C O N F I D E N T I A L

PROTOCOL

Targeting Central Pulsatile Hemodynamics in Chronic Kidney Disease

NCT02791906

Sponsor: National Heart, Lung and Blood Institute (NHLBI)

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Version 03

A. SPECIFIC AIMS

Heart Failure (HF) affects ~2% of the western population and ~10% of individuals aged >75 years. Not only is HF already an epidemic, but with the aging of the population, a dramatic further increase in its prevalence is expected. Given the enormous public health, clinical and societal burden of HF, finding effective prevention strategies for this condition is a top priority. Patients with CKD are at a markedly increased risk of HF, which is a major cause of morbidity and mortality in this population[1, 2]. The Chronic Renal Insufficiency Cohort Study, which included a large population of patients with early CKD, demonstrated that the most common cardiovascular endpoint was HF. Among 2,606 CRIC participants without HF at baseline, during a mean of 3.5 years of follow-up, 154 experienced new-onset HF.[3] This occurred despite a mean baseline blood pressure of 126/70 mmHg is this cohort. Thus, given the high prevalence of CKD in the adult US population (estimated at 12-13%[4]) and the high risk of HF in CKD, novel interventions to prevent HF in CKD are highly desirable.

Every heart beat, the left ventricle (LV) generates a pulse wave that travels forward in the arteries, gets partially reflected in the peripheral arterial tree and travels back to the heart, arriving while the LV is still ejecting blood. The reflected wave increases the late systolic workload of the LV. We will present substantial evidence that links the *loading sequence (increased late systolic load)* with the development of HF, diastolic dysfunction and LV hypertrophy independent of blood pressure levels. Therefore, wave reflections represent a potential therapeutic target to improve LV hypertrophy and diastolic dysfunction, and ultimately, the risk of HF in CKD.

Wave reflections can be markedly reduced by organic nitrates, which dilate middle-sized muscular arteries even at doses that do not substantially reduce brachial blood pressure. Our overarching hypothesis is that reducing arterial wave reflections with organic nitrates will reduce LV hypertrophy (a precursor to HF), fibrosis and improve myocardial function in subjects with CKD, independently of blood pressure reduction. Our ultimate goal is to perform an RO1-funded double-blind, parallel-arm, placebo-controlled randomized clinical trial that will enroll patients with CKD and randomize them to either sustained-release isosorbide mononitrate therapy (SR-ISMN) or placebo for 24 weeks.

In this R56-funded study, we aim to obtain key preliminary data for an RO1 re-submission to accomplish a phase IIb randomized controlled trial to test our hypothesis. We wish to obtain: (1) preliminary data regarding the variability in the change of our endpoints in response to our intervention in this specific population; (2) Preliminary data regarding the effects of ISMN on blood pressure vs. wave reflections at different doses; (3) Data supporting the feasibility and local expertise to implement a biomarker discovery approach using proteomics. Our specific aims are:

PRIMARY AIMS: to assess the variability in the response and to perform a dose-response assessment in the degree of change in central hemodynamics and cardiac endpoints with

ISMN therapy in this population. We propose to administer 3 different doses of SR-ISMN (30, 60 and 120 mg of SR ISMN daily) to 20 subjects with CKD, in order to accomplish the following co-primary aims:

1a. To assess the variability in the change in **LV mass** with ISMN administration **(in order to support aim 1a of our eventual RO1-funded trial**). LV mass will be measured with steady-state free precession cardiac MRI.

1b. To assess the variability in changes in **diffuse myocardial fibrosis** with ISMN administration, using T1-mapping MRI techniques.[5-12]

1c. To assess the variability in changes in **myocardial systolic and diastolic function** with ISMN administration, assessed via systolic strain (measured with displacement encoded MRI, DENSE) and early diastolic mitral annular velocity (measured with tissue Doppler echocardiography), respectively.

SECONDARY AIMS:

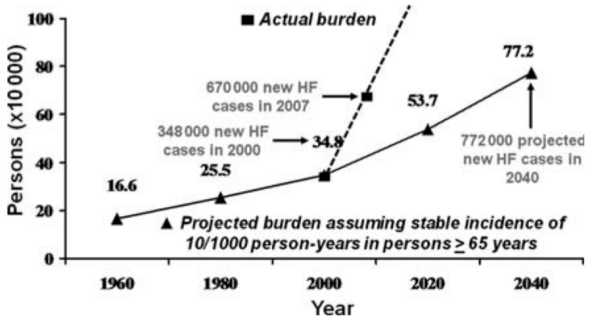
1) **To establish the feasibility** of reducing wave reflections while minimizing changes in brachial blood pressure. By performing assessments of the effects of 3 different doses of SR ISMN on office and 24-hour blood pressure and wave reflections, we aim to determine which dose of this drug best reduces wave reflections with the least impact on brachial blood pressure in this particular population. This will directly support secondary aim 1 of the RO1 application and will inform about the best dose to use in the definitive RO1-funded trial/application. We will also assess whether vitamin C reduces the tolerance to nitrate therapy over time.

