Protocol Version 7.1

Nitrite, Isoquercetin and Endothelial Dysfunction (NICE) Trial

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Abbreviations

AE	Adverse event
ADMA	Asymmetrical dimethylarginine
BP	Blood pressure
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CKD	Chronic kidney disease
CrCl	Creatinine clearance
CRF	Case Report Forms
CRP	C reactive protein
CVD	Cardiovascular disease
DSMB	Data and Safety Monitoring Board
eGFR	Estimate glomerular filtration rate
ET-1	Endothlin-1
FDA	Food and Drug Administration
FMD	Flow-mediated vasodilation
G6PD	Glucose-6-phosphate dehydrogenase
GRAS	Generally Recognized as Safe
HbA1c	Hemoglobin A1c
HDL-C	High density lipoprotein cholesterol
ICAM-1	intercellular adhesion molecule-1
ICF	Informed Consent Form
IL-1	Interleukin-1
IL1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IRB	Institutional review board
IR-nitrite	Immediate Release Sodium Nitrite
LDL-C	Low density lipoprotein cholesterol
MCP-1	Monocyte chemoattractant protein-1
NICE	Nitrite Isoquercetin Endothelial dysfunction
NO	Nitric Oxide
PAD	Peripheral Arterial Disease
SAE	Serious Adverse Event
TNF- α	Tumor necrosis factor- α
VCAM-1	Vascular adhesion molecule-1
vWF	Von Willebrand factor
WBC	White blood cell count

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1.0. TRIAL OVERVIEW

The proposed study is a randomized, double-blind, placebo-controlled, parallel-design clinical trial with a treatment and a control group. The overall objectives of the trial are to test the efficacy and safety of combination therapies. The treatment arm includes Immediate release sodium nitrite (IR-nitrite formula, 40 mg twice daily) and isoquercetin (225 mg, once daily). The control (placebo) arm tests placebo versions of IR-nitrite and isoquercetin. Effects will be measured based on endothelial dysfunction, inflammation, and oxidative stress among patients with albuminuric and non-albuminuric chronic kidney disease (CKD).

Sodium nitrite at 40 mg and 80 mg doses by mouth twice per day are considered safe and well tolerated based on previously conducted animal and phase 1 and 2 clinical trials.[4-7] The FDA has documented that the normal dietary dose of isoquercetin (as proposed in this trial) is considered Generally Recognized as Safe.

Eligible participants will be required to complete a screening visit, baseline/randomization visit, and follow-up visits conducted at day 3 (phone only), and in-person clinic visits on weeks 1, 6 and 12 following randomization. During the intervention period, Flow-mediated dilation (FMD), serum creatinine, urine albumin-to-creatinine ratio, biomarkers of endothelial dysfunction, inflammation, and oxidative stress will be measured at follow-up visits marked by 6 and 12 weeks post randomization (introduction to drug therapy).

The primary trial endpoint is the measurement of net change in endothelium-dependent FMD between the active treatment group and the placebo-control group over the 3-month intervention period. Secondary and exploratory endpoints include the expected differences in endothelial dysfunction biomarkers, estimated-glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratios between the intervention and control groups.

2.0. SPECIFIC AIMS

Chronic kidney disease (CKD) is highly prevalent in Louisiana and around the world.[1,2] Cardiovascular disease (CVD) and end-stage renal disease (ESRD) are the major causes of premature death in patients with CKD.[3,4] Randomized controlled trials have documented that renin-angiotensin system antagonists, blood pressure reduction, glycemic control, and lipid lowering can slow CKD progression and reduce CVD risk among CKD patients.[5,6] However, multiple risk factors contribute to the progression of CKD and development of CVD among CKD patients.[7] Previous works from our group and others indicate that endothelial dysfunction and inflammation may be the common etiologic pathway for the rapid progression of CKD and excess risk of CVD independent of traditional risk factors.[8-12] Nitric oxide (NO) deficiency may be a key component of

endothelial dysfunction in CKD patients and contribute to accelerate both CKD and CVD progression.[13-21] Until now, there has been no specific or effective pharmacological treatment for endothelial dysfunction and inflammation among CKD patients. Previous phase 2 trials suggest that sodium nitrite provides exogenous NO and improves endothelial function.[22-26] Dietary isoquercetin supplement (a natural flavonoid found in fruits and vegetables) has also been shown to reduce inflammation and oxidative stress.[27-33]

The overall objective of this proposed randomized placebo-controlled trial is to test the efficacy and safety of combination therapy with sodium nitrite (immediate-release sodium nitrite formula, 40 mg twice daily) and the dietary isoquercetin supplement (225 mg, once daily) on endothelial function, inflammation and oxidative stress among CKD patients, as compared to the placebo group.

The study plans to recruit 70 patients with albuminuric and non-albuminuric CKD from the greater New Orleans area in Louisiana. Participants will be randomly assigned to receive combination therapy with sodium nitrite and isoquercetin, or receive placebos of IR-nitrite and Isoquercetin for 3 months. The primary study outcome is endothelial function assessed by brachial artery flow-mediated dilatation (FMD). Secondary outcomes include biomarkers of endothelial dysfunction, inflammation, and oxidative stress. This trial is designed to have 80% statistical power to detect a 2.0% difference in FMD with a 2-sided significance level of 0.05.

2.1. Primary objective: *To test the efficacy of a combination therapy with sodium nitrite (IR-nitrite) and dietary isoquercetin supplement on endothelial function assessed by FMD among patients with CKD.*

Primary hypothesis: Compared to CKD patients receiving placebo IR-nitrite and Isoquercetin, FMD will be increased among CKD patients receiving the combination therapy with IR-nitrite and isoquercetin.

2.2. Secondary objectives: *To test the efficacy of the combination therapy with sodium nitrite and dietary isoquercetin supplement on biomarkers of endothelial function, inflammation, and oxidative stress among patients with CKD.*

Secondary hypothesis 1: Compared to CKD patients receiving placebo, the plasma levels of asymmetric dimethylarginine (ADMA), intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), E-selectin, endothlin-1 (ET-1), von Willebrand factorv (vWF), endothlin-1, and endostatin will be reduced among CKD patients receiving IR-nitrite and isoquercetin.

Secondary hypothesis 2: Compared to CKD patients receiving placebo, the plasma levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin 6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) will be reduced among CKD patients receiving IR-nitrite and isoquercetin.

Secondary hypothesis 3: Compared to CKD patients receiving placebo, the plasma levels of oxidized LDL and nitrotyrosines will be reduced among CKD patients receiving IR-nitrite and isoquercetin.

