Screening Number

All consented subjects will be given a unique screening number that will be used to identify the subject for all procedures that occur prior to randomization or treatment allocation. Each subject will be assigned only one screening number. Screening numbers must not be re-used for different subjects.

Any subject who is screened multiple times will retain the original screening number assigned at the initial screening visit.

Assignment of Treatment/Randomization Number

All eligible subjects will be randomly allocated and will receive a treatment/randomization number. The treatment/randomization number identifies the subject for all procedures occurring after treatment allocation/randomization. Once a treatment/randomization number is assigned to a subject, it can never be re-assigned to another subject.

A single subject cannot be assigned more than one treatment/randomization number.

Trial Compliance

Subjects will be directed to bring any used and unused bottles to each visit. The investigator must maintain a complete and current accountability record for the blinded investigational product.

Study drug will be dispensed starting at Randomization Visit and at Visit 1. Compliance with blinded trial medication will be assessed by tablet counts of returned medication. To facilitate this, subjects must be instructed to return all of the study drug packaging including unused study drug and empty packaging.

Any discrepancies between actual and expected amount of returned study medication must be discussed with the subject at the time of the visit, and any explanation must be documented in the source records.

Consultation should occur in a timely manner upon site learning of poor study drug compliance.The research site should conduct patient counseling regarding importance of study drug adherence, and the investigator can dispense study drug after such counseling, if in their medical judgment, the patient is able to comply moving forward with study treatment instructions.

Clinical Procedures/Assessments

Height

Height will be measured without shoes, using a stadiometer or other appropriate device.

Weight

Body weight will be measured using a standardized scale at each of the pre-defined time points outlined in the Trial Flow Chart.

Physical Examinations

Physical examination will be done at a clinical visit (inpatient or outpatients) preceding screening or randomization. The documentation will be reviewed by the investigator or qualified designee.

Vital Signs

Vital sign measurements include a triplicate measurement of sitting blood pressure and pulse rate. Site personnel should use the same blood pressure measuring device throughout the study for each subject. Other procedures should not be performed during the time of the blood pressure and pulse rate measurements.

Blood pressure assessment is to be completed after the subject has been seated for a 10 - minute rest. Whenever possible, BP measurements should be obtained using the same arm, same BP monitoring device, and same examiner at each visit. Systolic and diastolic blood pressures will be determined by averaging 3 replicate measurements obtained approximately 2 minutes apart.

Pulse Rate

Pulse rate is to be assessed after the subject has been seated for a 10 minute rest. Three measurements of the pulse rate must will be taken determined by averaging 3 replicate measurements obtained approximately 2 minutes apart.

Assessment of pulse rate can be manual (rather than using an automated device); however, when done manually,pulse rate must be measured in the brachial/radial artery for at least 30 seconds.

Quality of Life Measures

Kansas City Cardiomyopathy Questionnaire

The KCCQ-12 is a 12-item, self-administered questionnaire intended for the quantification of HF patients' perspectives of how their disease impacts their lives. The questionnaire requires, on average, 5 to 8 minutes for completion. The KCCQ-12 measures the impact of patients' heart failure, or its treatment, on 7 distinct domains using a 2 -week recall period: symptom frequency, symptom burden, physical limitation, health perceptions, social limitations, self-efficacy and symptom stability. The KCCQ-12 domains of self-efficacy and symptom stability will be reported but are not considered in the efficacy assessment as they address patient knowledge and recent changes in symptoms respectively.

In addition, there are 3 summary scores; a total symptom scale that combines the symptom frequency and the symptom burden scores, a clinical summary scale that combines the total frequency and physical limitation scores to replicate the NYHA Classification, and an overall summary score that includes the Total Symptom, PhysicalLimitation, Social Limitation and Quality of Life scores. The clinical summary score and its components address the most relevant concepts for the heart failure patients, showing good correlation with improvement following HF hospitalization.