2) We will gather preliminary data regarding the feasibility of using an **unbiased biomarker discovery approach** to identify biomarkers/pathways that change with ISMN administration in our 20 subjects. We will utilize a novel proteomic approach (Somascan) to measure levels of 1,129 candidate biomarkers at baseline and after ISMN. We eventually want to identify novel biomarkers/pathways that correlate with the cardiac response to ISMN.

3) To assess the variability in changes in **aerobic capacity** (peak oxygen consumption during maximal supine bicycle exercise test), **vasodilatory reserve to exercise** (reduction in systemic vascular resistance with exercise, measured with Doppler echocardiography), and **physical activity** (assessed via actigraphy) with ISMN administration.

B. BACKGROUND AND SIGNIFICANCE

Figure 1. The Epidemic of Heart Failure. Projected burden of HF in the US according to previous projectons (solid line) vs. actual burden (dotted line) From: Lam *et al. European journal of heart failure.* 2011;13:18-28



Heart failure (HF) is a major epidemic. Heart Failure (HF) affects ~2.4% of the US population and 10% of individuals aged >75 years. The burden of HF has increased dramatically over the last few years, vastly exceeding previous projections (Figure 1). According to the American Heart Association[13, 14] the number of new HF cases in the US increased from 348,000 in 2000 to 670,000 in 2007, representing a 93% increase over this time period. Not only is HF already an epidemic, but with the aging of the population, a dramatic further increase in its prevalence is expected.

Once established HF is a malignant disease, with a high mortality rate, poor quality of life and a high burden on society: Once HF ensues, mortality is high, with 50% of Medicare beneficiaries not surviving 3 years after an HF hospitalization.[15] Similarly, HF is associated with a markedly impaired quality of life.[16-19] It has recently been estimated that yearly costs to treat HF will double to ~70 billion from 2012 to 2030,[20] although direct costs could exceed $160 billion by 2030.[20]

HF is the main cardiovascular outcome in chronic kidney disease (CKD): Patients with CKD are at an increased risk of HF, which is a major cause of morbidity and mortality in this population[1, 2]. We have participated in the NIH-funded Chronic Renal Insufficiency Cohort Study, which included a large population of patients with early CKD. In CRIC, the most common cardiovascular endpoint was HF. Among 2,606 CRIC participants without HF at baseline, during a mean of 3.5 years of follow-up, 154 experienced new-onset HF (unpublished data; manuscript under peer-review). This occurred despite a mean baseline blood pressure of 126/70 mmHg is this cohort. Thus, given the high prevalence of CKD in the adult US population (estimated at 12-13%[4]) and the high risk of HF in CKD, novel interventions to prevent HF in CKD are highly desirable.

In summary, ***HF in an epidemic and the most common cardiac complication in CKD. New cost-effective strategies are needed for the prevention of symptomatic HF in CKD. Such interventions should target processes upstream of the development of myocardial remodeling and dysfunction.*** A series of animal studies support the notion that increased arterial wave reflections (which increase the late systolic load of the LV) lead to myocardial remodeling, fibrosis and dysfunction. In addition, observational studies in humans have shown that increased wave reflections are associated with left ventricular remodeling, myocardial dysfunction and an increased risk of HF. Yet, human experimental studies (i.e., randomized controlled trials) are lacking to prove the causal effect of wave reflections and exploit wave reflections as a clinically-relevant therapeutic target. Increased wave reflections constitute a prominent feature of patients with CKD, which is

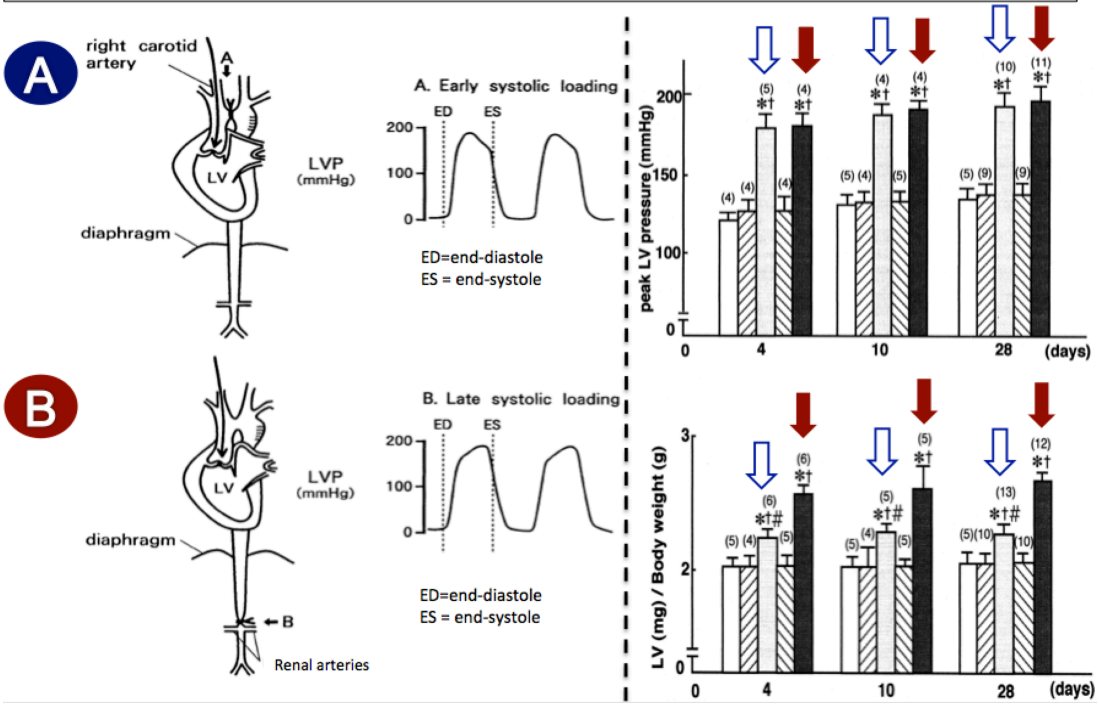
highly treatable. However, whether reducing late systolic load from wave reflections reduces maladaptive LV remodeling (a precursor of HF) is unknown. We wish to eventually perform a phase IIb randomized controlled double-blinded clinical trial to test this paradigm and address this knowledge gap. The trial would support a new pathophysiologic paradigm and identify a novel, inexpensive therapeutic intervention for HF prevention in CKD population. In order to propose a definitive trial, the NIH has awarded us a bridge grant, in order to generate preliminary data regarding dose-response and variability in study endpoints using nitrate therapy in CKD.