2.3. Safety objective: *To test the safety of the combination therapy with IR-nitrite and dietary isoquercetin supplement among patients with CKD.*

Safety hypothesis 1: The combination treatment is safe and well-tolerated. Blood levels of methemoglobin, nitrite, nitrate, and isoquercetin are within acceptable range in the treatment group.

Safety hypothesis 2: Compared to CKD patients receiving placebo control, eGFR will be increased and urinary albumin will be decreased among CKD patients receiving SR-nitrite and isoquercetin.

Impact: The proposed research is the first clinical trial to investigate the efficacy and safety of combination therapy with IR-nitrite and isoquercetin on endothelial function and inflammation among CKD patients. The findings from this study will help in the development of novel treatments targeting endothelial dysfunction and inflammation, primarily aiming to slow the progression of CKD and development of CVD among patients with CKD. This study will generate the necessary pilot data for an NIH-supported large phase-III clinical trial to test the efficacy of IR-nitrite and isoquercetin combined treatment and its effect on CKD progression.

3.0. BACKGROUND AND SIGNIFICANCE

3.1. Summary of Relevant and Significant Findings from Nonclinical Studies

Sodium Nitrite: Previous studies suggest that sodium nitrite supplement provides exogenous nitric oxide and improves endothelial function.[1-7] For instance, systemic intravenous administration of sodium nitrite (8.7 μmol/min) dilated the radial artery by 10.7% (95% confidence interval, 6.8-14.7%) under normal oxygenated conditions at supraphysiological and near-physiological concentrations in healthy male volunteers.[2] Nitrite enhanced cGMP production in a dose-dependent manner, suggesting nitric oxide-mediated vasodilatory effects.[2] Nitrite-induced radial artery dilation was maximal under conditions of normoxia and inhibited by hypoxia and hyperoxia.[2] A phase 2 clinical trial suggests that diabetic patients with peripheral artery disease receiving sodium nitrite (40 mg and 80 mg) by mouth had significantly higher flow-mediated vasodilation compared with those receiving the placebo. Furthermore, dietary sodium nitrite attenuates brain death-induced renal injury by regulating responses to ischemia and inflammation, ultimately leading to improvement in post-transplant kidney function in rats.[28]

Isoquercetin: Isoquercetin has been demonstrated to have a dose-dependent protective effects against oxidative endothelial injury in cultured human endothelial cells.[13] It has also been shown to protect venular endothelium from inflammatory products released by activated blood platelets and polymorphonuclear granulocytes in vitro.[14] Isoquercetin increases antioxidant activities and reduces oxidative stress in combating cadmium-induced toxicity in animals.[15] A small randomized, double-blind, placebo-controlled crossover trial reported that quercetin-3-glucoside (160 mg/d) reduced E-selectin by 27.7 ng/mL, interleukin -1β by 20.23 pg/mL, and the z score for inflammation by 20.33 in 37 healthy hypertensive or pre-hypertensive men and women.[16] Quercetin (500 mg four times per day) also reduced plasma malondialdehyde (a marker of lipid peroxidation) and tumor necrosis factor-α levels in a clinical trial among patients with sarcoidosis.[17] In addition, quercetin 500 mg + vitamin C 250 mg reduced interleukin-6 by 38% compared

to placebo in a randomized double-blind clinical trial among 60 male physical education students. A similar effect was not observed in the subjects who received quercetin 500 mg/day alone.[18] The mechanism of anti-inflammatory effects of quercetin/isoquercetin may be via down-regulation of the nuclear factor kappa B (NF-kB) pathway.[19-21] It has also been reported that quercetin is capable of inhibiting tumor necrosis factor-α production as well as tumor necrosis factor-α gene expression via modulation of NF-kB in human peripheral blood mononuclear cells.[22] Moreover, since NF-kB activation induces oxidative stress, the direct inhibition of NF-kB pathway by quercetin therefore reduces oxidative stress. A laboratory study also suggested that quercetin decreased lipid peroxidation and increased nitric oxide production and antioxidant enzyme activity.[23]

3.2 Description of and Justification for the Route of Administration, Dosage, Dosage Regimen, and Treatment Period

Sodium nitrite: Oral administration of IR- nitrite will be used due to its increased safety and practicality for routine clinical use, when compared to intravenous therapy. Patients will receive IR-nitrite for three months, as empirical evidence suggests that this time frame is sufficient to show that changes in endothelial dysfunction (measured by FMD) can be detected. During the treatment portion of the trial, the following factors will be monitored with these goals in mind: methemoglobin, nitrite, and nitrate (aiming to keep methemoglobin <3%, plasma nitrite within the normal range of 1.3-13 μmol/L, and plasma nitrate within the normal range of 4.0-45.3 μmol/L).[50]

Isoquercetin: The effective oral dose of 225 mg isoquercetin (supplied by Quercegen Pharmaceuticals), will be administered to participants daily. This dose is below the threshold of the FDA's acceptable daily intake of 293 mg/person/day and the dose for no-observed-adverse-effect level (539 mg/kg/day).[24] Isoquercetin has been selected over quercetin due to the fact that the absorption rate of isoquercetin is roughly 2 times higher in comparison to quercetin[11,12] and the effective dose of isoquercetin is half that of quercetin in combination with vitamin C.[18]. It is also less likely that participants would have any side effects with isoquercetin. Expected clinical symptoms including headaches and plasma isoquercetin levels will be closely monitored throughout patient participation.

3.3. Pharmacology and Description of the Investigational Products

Immediate Release Sodium Nitrite (IR-nitrite): The chemical formula appears as $NaNO_2$. IR-nitrite is also referred to as TV1001, a product of TheraVasc Inc. (pharmaceutical company). IR-Nitrite is absorbed from the stomach. The half-life elimination is approximately one hour, with peak effect occurring at roughly 25 minutes, while duration of action is close to 10 hours. More detailed information on sodium nitrite, including the

investigator's brochure, the compound's chemistry, manufacturing and control data, and pharmacology/toxicology data is described in the FDA approved IND Application (IND #108526, Protocol TheraVasc-TV1001-002, submitted by TheraVasc, Inc. on March 14, 2011, serial #0001, ClinicalTrials.gov identifier: NCT01401517). Please note, the IND# specific to the sodium nitrite for packaging label given by TheraVasc is #108526 and appears on the Investigator's Brochure as such. The IND associated with the NICE trial by the FDA is #127397.