Visual Analogue Scale

The VAS records patient self-rated health on a 20 cm vertical VAS with endpoints labeled 'the best health you can imagine' and 'the worst health you can imagine' and is scored on a 0 to 100 scale.

Vital Status Assessment

Vital status is collected at Week 2, Week 8 and Final Phone Contact. This assessment can also be performed at any point in the trial when vital status is in question, unless subject has specifically withdrawn consent for further follow-up.

Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below.

Laboratory tests are specified in Table 2.

NT-proBNP, TNF-α, hsCRP, IL-1β, IL-6, IL-18 measurements assessed by the central lab will be blinded after Visit 2.

Table 2. Laboratory Tests

Hematology	**Chemistry**	**Other**
Hematocrit (Hct)	Bicarbonate	Natriuretic Peptide (NTproBNP)
Hemoglobin (Hb)	Blood glucose	Serum β-human chorionicgonadotropin†
Mean corpuscular volume(MCV)	Blood urea nitrogen (BUN)	TNF-α
Mean corpuscular hemoglobin (MCH)	Calcium	hsCRP
Mean corpuscularhemoglobin concentration(MCHC)	Creatinine	IL-1β
Platelet count	Chloride	IL-6
Red blood cells (RBC)	Potassium (K)	IL-18
Red cell distribution width(RDW)	Sodium (Na)	
Reticulocytes		
White blood cells (WBC)		

Flow Mediated Dilation (FMD)

A blood pressure cuff will be placed on the arm, distal to the ultrasound Doppler probe on the brachial artery. Simultaneous measurements of brachial artery blood velocity and vessel diameter will be performed using a linear array transducer operating in duplex mode, with an imaging frequency of 14 MHz and Doppler frequency of 5 MHz (Logic 7, GE Medical Systems, Milwaukee, WI). All measurements will be obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume will be maximized according to vessel size and centered within the vessel on the basis of real-time ultrasound visualization. The brachial artery will be insonated approximately midway between the antecubital and axillary regions, and measurements of diameter and blood velocity (Vmean) will be obtained continuously at rest and for 2 minutes after cuff deflation. End-diastolic, ECG R-wave-gated images will be collected via video output from the Logic 7 for off-line analysis of brachial artery vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, IA). During FMD, non-invasive arterial blood pressure may be assessed. Systemic arterial blood pressure (ABP) will be measured non-invasively on a continuous basis using finger photoplethysmography (Finometer,Ohmeda, Madison, WI, USA). The finometer cuff will be placed on a finger and supported at heart level.

6 MWT (Six-minute walk test)

Six-minute walk test distance will be performed according to published guidelines for this patient group by trained research staff. The 6MWT will be performed indoors, along a flat, straight corridor with a hard surface. The subjects will wear comfortable clothing and shoes. The subjects will be instructed on the objectives of the test and be provided instructions on how the test will be carried out.

Cardiac and Inflammation Marker Investigations

Cardiac and inflammation marker investigations will examine the impact of vericiguat on HF and peripheral vascular disease progression and identify candidate markers that may predict drug response to the treatment.

Marker blood samples will be collected at the time points indicated in Study Flow Chart.

Investigators will not receive the results of analyses during the study, and no alerts will be sent.

Other Procedures

Withdrawal/Discontinuation

Subjects who discontinue/withdraw from treatment prior to completion of the treatment regimen should be encouraged to continue to be followed for all remaining study visits.

When a subject discontinues/withdraws from participation in the trial, all applicable activities scheduled for the Premature Treatment Discontinuation visit should be performed at the time

of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements.

Blinding/Unblinding

STUDY TREATMENT IDENTIFICATION INFORMATION IS TO BE UNMASKED ONLY IF NECESSARY FOR THE WELFARE OF THE SUBJECT. EVERY EFFORT SHOULD BE MADE NOT TO UNBLIND THE SUBJECT UNLESS NECESSARY.