Wave reflections in the arterial tree: Every heartbeat, the LV generates a pulse wave that travels forward in the arteries and gets partially reflected at sites of impedance mismatch, such as points of branching or change in wall diameter or material properties along the arterial tree. **Wave reflections occur predominantly in the bifurcations in middle-sized muscular arterial segments[21-23]** and are conducted back to the heart, merging into a discrete reflected wave.[21-23] The source of wave reflections is thus different from the resistance microvasculature, which determines mean arterial pressure.[21-23]

The reflected wave affects LV afterload and alters the loading sequence. Due to the wave transit time from the heart to reflection sites and back to the proximal aorta, wave reflections arrive back at heart while the LV is still ejecting blood in mid-to-late systole.[23, 24] Wave reflections thus increase the **late systolic workload of the LV and profoundly impact the LV loading sequence** (late relative to early systolic load).

Late systolic loading from wave reflections promotes LV hypertrophy in rats. For any given level of systolic (peak) blood pressure, prominent late-systolic loading has been shown to exert deleterious effects on LV structure and function in animal models.[23, 25, 26] Kobayashi *et al*[25] used a Wistar rat model and performed constriction of either the ascending aorta (intervention A, top left and middle panels in Figure 2) or suprarenal abdominal aorta (intervention B, bottom left and middle panels in Figure 2). Analysis of aortic input impedance (which represent the mechanical pulsatile load that the arterial tree imposes on the LV; not shown in Figure 2) demonstrated that constriction of the ascending aorta increased LV load in early systole, whereas constriction of the descending aorta caused prominent late systolic loading from a reflected wave that originated at the distal aortic constriction site, arriving at the heart in late systole.[25] The middle top and bottom panels in figure 2 show an example of the LV pressure profile induced by proximal aortic constriction (early systolic peak) and descending aortic constriction (late systolic peak), respectively. The 2 bar graphs on the right of figure 2 show peak LV pressure and LV mass in rats subjected to ascending aortic constriction (hollow blue arrows), descending aortic constriction (solid red arrows) and sham interventions (no arrows). It can be seen

Figure 2. Effect of Early versus Late Systolic Pressure Overload in Rats



that, despite an identical peak LV pressure in rats that underwent ascending vs

Figure 4. Effect of early systolic versus late systolic proximal aortic balloon inflation on relaxation. For any given LV pressure increase induced by balloon inflation, the delay in relaxation (increase in tau) was much more pronounced by late systolic proximal aortic balloon inflation than by early systolic inflation. From: Gillebert TC, Lew WY. *AJP Heart Circ Physiol.* 261: 805-13.

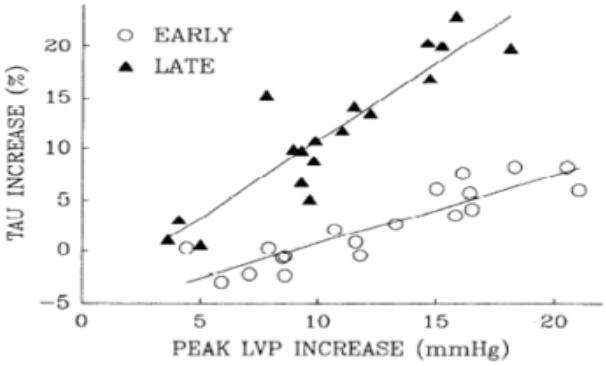


Figure 5. Time-resolved wall stress in a human during ejection. The shaded area represents late systole. From Chirinos JA, Segers P, Gupta A et al. *Circulation.* 2;119(21):2798-807