Nitrite is a major intravascular storage pool for nitric oxide. Sodium nitrite has been used as a nitric oxide-donor to improve endothelial dysfunction in the treatment of cardiovascular disease (CVD).[1-7]

In brief, sodium nitrite at 40 mg and 80 mg doses by mouth twice per day are safe and well tolerated in animal as well as human phase 1 and 2 clinical trials.[4-7] Its biological effect in improving endothelial dysfunction has been observed in both animal studies and phase 2 clinical trials.[4-6] The major adverse effects associated with sodium nitrite are methemoglobinemia and hypotension. However, these potential adverse effects were not observed in patients with peripheral artery disease who received 40 mg or 80 mg immediate release sodium nitrite by mouth twice per day, or in patients with diabetes mellitus and active or healed foot ulcers who received a single dose of 80 mg IR-nitrite in clinical trials.[4,7]

Isoquercetin: Isoquercetin (quercetin 3-glucoside or quercetin-3-O-glucoside) is a natural dietary flavonoid of fruits and vegetables. In a human study, isoquercetin was shown to peak at 30 minutes after ingestion of 156 mg, with a peak plasma concentration of 5 µmol/L. The half-life elimination is reported as 18.5 hours, and urinary excretion is measured as 3.6%.[8] Isoquercetin has the same therapeutic effects in vivo as quercetin, but with better bioavailability, resulting in potentially increased efficacy compared to quercetin.[9] In a study of male participants, the absorption rate was noted to be 52% for quercetin 3-glucosides and 24% for quercetin.[10] Rats given quercetin and isoquercetin achieved plasma concentration 2.5 to 3 times higher with isoquercetin compared to quercetin.[11,12]

The FDA has documented isoquercetin to be Generally Recognized as Safe (GRAS) in 2007 with an acceptable daily intake of 293 mg/person/day. Detailed information on isoquercetin and quercetin, such as their chemical and pharmacological properties, as well as safety and toxicology data is described in the GRAS application (please see attached letter on GRAS for isoquercetin and quercetin). Isoquercetin and quercetin have been shown to reduce inflammation and oxidative stress in animal studies and phase 2 clinical trials.[13-23] No-observed-adverse-effect level of enzymatically decomposed rutin (containing 95% isoquercitrin) in Wistar rats was estimated to be 1% (539 mg/kg/day, i.e. 38 g for a 70 kg individual) for acute or chronic use. Adverse effects of higher doses in rats included mostly (benign) chromaturia.[24] This trial will administer a 225 mg daily dose as this is below the FDA's threshold of the acceptable daily intake of 293 mg/person/day as well as the previously discussed dose of539 mg/kg/day, which showed no-observed-adverse-effect level.

Vitamin C: Vitamin C is also referred to asL-ascorbic acid or ascorbate. Participants will receive vitamin C 55.8mg by mouth daily, as a stabilizing, inactive ingredient in the

Isoquercetin capsules, to increase the absorption of isoquercetin and reduce methemoglobin, oxidative end products of nitric oxide, and nitrosamine resulting from sodium nitrite treatment.[25-27]

3.4. Safety of Investigational Products

Sodium nitrite: Sodium nitrite has been used as a nitric oxide-donor to improve endothelial dysfunction in the treatment of cardiovascular disease (CVD).[1-7] Nitric Oxide (NO) deficiency may be a key component of endothelial dysfunction in patients with chronic kidney disease (CKD) and contribute to accelerate both CKD and CVD progression.[29-38] Therefore, sodium nitrite may correct nitric oxide deficiency and improve clinical CKD and CVD outcomes in CKD patients. However, the increased NO production associated with sodium nitrite supplementation also potentially dilates vessels and causes side effects such as hypotension and dizziness. Headaches, when occurring as a side effect, are transient and reversible. In addition, nitrite can react with oxyhemoglobin to form methemoglobin, which may cause tissue hypoxia. The reaction of nitrite with secondary amines under acidic conditions may form carcinogenic nitrosamines. Increased oxidation of NO may also induce formation of nitrosamines. Vitamin C (an antioxidant) can reduce methemoglobin to hemoglobin as well as prevent nitrosamine formation.[25-27] According to 9 Code of Federal Regulations (CFR) 424.22, 550 ppm (550ppm = 550mg/kg) of Vitamin C has been used in cured meats (containing nitrite) to reduce nitrosamine formation within the food industry, according to the regulation of U.S. Department of Agriculture. Therefore, only 0.044 mg of vitamin C is needed for 80 mg of sodium nitrite (used in this study, sodium nitrite 40 mg BID) to prevent nitrosamine formation.

Other antioxidants, such as vitamin E and erythorbic acid, have also been used for the same purpose.
For the purposes of this trial, IR-nitrite will be given orally, which was demonstrated as safe in a phase 2 clinical trial, without any additional vitamin C supplement.[4] Patients in both arms of the trial will receive a dose of 55.8 mg of vitamin C supplied (more than 550 ppm vitamin C for sodium nitrite) as a filler ingredient within the isoquercetin capsule. The combination of vitamin C and isoquercetin used in this study may further increase the effect of antioxidants, thereby reducing nitrosamine formation. In addition, as absorption time of IR-nitrite is roughly 30 minutes, participants will be asked to take their dosage of sodium nitrite 30 minutes before meals to avoid a reaction with potential amines in food. In order to report and treat any potential side effects, we will closely monitor methemoglobin and nitrite related oxidation product such as nitrotyrosines through blood draws at visits.
A dose of 40 mg of IR-nitrite twice per day will be used for the treatment arm patients, which is a 50% reduction of the FDA-approved dosage of 80 mg twice per day. The 80mg BID dose was well-tolerated in a clinical trial studying patients with peripheral artery disease (PAD).[4] The reduced dosage is selected due to increased safety considerations amid patients with declined kidney function, as the proposed cohort of this clinical trial.