For emergency situations where the investigator or sub-investigator needs to identify the drug used by a subject and/or the dosage administered he/she will contact the investigational pharmacy and make a request for emergency unblinding. The investigator or sub-investigator will enter the intensity of the adverse experiences observed, their relation to study drug, the reason thereof, etc., in the medical chart.

Visit Requirements

Visit requirements are outlined in Trial Flow Chart.

Screening

Procedures for the Screening visit can be performed over several days. Alternatively, the screening visit and randomization visit can take place on the same day and subjects can be randomized during this visit if all required tests for eligibility criteria are available.

Additionally, evidence of a LVEF <45% within 12 months prior to randomization by any method (most recent measurement must be used) must be provided.

Subjects who sign the ICF and, who for any reason (e.g. failure to satisfy the selection criteria), do not continue on to randomization will be considered a screen failure.

Treatment Period

Visit requirements are outlined in Trial Flow Chart. Total treatment period will be 12 weeks.

Randomization Visit

At the randomization visit, subjects who fulfill all inclusion/exclusion criteria will be randomized to either vericiguat or placebo on a background of standard of care HF treatment.

As noted above, the Screening visit and the Randomization visit can occur on the same day, if all required tests for eligibility criteria are available at screening visit.

All procedures and assessments specified for this visit will be done after screening visit procedures are performed.

Eligible subjects will be provided a blood pressure machine and cuff to perform at-home blood pressure assessment required at FU phone call A (performed at week 2) and FU phone call B (performed at week 8).

Unscheduled Visits

In the event a subject requires an unscheduled visit for dose modification, the following assessments will be performed:

- Adverse events
- Dose assessment
- Clinical events
- Concomitant medication: record any new or ongoing medication or changes in dosage
- Blood pressure and pulse rate (3 measurements, each approximately 2 minutes apart) prior to study drug dosing

Upon interruption of the study drug due to intolerability, intake should be resumed as soon as medically justified to the discretion of the investigator.

Investigators should make every attempt to resume study drug treatment in all subjects after interruption of study drug as soon as medically justified.

Follow-Up Phone Calls

Patients will be contacted at specific timepoints mentioned in study Flow Chart to assess for clinical events,adverse events, blood pressure measurements and vital status (if applicable) and titrate the investigational drug doses accordingly.

Final Visit

Final Visit's study procedures are outlined in Study Flow Chart.

Final Phone Contact

All subjects will be contacted by phone after 14 days of the Final visit to assess for clinical events, adverse events and vital status (if applicable).

Procedures performed for research purposes only:

Statistical Methods, Data Analysis and Interpretation

Statistical Methods

General

Standard descriptive statistics (proportions and frequencies for categorical factors; means, standard deviations, and designated percentiles for numeric factors) will display baseline characteristics by randomized treatment group. If a substantial imbalance is identified in a particular baseline factor, the ITT analyses described below will be accompanied by sensitivity analyses to investigate the impact of inclusion of this factor as a covariate. Outcomes with substantial skewness may be transformed prior to subsequent analyses to better approximate normality. Analyses evaluating the effect of the treatment on study outcomes will be performed in accordance with the intention-to-treat principle, with subjects analyzed according to their randomly assigned treatment irrespective of the achieved dose of Vericiguat. Unless specified otherwise, hypothesis tests will be performed with 2-sided α=0.05, without adjustment for multiple comparisons.

Primary Analysis

The effect of administration of Vericiguat on the primary FMD outcome will be estimated by applying an analysis of covariance to compare the mean FMD levels at 12 weeks between the Vericiguat and placebo groups, with the baseline FMD levels included in the model as a covariate.

Secondary Analyses

The effect of administration of Vericiguat on the secondary 6-minute walk test and KCCQ outcomes will be estimated by applying separate analyses of covariance to compare the mean levels of these outcomes at 12 weeks between the Vericiguat and placebo groups, with the baseline level of the outcome included as a covariate. Separate analysis of covariance models will also be used to estimate the effects of the intervention on C-Reactive Protein and Interleukinς 1β, 6, 18. If these markers exhibit heavy positive skewness, as we expect, they will be log transformed prior to analysis.