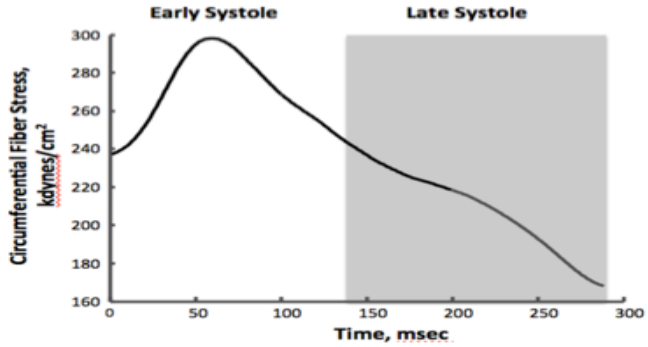


Table 1. Early and late systolic stress as predictors of early diastolic mitral annular relaxation velocity in a multivariate model (R2=0.46) among 1,215 adults in the general population (Asklepios study cohort). From Chirinos JA, Segers P, Rietzschel ER *et al.* Hypertension. 61(2):296-303

Independent variables	Standardized Coefficient β	*P* value
(Constant)		<0.0001
Late ejection-phase STI (kdynes·cm^{-2}·s)	-0.25	<0.0001
Early ejection-phase STI (kdynes·cm^{-2}·s)	0.18	<0.0001
Age (years)	-0.34	<0.0001
Male gender	-0.17	<0.0001
Body height (m)	0.068	0.06
Body weight (kg)	-0.38	<0.0001
Total cholesterol (mg/dl)	-0.067	0.005
HDL-cholesterol (mg/dl)	0.073	0.008
Triglycerides (mg/dl)	-0.027	0.27
Estimated GFR, mL·min^{-1}·1.73 m^{-2}	-0.008	0.71
High-sensitive CRP (ln-transformed; mg/dl)	-0.002	0.91
Current smoking	0.025	0.26
Diabetes mellitus	0.005	0.84
LV sphericity	0.14	<0.0001
Antihypertensive medication use	-0.027	0.23
Heart rate (bpm)	-0.017	0.47

descending aortic constriction (top right panel in figure 2), rats that underwent descending aortic banding (and were thus exposed to greater late systolic load) demonstrated much greater LV hypertrophy than those undergoing ascending aortic banding (which were exposed to increased early systolic load) (right bottom panel in figure

2). Rats that underwent descending aortic
banding also demonstrated a greater amount of myocardial fibrosis[25] (not shown).

To date, we have only observational data in humans supporting these causal findings.

Since our initial application, we have published a study demonstrating an independent association between reflected wave amplitude an LV hypertrophy in the general population.[27] Furthermore, Hashimoto *et al*[28] reported an observational study in which the reduction in wave reflections correlated with regression of LV mass independently of blood pressure reduction. The association between reflected wave magnitude reduction and LV mass (i.e., hypertrophy) reduction was also independent of age, sex, and use of renin-angiotensin system inhibitors (β=0.41, *P*=0.001). Of note, despite the fact that standard antihypertensive therapies reduce wave reflections in some patients (those on the negative side of the *x* axis in figure 3), the change is highly unpredictable, with reflection magnitude actually increasing in some subjects (see those demonstrating a positive change in the *x* axis of Figure 2). In contrast, organic nitrates consistently and markedly blunt wave reflections, which occurs at doses that do not cause significant changes in brachial blood pressure (see also our preliminary data in CKD regarding the lack of changes in brachial BP).[29, 30] Of note, organic nitrates are not part of the routine therapy for CKD or hypertensive patients. Similarly, the paradigm of targeting wave reflections with therapy has not been implemented in clinical practice due to the lack of: (a) experimental data proving a cause effect relation between wave reflections and LV hypertrophy / failure and; (b) Efficacy data for organic nitrates for this purpose. ***Late systolic load promotes diastolic dysfunction in humans*** Gillebert *et al* [31] used a canine model to study the effect of the **timing** of systolic load on LV relaxation by inflating balloons in the ascending aorta during either early vs. late systole. Their study demonstrated that, for a given increase in peak (systolic) LV pressure, late systolic inflation (triangles in figure 3) prolonged *tau* (i.e. impaired LV relaxation assessed with the gold standard measure) much more than early systolic inflation (circles in figure 3), demonstrating that late systolic load can lead to diastolic dysfunction.[31] In support of these findings, cross-sectional observational studies in humans have independently linked wave reflections and late systolic load to diastolic dysfunction in humans.[32, 33] Recently, we developed a non-invasive technique to characterize the time course of LV systolic wall stress in humans, by means of assessing time-resolved LV geometry with speckle-tracking echocardiography and time-resolved central pressures using carotid arterial tonometry.[34] In 2009, we published our first characterization of time-resolved LV wall stress in normotensive and hypertensive humans.[34] A time-resolved stress curve can be used to separate early and late systolic wall stress (i.e., early vs. late myocardial systolic load), which can be quantified as the area under the time-resolved stress curve (stress-time integral, STI) in the first and second halves of ejection, respectively (figure 5). More