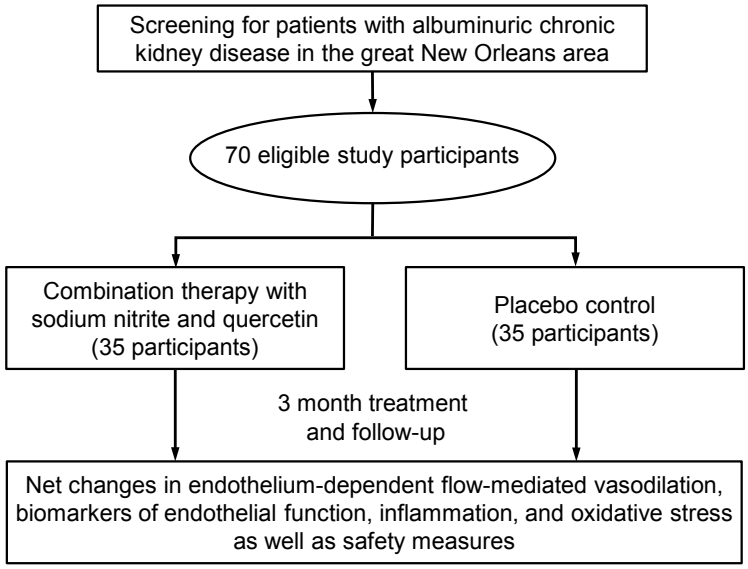
Isoquercetin: Isoquercetin/quercetin have anti-inflammatory and antioxidant effects, which have been documented in both phase 2 clinical trials and animal studies.[13-23] Inflammation and oxidative stress are increased in CKD patients and predict CKD and CVD progression.[39-49] Therefore, CKD patients may benefit from isoquercetin treatment through the reduction of inflammation and oxidative stress and ultimately improved CKD clinical outcomes. The Isoquercetin capsule additionally contains 55.8 mg of vitamin C and 4.5mg of vitamin B3 to increase absorption and stability of isoquercetin.

4.0. INVESTIGATIONAL PLAN

4.1. Study Design - Overview

The proposed study is a randomized, double-blind, placebo-controlled, parallel-design trial. Seventy (70) patients with chronic kidney disease who meet eligibility criteria will be recruited in the greater New Orleans area. Eligible participants will be randomly assigned to the intervention (combination therapy with IR-nitrite and isoquercetin) and placebo (placebo IR-nitrite and Isoquercetin) groups. The intervention will last for 3 months. The primary, secondary, and safety outcomes will be measured throughout the proposed time frame (Figure 1).

Figure 1. Overview of the Study Design



4.2. Study Population

The study plans to recruit 70 patients with albuminuric and non-albuminuric chronic kidney disease (CKD) in the greater New Orleans area including Tulane hospital, Louisiana State University Medical Center, and Ochsner Health System. Thirty-five (35) patients will be randomly assigned to the active combination treatment group and 35 to the placebo control group.

4.3. Inclusion Criteria

a. Men and women aged 21-74 years old of all race/ethnicity backgrounds
b. eGFR<60 ml/min/1.73m^2 or urinary albumin-to-creatinine ratio ≥30 mg/g or protein to creatinine ratio ≥150 mg/g
c. Systolic BP ≥120 and <180 mmHg and/or diastolic BP ≥70 and <110 mmHg

4.4. Exclusion Criteria

a. Allergic to organic nitrite, isoquercetin, and/or vitamin C
b. Institutionalized (e.g., prisoner, nursing home or skilled nursing facility resident)
c. Unable or unwilling to give consent
d. Known HIV infection and/or AIDS
e. Pregnant or lactating women
f. Currently on dialysis
g. Previous or current organ or bone marrow transplant
h. Receiving immunosuppressive treatment or other immunotherapy
i. Receiving chemotherapy or alkylating agents for systemic cancer
j. Recent acute myocardial infarction, cerebrovascular accidence or transient ischemic attack, or hospitalization in 3 months
k. Acute kidney injury within 3 month
l. Currently taking a phosphodiesterase-5 enzyme inhibitor, such as Viagra
m. Chronic headaches
n. Chronically receiving fluoroquinolones, cyclosporin (neural, sandimmune), nitrate drug, NSAIDS (except aspirin ≤ 81 mg daily) , allopurinol or Uloric (febuxostat), meperidine and related central nervous system (CNS) depressants, oral glucocorticoids, and not willing or able to stop during study period.
o. Active infection (i.e. systemic or osteomyelitis)
p. Class III or IV heart failure
q. History of hemolytic anemia including sickle cell disease
r. Hemoglobin <10
s. History of chronic obstructive pulmonary disease (COPD)
t. Have a positive screen for glucose-6-phosphate dehydrogenase (G6PD) deficiency at screening
u. Involvement in other clinical trials
v. Current alcohol or other substance abuse
w. Current smokers
x. Unwillingness to stop flavonoid or nitrite/nitrate supplementation

4.5. Clinical Screening visit

All subjects will undergo screening visit to assess their eligibility.

The following information will be obtained at the clinical visit:

- Informed consent for the screening visit
- Detailed medical history and use of medication and dietary supplement
- Blood pressure and pulse (measured using a standardized automatic blood pressure monitor); height and weight

- CBC; BMP (Serum creatinine (eGFR)), urine albumin, and plasma aglucose-6-phosphate dehydrogenase measured via serum samples- roughly 15mL of blood. Exception: should CBC, BMP and UA results be available within three months, these labs will not be drawn.
- Spot urine pregnancy test for women at child-bearing age without surgical sterilization (such as tubal ligation).
- Information about heart function including review of medical records, assessment of clinical symptoms, and a full physical exam as needed.

4.6. Randomization Procedures

Eligible participants will be assigned, using a block stratified randomization method, to the active treatment or placebo control groups. The randomization will be stratified by sex and use random block sizes of 4 or 6 in order to maintain balance among groups over time. A statistician, who is not involved in the clinical operation of the trial, will prepare the randomization allocation using the SAS program. The randomization allocation will be concealed in a sealed envelope, and stored securely with the statistician. The sealed envelope will be opened only by the investigational (research) pharmacist who is not involved in the clinical operation of the trial. After eligibility and consent have been ascertained, the study nurse/coordinator will order the study drugs from Tulane pharmacy. The research pharmacist will match the patient identifier to the subsequent randomization allocation, and dispense the study drugs according to the assignment. Drug packs will be labeled with random pack numbers, and each patient will receive one bottle at randomization with a 45-day supply, and one bottle at their week-6 visit. Periodic quality control checks will be run between the clinical research nurse and the investigational pharmacist to ensure the same number of patient identifiers have been placed into allocated positions. Once the assignment has been made, trial participants are considered to be officially randomized, and every effort will be made to obtain complete follow-up information.

Study participants, coordinators and investigators will be blinded to treatment assignments. In addition, ultrasound technicians and clinical laboratory technicians will be blinded to the intervention assignments. Separate rooms at the study clinic will be utilized for ultrasound measures, blood draws, and study intervention. All biochemical measures will be conducted at Tulane University Medical Center Clinical Laboratory. Ultrasound readings will be performed at the University of Pennsylvania Hospital Cardiovascular Center, where all technicians will be unaware of the study participants' study arm assignments.