We will also provide scatter plots with Pearson and Spearman partial correlations with adjustment for randomized treatment group to describe the association of the change in FMD from baseline to 12 weeks with changes in individual inflammatory markers during the same time interval. We will also provide Pearson and Spearman correlations relating changes in FMD to changes in inflammatory markers separately within the two randomized groups. Positively skewed inflammatory markers will be log transformed prior to this analysis.

Power analyses

We expect to enroll and randomize 24 study subjects. With an anticipated drop-out rate of 15%, data from 20 subjects will be available for analysis.

Primary Outcome.

Flow-Mediated Dilation (FMD): Output from FMD testing is maximal change in brachial artery diameter across a 2-min period following 5 min of supra-systolic arterial occlusion, expressed as % change from pre-occlusion baseline. We hypothesize that the treatment will lead to a 5.1% increase in FMD relative to the control group. We anticipate a standard deviation (SD) in %FMD 2.4% in patients with HFrEF. With this SD, 10 evaluable patients per treatment group will provide at least 80% power with 2-sided α=0.05 to detect an increase in mean %FMD of 3.18% relative to the control group. The minimum detectable effect of 3.18% is well under the hypothesized effect size of 5.1%, indicating that the design has excellent statistical power to evaluate the hypothesized treatment effect on the primary outcome. This power calculation conservatively assumes a 0 correlation between the baseline and 12-week FMD values; if the serial correlation is at least 0.5, the study will have at least 80% power to detect an 2.76% increase in %FMD.

Secondary Outcomes

6MWT: We anticipate SD of 100 m in the 6MWT. With this assumed SD, 10 evaluable patients per treatment group will provide at least 80% power with 2-sided α=0.05 to detect an increase in 6MWT distance of 115 m so long as the serial correlation between the baseline and 12 week 6MWT values is at least 0.5.

Kansas City Cardiomyopathy Questionnaire (KCCQ): We anticipate an SD for the change in the KCCQ of 19.9 points. Based on this assumed SD, 10 evaluable patients per treatment group will provide at least 80% power with 2-sided α=0.05 to detect an improvement in mean KCCQ of 24.4 points. It is anticipated that KCCQ score changes will be smaller. The absolute differences may be hypothesis generating and provide pilot data for future targeted investigations.

hsCRP: We project a median and interquartile range from the baseline hsCRP values of 3.68 mg/L and (1.14-8.41 mg/L), respectively. Under the assumption that hsCRP is lognormally distributed, we estimate an SD of 1.323 for log transformed hsCRP. Based on this assumed SD, 10 evaluable patients per treatment group will provide at least 80% power with 2-sided α=0.05 to detect an 83% reduction in the geometric mean hsCRP for the treatment group compared to the control group.

Interleukins 1β, 6, 18 and TNF-α: We also assume an SD of 1.323 for log transformed IL-1β, IL-6, IL-18, TNF-α. Hence, we also expect that 10 evaluable patients per treatment group will provide at least 80% power with 2-sided $\alpha=0.05$ to detect an 83% reduction in the geometric mean of these biomarkers for the treatment group compared to the control group.

We note that with 10 patients per group, our proposed design provides excellent statistical power to detect the hypothesized treatment effect on the primary outcome of FMD. Our statistical power will allow to detect large effects on the secondary outcomes. Hence, analyses of the secondary outcomes will be exploratory.

Correlational analyses

A sample size of 20 evaluable subjects is sufficient to detect a partial correlation of 0.59 with 80% power and 2-sided type-1 error of 5%.

Multiplicity. In this preliminary study, all hypothesis tests will be performed on a comparisonwise basis, without adjustment for multiple comparisons.