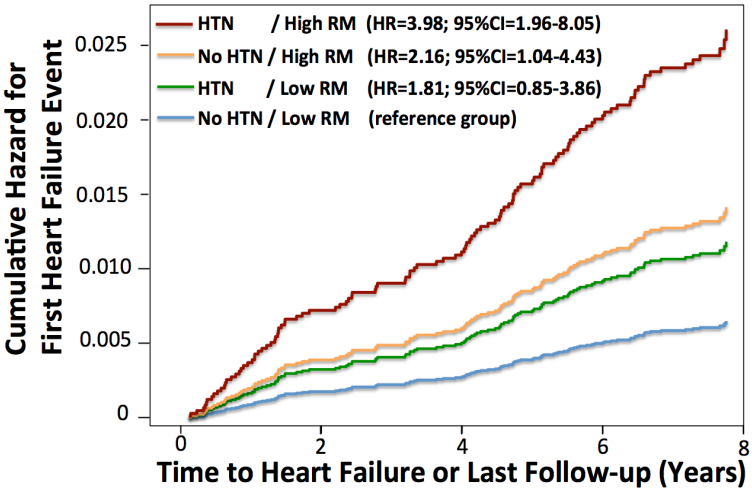
recently[35, 36] we applied this technique to a large population-based study (Asklepios study), in which we have collaborated over the last few years.[34, 36-39] We assessed the relationship between the myocardial loading sequence (early vs. late wall stress) and diastolic function.[35] After adjustment for multiple covariates, late systolic load was independently associated with lower mitral annular relaxation velocities, in sharp contrast to early systolic load which was associated with higher mitral annular relaxation velocities, in a multivariate model (Table 1), implicating the loading sequence as an independent correlate of myocardial relaxation in humans. This model explained 46% of the variability in mitral annular diastolic (relaxation) velocity. However, available observational data in humans cannot prove a causal relationship between late systolic load and diastolic dysfunction and this approach is not currently applied clinically in patients with diastolic dysfunction.

The magnitude of wave reflections strongly predicts new-onset HF. Based on the data presented above, we hypothesized that wave reflections can predict new-onset HF in the general population. To test this hypothesis, we derived aortic pressure waveforms using a transfer function applied to the radial waveform recorded at baseline with arterial tonometry from 5,934 participants in the Multiethnic Study of Atherosclerosis, who were free of clinically apparent cardiovascular disease. The central pressure waveform was used to assess reflection magnitude.[40, 41] During 7.61 years of follow-up (and after adjustment for blood pressure, age, gender, body mass index, diabetes, ethnicity, antihypertensive medication use, total and HDL-cholesterol, current smoking, heart rate and glomerular filtration rate), reflection magnitude strongly predicted HF (Hazard ratio per 10%-increase = 2.69; 95%CI = 1.79-4.04; P<0.0001) and **was a stronger predictor than blood pressure and all other modifiable risk factors listed above**.

When we stratified the population based on the presence or absence of hypertension and the presence or absence of high reflection magnitude (Figure 6), we found that, compared to non-hypertensive subjects with low reflection magnitude (lowest risk category), hazard ratios for hypertensive subjects with low reflection magnitude, non-hypertensive subjects with high reflection magnitude and hypertensive subjects with high reflection magnitude were 1.81, 2.16 and 3.98, respectively (95%CIs are shown in Figure 6).

Furthermore, we compared the strength of the association between reflection magnitude and incident HF using multiple parameters, including standardized hazard ratios, c-statistics and measures of model fit (Akaike's information criterion, Bayesian information criterion). We also assessed the incremental information provided when various predictors were added to a model containing all other predictors of heart failure, in terms of discrimination (integrated discrimination improvement) and reclassification (net reclassification improvement). Reflection magnitude was associated with the largest Wald statistic of all predictors (including age), the greatest reduction in Akaike's information and Bayesian information criteria (indicating improvement in model fit) and the greatest increases in integrated discrimination improvement (with a 48% increase in discrimination slope achieved when reflection magnitude was added to a base model containing all other predictors of HF and multiple confounders listed at the bottom of Table 2). With the exception of age, a non-modifiable risk factor, reflection magnitude was associated with the greatest net reclassification improvement for prediction of HF. Therefore, reflection magnitude was the most robust and strong independent modifiable predictor of incident HF. More recently we demonstrated that, for any given level of systolic and diastolic blood pressure and various other risk factors and confounders, a greater area under the pressure curve in late systole (relative to early systole) is strongly predictive of incident HF in the same cohort.[42] Our findings from these studies in a large community-based sample with careful follow-up and event adjudication, implicate late systolic load from arterial wave reflections as a **novel strong predictor for the risk HF**, Based on the strength of this prediction, a high reflection magnitude has been proposed to represent a novel form of stage B HF.[41] Yet, although available data clearly implicate late systolic load from arterial wave reflections as a novel **marker** of the risk of HF**,** available data do not prove a causal role in humans. We believe that the strong biologic plausibility (based on animal models) and the supportive human observations **support the need for a randomized controlled trial specifically designed to test this paradigm**. The lack of such data is a major gap in the field and in the translation of the paradigm to human therapeutics. We aim to fill this knowledge gap, which would significantly advance the field. Furthermore, If the remarkably strong association demonstrated between wave reflections and HF is *causal*, then **targeting wave reflections could provide large reductions in HF-related morbidity and mortality**, a