Placebo and active medications will be stored in the Research Pharmacy at Tulane University Medical Center. Research pharmacists will receive written orders for the coordinating drug pack(s) to be dispensed to the patient based on their randomization assignment. Once dispensed by designated research pharmacists, the drug pack(s) will be given to the patients by study staff. Drug Accountability Logs will be kept by the research pharmacists, and coordination of inventory will be managed by the pharmacists and study nurse.

4.7. Cohort Follow up and Clinic Visits

Study participants will be recruited from hospitals and clinics in the greater New Orleans area. Following the pre-screening visit, interested patients meeting the eligibility criteria will be invited for a clinical screening visit.

Table 1. Study visits and data collection

	Screening	Baseline/ Randomization	Follow-up visits		
			Day 7	Week 6	Week 12
Consent form	X				
Medical history and medications	X		X	X	X
Blood pressure, pulse, weight	X	X		X	X
Height	X				
eGFR and/or urine albumin-Cr ratio	X	X	X	X	X
Glucose-6-phosphate dehydrogenase (G6PD)	X				
CBC, BMP	X		X	X	X
Methemoglobin, nitrite, nitrate, isoquercetin, renal function and chemistry panels		X	X	X	X
Lipid Panel		X			X
Flow-mediated dilation		X		X	X
Biomarkers for endothelial dysfunction, inflammation, and oxidative stress		X		X	X
Adherence and adverse events			X	X	X

CBC: complete blood count; eGFR: estimated-glomerular filtration rate

Baseline/randomization visit

After eligibility is confirmed, patients will be invited to attend a baseline/randomization visit. At this time, study participants will receive their randomization assignments and begin treatment or placebo therapy. Patients will receive 50 tablets of all medications prescribed based on their randomization assignment to facilitate compliance and follow-up.

As a safety precaution, given the immediate absorption rate of the IR-Sodium Nitrate, all patients (regardless of treatment or placebo randomization assignment) will be asked to take both pills in clinic for the first time, and will be monitored for 30 minutes following their dose for potential side effects (see section 4.9 for further detail).

The following information will be obtained at the clinical visit:

- Contact Information confirmed

- Questions regarding medications and dietary supplement(s) that the subject is currently taking, and discussion of other health related events.
- Vital Signs (blood pressure and pulse), and Anthropometric measure (height, weight).
- Symptoms or health problems since the previous clinic visit.
- Blood Samples (approximately 2-4 tablespoons) for:
    - Routine tests, including chemistry and renal function panel, lipid panel, complete blood counts.
    - Methemoglobin, nitrite, nitrate, isoquercetin.
    - Biomarkers related to endothelial dysfunction, inflammation, oxidative stress, and other related biomarkers.
- Urine sample to test urine protein, creatinine, and other related biomarkers.
- Forearm ultrasound to measure vascular function (see below for details.)

Forearm Ultrasound Test (Brachial Artery Reactivity Test)

Endothelium-dependent flow-mediated vasodilation (FMD) of the brachial artery is induced by the elaboration of endothelium-derived NO. Endothelium-dependent FMD in response to reactive hyperemia will be evaluated non-invasively by use of high-resolution ultrasound (113-115). Ultrasound measurements will be performed according to a standard method. Briefly, with subjects in a supine position, at rest, and in a quiet temperature-controlled room (22°C to 25°C), B-mode scans of the right brachial artery will be obtained in longitudinal sections 2 to 8 cm above the elbow by use of a 7.5-MHz linear array transducer and a Hewlett Packard 5500 duplex ultrasound machine. To ensure consistency of the image with serial scans, the transducer position will be marked on the skin. The end-diastolic arterial diameter will be measured in ECG-gated end-diastolic frames from 1 media-adventitia interface to the other at the clearest section. Reactive hyperemia will be induced by inflating a pneumatic cuff to suprasystolic pressures at the level of the forearm, distal to the brachial artery. After 5 minutes, the cuff will be deflated, resulting in a brief episode of reactive hyperemia. Measurements of brachial artery diameter will be made 1 minute before application of the cuff and every 60 seconds after cuff deflation for 5 minutes. Images will be recorded on a MOD (Magneto Optical Disk) for quantitative analysis at the University Of Pennsylvania Vascular Research Center, where there is a reading center for several large national studies on endothelial dysfunction. Endothelium-dependent FMD will be calculated as the maximal percentage change in vessel size during hyperemia.

Patients will be asked a series of questions by study staff to confirm eligibility in continuing the BART exam process. Should a patient report he has taken Viagra within 8 hours of the BART exam, the exam will be postponed to a time that can be >8 hours from their last dose of the medication so as to avoid potential increased vasoactive side effects. Patients will also be reminded that Viagra is an exclusionary medication during their participation in the study.

Follow-up visits

At weeks 6 and 12 after randomization, study participants will be assessed for the following information at clinical visits:

- Detailed medical history and use of medication and dietary supplement(s)
- Blood pressure, pulse, anthropometric measures (weight)
- Flow-mediated dilation
- Urine albumin/cr ratio
- Blood Samples (approximately 2-4 tablespoons) for:
    - Routine tests, including chemistry and renal function panel, lipid panel, complete blood counts.
    - Methemoglobin, nitrite, nitrate, isoquercetin.
    - Biomarkers related to endothelial dysfunction, inflammation, oxidative stress, and other related biomarkers.

Patients will also receive 50 additional tablets of all required medications to last until the week 12 visit.

Safety monitoring visits

Study staff will contact the participants by phone or email three days after the first dose of therapy to confirm that no adverse events have occurred. At day 7 after starting treatment, medical history, medication, blood pressure, pulse, blood methemoglobin, nitrite, nitrate, isoquercetin, blood chemistry panels, and CBC will be assessed during the in-person visit for safety monitoring. Clinical adverse events will also be evaluated.

A one-week window will be allowed for patients experiencing difficulty with scheduling their follow-up visits for weeks 6, and 12. This will allow the patients to complete their visits one week prior to or following their scheduled visit date to ensure compliance.

4.8. Study Treatment

The treatment group will receive combination therapy with IR- nitrite and isoquercetin. An immediate-release formulation of sodium nitrite (IR-nitrite or TV1001IR) will be administrated. The minimal effective dose of IR-nitrite (40 mg twice daily) will be administered.

As a naturally occurring compound, a small amount of isoquercetin exists in the human diet. Isoquercetin will be given at a dose of 225 mg daily, which is below the threshold of the FDA's acceptable daily intake. This dose falls within the normal dietary intake range. The Isoquercetin capsule additionally contains 55.8mg of vitamin C and 4.5mg vitamin B3 to increase absorption and stability of isoquercetin.

Patients randomized to the placebo arm will receive IR-nitrite in its placebo form (with no notable difference in appearance of the tablet). A placebo dose of Isoquercetin

(225mg per day) will also be administered to placebo patients. All therapy for both selected arms will be taken orally.

Temporary Stops

In the interest of maintaining safety and best clinical practices, a temporary stop will be available to all enrolled patients should specific circumstances arise. Should a patient begin a temporary dose of antibiotics or steroids, which may be considered an interaction risk by the study physician, treatment may be stopped on a temporary basis. Enrolled participants will be advised to inform study staff any infection, arthritis pain, and other conditions requiring systemic steroid treatment. Patients will also be asked to note any persistent or intolerable symptoms that could be related to the study drugs pending evaluation by the study physician. In addition, participants will have the option to temporarily stop the study drugs due to symptoms that are potentially related to study drugs, following an evaluation by the study physician.

In the event of the temporary stop, patients will continue to follow up with their protocol visits as scheduled, and side effects and additional medications will be assessed at that time. The study participant is to restart the study medications immediately upon conclusion of the contraindicated treatment or once related side effects subside based on the evaluation of the study physician.

All study drugs and placebos will be directly shipped to Tulane inpatient pharmacy. The designated research pharmacist will fill and dispense the study drugs according to written orders from the study physician and nurse.

4.9. Monitoring Participant Symptoms and Laboratory Values

The following safety parameters will be assessed: medical and medication history; concomitant medication usage; physical examination; blood pressure; pulse; clinical chemistries (including glucose, urea nitrogen (BUN), creatinine, calcium, phosphorus, bicarbonate, cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), complete blood count (CBC), side effects, and adverse events. The plasma levels of methemoglobin, nitrite, nitrate, and isoquercetin will be monitored.

Side effects will be recorded via a questionnaire, and adverse events will be assessed at 1-week, 6-week, and 12-week follow-up visits. Blood methemoglobin, nitrite, nitrate, and isoquercetin, chemistry panels, as well as CBC, will be assessed at baseline, 1 week, 6 weeks, and 12 weeks. Plasma nitrite and nitrate will be measured using the Griess reaction coupled to high-performance liquid chromatography separation with the ENO-20 analyzer (Eicom, Kyoto, Japan). Plasma isoquercetin level will be determined by HPLC at Dr. David Nieman's lab at Appalachian State University, North Carolina. Blood methemoglobin and G6PD will be measured at Tulane Medical Center Clinical Lab, New Orleans, LA.

As an added precaution, and due to the immediate absorption rate of the IR-Sodium Nitrate (30 minutes or less), participants will be asked to remain in the clinic for up to 30 minutes following their dose administration at the randomization visit. According to the published Investigator's Brochure from drug manufacturer, TheraVasc, decreased SBP and DBP have been noted to be caused by the Sodium Nitrate, as well as a compensatory increased pulse rate. Patient's blood pressure will be taken by the automatic machine prior to their leaving clinic. Patients will also be asked if they are experiencing any dizziness, fatigue, or headache, due to the vasodilating nature of the medication. Should patient's BP measure ≤100/60mmHg, the patient will be asked to remain in clinic until BP remains stable, a nursing or physician assessment will be performed prior to the patient's leaving clinic.

As an addition to the safety monitoring, inclusion criteria has been carefully selected to include patients with a BP of ≥120/70mmHg, in order to minimize the risk of hypotension resulting from the study medication. Increased vasoactivity effects of sodium nitrite have been noted only with intravenous doses of sodium nitrite, and with this method, a mean blood pressure decrease was noted to be 7mmHg. This level is not anticipated with the proposed study patients, as the dose will be given orally rather than intravenously, however, side effects and measurements will be monitored to ensure utmost concern for safety.

Other serious adverse events noted by the Investigator's Brochure are not relevant for monitoring at the dose prescribed for study patients, as those side effects resulting from Methemoglobinemia are associated with much larger doses of IV administered sodium nitrite. The minimally effective dose (40 mg BID) of IR-Sodium Nitrite has been chosen for use in the study in order to minimize vasoactivity associated side effects. Per previous report, there was 0% new hypotension and no apparent orthostatic change in pulse or blood pressure throughout the study in the 40 mg BID group.[4]

4.10. Endpoint Definitions and Documentation

Primary endpoints

The primary outcome of interest is the net change in endothelium-dependent flow-mediated vasodilation (FMD) between the active treatment group and the placebo-control group over the 3-month intervention period. FMD will be measured using high resolution ultrasound on the brachial artery at baseline, 6 weeks, and 12 weeks according to a standard method, and images will be analyzed quantitatively at the vascular laboratory of the University of Pennsylvania. FMD will be calculated as the maximal percentage change in vessel size during hyperemia.

Secondary endpoints

The secondary outcomes include endothelial dysfunction biomarkers (asymmetrical dimethylarginine [ADMA], intercellular adhesion molecule-1 [ICAM-1], vascular adhesion molecule-1 [VCAM-1], E-selectin, endothlin-1 [ET-1], von Willebrand factor [vWF], endothlin-1, and endostatin), inflammatory biomarkers (C-reactive protein

[CRP], tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β], interleukin-6 [IL-6], and monocyte chemoattractant protein-1 [MCP-1]), and oxidative stress biomarkers (oxidized LDL and nitrotyrosines). All biomarkers will be measured using ELISA assays according to a standard protocol.

Exploratory endpoints

The estimated-glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio will be measured and compared between the intervention and control groups.

4.11. Drug Compliance Monitoring

Participants will be instructed to return unused study medications and empty packaging at each study visit. All returned capsules/tablets will be counted and recorded to the corresponding case report forms. Compliance will be calculated as the number of capsules/tablets taken divided by the number of capsules/tablets expected. If a participant has taken fewer capsules/tablets than expected, the study staff will counsel the participant on the importance of compliance. The research pharmacy at the investigators' institute will be responsible for receipt and proper storage of study medication, as well as for maintaining records of product delivery to the site, dispensing of product to each patient, and return of product at the end of the study.

4.12. Central Bio-bank and Data Storage

In addition to the safety laboratory testing, blood samples will be collected during the trial and stored in a central facility (Tulane National Primate Research Center). These samples will be used after the trial is complete to increase the understanding of the study results. These samples will also be used to research how the study drug is processed by the body. It is possible that new tests may become available in the future, which could be useful in understanding kidney and heart disease. Stored blood and urine may be used later to identify new risk factors for kidney disease.