Figure 6. Hazard curves for incident of Heart Failure among 5,958 MESA participants stratified according to the presence or absence of hypertension (prevalence=45%) or the presence or absence of "high" reflection magnitude (top 45% of the population). Hazard curves are adjusted for other significant predictors of HF in this population. From: Chirinos JA et al, *J Am Coll Cardiol*; 60:2170-2177.



highly desirable goal from the clinical and public health perspective. In order to design a definitive proof of concept trial, we must first establish the optimal dose of ISMN to reduce wave reflections independently of blood pressure and to assess the variability in study endpoints in response to ISMN in this particular population, such that adequate power calculations can be made.

C. RESEARCH DESIGN AND METHODS

C.1. Overview of study design

This is a pilot study in which we will study 20 subjects with CKD. We will perform a run-in phase in which the dose of sustained-release ISMN (SR-ISMN) will be increased from 30 to 120 mg over weeks. We will administer 30 mg for 1 week, followed by 60 mg for 1 week, followed by 120 for 1 week. At the end of each week, we will perform detailed assessments of blood pressure and central hemodynamics. We will assign half of the participants to receive vitamin C (500 mg three times daily) to assess whether this intervention reduces nitrate tolerance.

After the initial 3-week run in phase, we will randomly assign subjects to receive either 60 or 120 mg of SR-ISMN for additional 21 weeks (for a total of 24 weeks of therapy). Half of the subjects will continue to receive vitamin C (500 mg three times daily by mouth) throughout this 21-week period. We will assess the effect of our interventions on late systolic load, LV mass, LV fibrosis, diastolic relaxation and quality of life.

After subjects are identified and demonstrate an interest in the research study, inclusion and exclusion criteria will be assessed and if eligible, the patients will undergo written informed consent and enrolled in the study.

C.2 Study Sites

The two sites for this trial will be the Hospital of the University of Pennsylvania and the Penn Presbyterian Medical Center. Both sites share a single Institutional Review Board.

C.3. Study Population

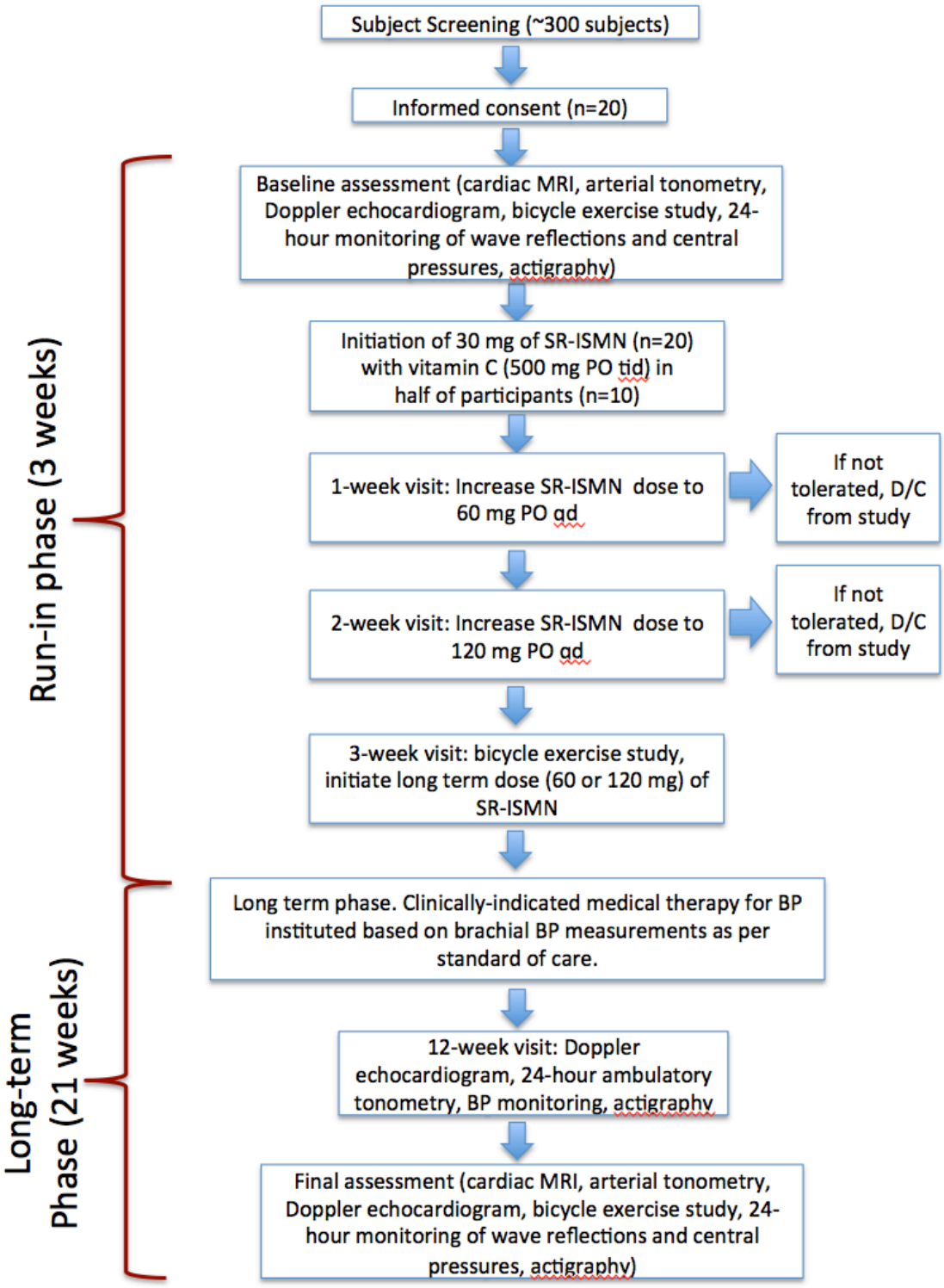
Subjects will be recruited from referrals as well as medical record searches (PennSeek). We will enroll adults (age 18 or older) who meet the following criteria:

Inclusion criteria: (1) Chronic kidney disease stage 3 (defined as an estimated glomerular filtration rate [GFR] of 30-59 ml/min/1.73m^2), estimated by the CRIC GFR equation[43] (2) An elevated left ventricular mass index (defined as >60 g/m$^{1.7}$ in women and 80 g/m$^{1.7}$ in men)[38] or LV posterior wall thickness[44] >1.4 cm documented in a clinically indicated echocardiographic or MRI examination within the previous 24 months; (3) Stable medical therapy as defined by no addition, removal or change in dosage >100% of ACE

inhibitors, angiotensin receptor blockers, beta-blockers, or calcium channel blockers for >30 days; (4) Current therapy with an ACE inhibitor, hydralazine or a statin, all of which have been shown to reduce nitrate tolerance. [45-53]

Exclusion Criteria: (1) A clinically- indicated stress test demonstrating significant myocardial ischemia within 1 year of enrollment, not followed by coronary revascularization; (2) Rhythm other than sinus (i.e., atrial fibrillation); (3) Non-cardiac condition limiting life expectancy to <1 year; (4) Current or anticipated future need for long acting organic nitrate therapy; (5) Severe aortic or mitral valve disease; (6) Hypertrophic cardiomyopathy; (7) Known infiltrative or inflammatory myocardial disease (amyloid, sarcoid); (8) Pericardial disease; (9) Primary pulmonary arteriopathy; (10) History of myocardial infarction, unstable angina, percutaneous transluminal coronary angiography (PTCA) or coronary artery bypass grafting (CABG) within 60 days, or requirement for either PTCA or CABG at the time of consent; (11) Resting heart rate (HR) >100 bpm: (12) A reduced LV ejection fraction (EF<50%); (13) Known severe liver disease (AST >3x normal, alkaline phosphatase or bilirubin >2x normal); (14) Allergy to ISMN; (15) Current therapy with phosphodiesterase inhibitors, such as sildenafil, vardanafil or tadalafil, since the combination of ISMN and phosphodiesterase inhibitors can result in severe hypotension; (16) Therapy with rosiglitazone, since its combination with ISMN is not recommended; (17) Current pregnancy or a positive urine pregnancy test; women who become pregnant during the study will be discontinued from the trial; (18) Therapy with warfarin (as vitamin C may interact with warfarin):[54, 55] (19) History of kidney stones (as vitamin C may increase the excretion of oxalate); (20) History of G6PD deficiency (as vitamin C may precipitate hemolysis in these patients);[54, 55] (21) Systolic blood pressure <110 mmHg or diastolic blood pressure <40 mmHg; (22) Contraindications to a cardiac MRI: (a) Central nervous system aneurysm clips; (b) Implanted neural stimulators; (c) Implanted cardiac pacemaker or defibrillator; (d) Cochlear implant; (e) Ocular foreign body (e.g. metal shavings); (f) Other implanted medical devices: (e.g. drug infusion ports); (g) Insulin pump; (h) Metal shrapnel or bullet; (i) Claustrophobia; (j) Extreme obesity rendering the patient unable to fit into narrow-bore scanners; (k) Unwillingness of the patient to undergo a cardiac MRI.

Flow of subjects in the study.