The key that connects code numbers to the participants' identifying information will be kept by the study staff. It will be stored in a locked file within a password-protected computer and be accessible for study use only. The study records will also be stored in a locked area at the Tulane clinical site. Only the study staff members will have access to the identifying information.

4.13. Adverse Events (AEs) and Serious Adverse Events (SAEs)

For the purpose of this trial, an Adverse Event (AE) is defined as any untoward medical occurrence, including any clinically significant abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease associated with the use of a drug in humans, whether or not considered drug-related. Serious adverse events (SAEs) are defined as any undesirable experience associated with the use of a medical product that meets any of the following criteria: fatal or life-threatening; resulting in significant or persistent disability; requiring or prolonging hospitalization (greater than 24-hours); resulting in a congenital anomaly/birth defect; important medical events that investigators judge to represent significant hazards or harm to research participants. Any AE that meets the criteria outlined as an SAE will be documented and reported as such.

In addition, a select list of important events, regardless of whether they resulted in hospitalization, will also be considered SAEs, including: injurious falls, syncope, and unexpected events which the investigator believes that the study intervention caused or contributed to. Participants will be queried for SAEs and selected AEs at each clinic visit.

Situations that may require temporary reduction or elimination of a study medication include side effects such as hypotension, acute kidney injury, methemoglobinemia, and other illnesses. All AEs and SAEs are provided to the site clinician for his/her action and signature. The study site will be responsible for timely reporting to the Data Safety Monitoring Board (DSMB). The study site will provide reports of SAEs for review by the DSMB at their meetings. Causality of AEs and SAEs will be evaluated by the PI and site physician to determine whether they are probably, possibly, or not related to study intervention. Probably or possibly related events will be considered drug-related for regulatory reporting purposes, and will be captured as such.

Expected events: Headaches may be caused by sodium nitrite or isoquercetin. Dizziness, lightheadedness, and hypotension may be caused by combination of sodium nitrite and isoquercetin. Blood pressure and pulse will be closely monitored, as a decrease in these vital signs may cause the aforementioned side effects. Sodium nitrite may also cause methemoglobinemia. Participants with history of headaches and with systolic blood pressure below 120 mm Hg will be excluded due to safety concerns. Clinical safety alerts (including SBP<90, pulse >100 or <45, serum creatinine increase by at least 50% to a value >=1.5 mg/dL, or methemoglobin >3%) are to be provided to the site clinician for his/her decision of action. Study staff will send safety alerts to the participants' designated physician, noted at the screening visits. The site clinician will review all lab results regularly and determine whether results should be sent to the participants' treating physician for further review or treatment.

The site physician and nurse will track AEs and their progress through the resolution, and determine whether post-study follow-up is necessary. SAEs will be followed until resolution, stabilization, or until it is determined that study participation is not the cause.

4.14. Data Coordination

All data will be double-entered into a computer database. Subject identifiers and contact information will be kept in a separate file and not eligible for data entry. The computer database will be protected by a series of passwords available only to study investigators and key study personnel. The link between patient identity and a unique study identification number will be destroyed after the study is complete. Published reports will contain only aggregate and statistical results, with no disclosure of individual results or patient identifiers. These safeguards minimize the chances of a breach of confidentiality or invasion of privacy.

We will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source data/documents.

4.15 Quality Control and Quality Assurance

Personnel training and certification: A comprehensive training session will be conducted with all study personnel and encompass all aspects of the study including communication, principles of good clinical practice, study implementation and procedures, data entry and verification, and specimen testing/handling. All clinical technicians have been trained and certified for standard measurements of weight, height, and BP in ongoing studies. The ultrasound technologists are also certified in their field. Flow-mediated dilation (FMD) results will be read by trained personnel.

Quality control: All clinical equipment will be checked regularly and standardized by the study staff. Prior to completion of each visit, all questionnaires, forms, and reports of laboratory studies will be checked for completion and accuracy. All lab equipment will be checked regularly and standardized by study staff.

4.16 Assessment of Efficacy

Specification of the efficacy parameters:

Please see the section "Endpoint Definition and Documentation" of this protocol for details. In brief, we will assess the net changes of flow-mediated dilatation (FMD) (primary endpoints) between the active treatment group and the placebo-control group over the 3-month intervention period. In addition, the secondary outcomes, including biomarkers of endothelial dysfunction, inflammation, and oxidative stress, as well as exploratory endpoints including eGFR and urinary albumin, will be measured and compared between the intervention and control groups.

Methods and timing for assessing, recording, and analyzing efficacy parameters:

Flow-mediated dilatation (FMD) will be measured using high resolution ultrasound on the brachial artery at baseline, 6 weeks, and 12 weeks according to a standard method and images will be analyzed quantitatively at the vascular laboratory of the University of Pennsylvania. FMD will be calculated as the maximal percentage change in vessel size during hyperemia. All biomarkers will be measured using ELISA assays according to a standard protocol using specimens collected at baseline, 6 weeks, and 12 weeks. eGFR and urinary albumin will be measured following a standard protocol using specimens collected at baseline, 6 weeks, and 12 weeks.

5.0. DATA ANALYSIS PLAN

5.1. Statistical Analysis

The primary outcome of interest is the difference in changes of flow-mediated dilation between the active intervention and control groups over the 3-month intervention period. The changes of FMD during the intervention will be calculated for each participant. A mixed-effects model will be used to assess the effects of the active

treatment on the slope of FMD, in which participants will be assumed to be random effects and treatment will be assumed to be an estimable fixed effect. PROC MIXED of SAS version 9.2 (SAS Institute Inc, Cary, NC) will be used to obtain point estimates and standard errors of the treatment effects and to test for differences between treatment arms. An autoregressive correlation matrix will be used to correct within-subject correlation for repeated measurements.

The intention-to-treat (ITT) analysis will be used in all primary analyses. In this analysis strategy, all subjects are compared in the treatment groups to which they were originally randomized, regardless of any treatment that they subsequently received. In addition, the per protocol analysis (PPA) will be used in the secondary analyses. In this analysis strategy, subjects who are not fully compliant with the study protocol will be excluded. By focusing only on the fully compliant subjects, one can determine the maximal efficacy of a treatment.

The same analysis approaches will be used for the secondary and exploratory study outcomes. The level of significance will be 0.05 for a 2-sided test.

Any deviation(s) from the original statistical plan will be described and justified in the updated study protocol and final report.

5.2. Sample Size and Power

The proposed trial has sufficient statistical power to detect a small but clinically meaningful effect of combination treatment on FMD. Sample size has been calculated based on the following:

- 80% statistical power;
- a 2-sided significance level of 0.05;
- a standard deviation of 4.6%;
- A detectable difference of 2.8% between intervention and control groups with an average of 3 repeated measurements (at baseline, week 6 and week 12); and
- A correlation between FMD measures over time of 0.55.

Based on these assumptions, thirty (30) participants are required in each treatment arm, for a total of sixty (60) participants. To take into account for withdrawal and loss to follow-up, seventy (70) patients will be recruited (35 in each arm of treatment).

5.3. Missing and Spurious Data

All data collection will follow a rigorous quality control process. Spurious and missing data will be checked against the original case report forms (CRFs). As a last resort, should questionable data not be resolved from the CRFs, such data will be coded as missing. When conducting multivariable analyses, multiple imputation for missing data will be conducted using the Markov chain Monte Carlo method.

5.4. DSMB Analyses

Due to the potential risks to study subjects, and the fact that this is a Phase II clinical trial, the study will have a formal, and independent Data and Safety Monitoring Board (DSMB). A group of experts in Biostatistics, Clinical Nephrology, and Cardiology from Tulane University will comprise an internal DSMB. The board will regularly review trial progress, including unblinded interim results, and can recommend that the trial prematurely end if participants are at risk or if it becomes clear that the trial will not be able to yield statistically significant results. Study investigators will prepare accurate and timely data tables and reports for the DSMB meetings.

If methemoglobinemia is shown to be significantly increased in the treatment group ($p<0.01$), the trial will be discontinued. As an extra protective measures, should individual patients develop methemoglobinemia, they will be withdrawn from the trial.

6.0. HUMAN SUBJECTS CONSIDERATION

Recruitment and informed consent: Study participants will be identified by research personnel employed by the proposed project, based on screening of patients with evidence for albuminuric and non-albuminuric chronic kidney disease. Patients who meet eligibility criteria to participate in this project are required to undergo an informed consent procedure. The consent outlines all of the participation procedures of the study.

6.1. Informed Consent

Prior to signing the informed consent (ICF), the Research Coordinator will review the details of the consent form orally with the potential participant in a private setting, and answer any questions that the participant has concerning participation. The original signed ICF is stored in the participant study file at the clinical center, and a copy of the signed consent form is given to the participant. Specifically, the following must be accomplished during the informed consent process:

- The participant must be informed that participation in the study is **voluntary** and that refusal to participate will involve no penalty or loss of benefits or negative impact on their medical care.
- The participant must be informed of the **purpose** of the study and that it involves **research**.
- The participant must be informed of any **alternative procedures,** if applicable.
- The participant must be informed of any reasonably foreseeable **risks**.
- The participant must be informed of any **benefits** from the research.
- An outline of safeguards to protect participant **confidentiality** must be included, as well as an indication of the participant's right to withdraw without penalty. This should be balanced with a discussion of the effect withdrawals

have on the study, and the responsibility a participant has, within limits, to continue in the study if he or she decides to enroll.

- The participant must be informed **whom to contact** for information about research subjects' rights, information about the research study, and in the event of research-related injury.
- The participant must be informed as to whether or not **compensation** is offered for participation in the study and/or in the event of a medical injury.
- The participant must be informed that he/she will be notified of any significant **changes** in the protocol that might affect their willingness to continue in the study.

6.2. Risks

Taking IR-nitrite and isoquercetin may cause one or more side effects including headaches, dizziness, hypotension, and methemoglobinemia. In general, the side effects are considered transient and reversible. The use of the minimally effective dose of IR-nitrite and Isoquercetin is expected to decrease the risk of side effects in study participants. There may be other unknown risks, and for this reason, participants will be closely monitored for side effects. The study physician will be informed about any side effects potentially related to intervention, and take action accordingly.

As a result of venipuncture, there is a risk of bruising (the appearance of a black and blue mark) or pain at the site. There is also a small risk of infection, lightheadedness, and/or fainting. Trained and certified staff members will draw blood to minimize complications. Moreover, a physician will be available to evaluate patients as needed.

For patients with diabetes taking insulin, fasting before specified study visits may cause hypoglycemia. This may result in confusion, weakness, or more serious conditions and will be treated by providing the patient with high-fructose food or drinks. Participants will be advised to hold their diabetes drugs the day before the visit if fasting is required.

There is no major risk reported for the forearm ultrasound test. Temporary decrease of blood flow through the artery in the participant's arm during the forearm ultrasound test may cause temporary numbness and tingling in the forearm and hand. The risk of numbness involved is slightly greater than the risk during routine blood pressure measurements.

Additional risks of the study relate to unauthorized disclosure of medical information or laboratory data. Subject identifiers and contact information will be kept in a separate file and not entered into the computer database to prevent this occurrence.

6.3. Benefits

Potential benefits for the participants on the active combination treatment group may exist, as combination therapy may provide improvement of endothelial dysfunction, inflammation, and oxidative stress. Progression of CKD to ESRD is the major cause of

premature death in patients with CKD. The proposed study will test a novel treatment targeting the improvement of endothelial dysfunction and inflammation in CKD patients. This study will also provide data to test the effect of combination therapy on the delay of CKD progression. There may be substantial benefits to medical knowledge, and since there is very little risk to individual subjects, the risk to benefit ratio is favorable.

6.4 Subject Withdrawal Criteria and Procedures

Subject withdrawal from the trial

Participants will be withdrawn if there are significant changes in safety parameters or significant AEs related to treatment with study medications (i.e. an imbalance in the safety profile in subjects receiving the active drug vs. placebo), if there is any safety issue raised by the DSMB, or if a participant is unwilling to continue to participate in the study.

Data to be collected for withdrawn subjects

Participants who are withdrawn from the study will be required to sign and date the withdrawal form. The data collected before withdrawal will be used in the final data analyses.

Replacement of withdrawn subjects

Subjects withdrawn from the trial will not be replaced using further recruitment methods. In order to ensure adequate numbers of participants to complete the study and measure results based on acceptable statistical power, we plan to recruit an additional 15% of study participants for the trial.

Follow-up for withdrawn subjects

Participants will be allowed to contact study staff/site if they have any concerns related to the study.

6.4. Payments and Costs

Participants will be paid $25 for each completed visit. If a participant withdraws or does not complete all protocol study visits, payment will be adjusted accordingly. The study will not provide insurance for any study related medical or non-medical issues, which will be clearly stated in the participant's ICF. There will be no costs to the subject for participating in this research study

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