C.4. Baseline assessments

Arterial tonometry: We will use a commercially available system (SphygmoCor, AtCor Medical) that uses a high-fidelity applanation tonometer (Millar Instruments; Houston, TX).[23] The tonometer will be used to record carotid, radial and brachial arterial pressure waveforms. Brachial systolic and diastolic pressures will be measured with a validated oscillometric device. Brachial diastolic and mean pressures will then be used to calibrate the carotid pressure waveform.[56] Carotid-femoral and carotid-radial pulse wave velocity measurements will be performed. Heart to ankle and heart to femoral PWV will also be measured using a VaSera device (Fukuda Denshi; Tokyo, Japan), which uses inflatable cuffs around the thighs, ankles and arms and a phonocardiographic signal to assess the timing of the pulse at the heart, the femoral artery and the posterior tibial artery.

Doppler echocardiography: will be performed with pulsed-wave Doppler interrogation of flow velocities in the LV outflow tract, mitral inflow and mitral annular velocities. Flow volume will be computed by multiplying LV outflow tract flow velocity by the LV outflow tract cross-sectional area measured with 3D echocardiography.[21, 22]

Reflection magnitude will be computed using linear wave separation analysis[21, 22, 56, 57] using central pressure and flow waveforms measured via arterial tonometry and Doppler echocardiography, respectively.[21, 22]

Bicycle Exercise Test: This is a test in which the subject will ride a special bicycle while lying on his/her back. We will ask the subject to ride the bike as long as possible, then rest, then ride the bike again for an additional 6 minutes. During each exercise event, the subject will breathe through a mouthpiece to measure oxygen consumption and CO_2 production. The length of this test will vary based on their exercise capacity. This will occur during the baseline assessment, at the end of the titration phase (3 weeks) and at the end of the long-term phase (24 weeks).

Activity Monitor: Patients will be given an activity monitor to wear during the length of the titration phase, and then for 2 weeks prior to the 12- and 24- week visits (for the latter, the device will be mailed to the subjects 2 weeks prior to the study visit). This device, manufactured by ActiGraph, is a portable, wearable, activity monitor that subjects can wear around their belt. The device records activity information such as the number of steps taken and intensity of the physical activity.

Cardiac MRI: The cardiac MRI protocol will include true-FISP cine acquisitions of the LV for assessment of LV mass. We will assess diffuse myocardial fibrosis by assessing the extracellular volume fraction, computed with the post-gadolinium (0.1 mmol/kgDotarem)

normalized myocardial 1/T1 change and the blood 1/T1 change, after correction for hematocrit.[58, 59] T1 mapping will be performed using a modified Look-Locker inversion recovery (MOLLI)[60-62] with image analysis using CMR42 software (CVI42; Canada) and QMass software (Medis Medical Imaging Systems, The Netherlands.

24-hour blood pressure monitoring: will be performed as using a MobileOGraph device (APC cardiovascular, London, UK) or an Oscar 2 device (SunTech Medical) which measures time-resolved brachial arterial pressure estimates arterial wave reflections in an ambulatory fashion based on analysis of the brachial pressure waveform obtained with a cuff. This test will be used to assess potential increases in wave reflections in the nitrate-free period over the nighttime. The Oscar 2 device is FDA approved, but we will use a more advanced version of the device that can record the time-resolved pulse wave from the cuff while blood pressure is being taken. This particular use of the cuff is not FDA approved at present, but we won't be used to guide any clinical decision making. We will also use a novel watch device produced by Microsoft (which is not yet commercially available or FDA-approved), which continuously measures the radial pulse via a mounted tonometer. The device also measures transit time from the heart to the wrist in an ambulatory fashion.

Blood Draw: Blood samples will be obtained in order to measure serum creatinine and hematocrit prior to each cardiac MRI. In addition, samples will be centrifuged and frozen at -80 degrees C to perform proteomics analysis using the Somascan platform, which measures ~1300 plasma proteins. We will also collect a spot urine sample (~50 cc) in every study visit.

C.5. Run-in phase and ISMN initiation

Subjects will first undergo a run-in phase to assess within subject responses to ISMN at doses of 30 mg, 60 mg and 120 mg daily (with or without ascorbic acid), which will be better powered than between-arm comparisons. Half the subjects will be randomly assigned to receive ascorbic acid (500 mg by mouth three times daily).

- After baseline assessments, ISMN will be initiated at a dose of 30 mg daily (with our without ascorbic acid).
- **1-week visit:** Subjects will undergo a brief visit for peripheral, central BP and arterial stiffness measurements (arterial tonometry and VaSera device measurements) after ~1 week (5-9 days). ISMN will be increased to 60 mg daily. Blood (20 cc) and urine (~50 cc) will also be sampled and frozen.
- **2-week visit:** Subjects will undergo a brief visit for peripheral, central BP and arterial stiffness measurements (arterial tonometry and VaSera device

measurements) after ~1 week (5-9 days). ISMN will be increased to 120 mg daily. Blood (20 cc) and urine (~50 cc) will also be sampled and frozen.

- **3-week visit:** Subjects will undergo a brief visit for peripheral, central BP and arterial stiffness measurements (arterial tonometry and VaSera device measurements) after ~1 week (5-9 days). Subjects will also undergo a Doppler echocardiogram during this visit. A 24 hour ambulatory BP monitoring Blood (20 cc) and urine (~50 cc) will also be sampled and frozen.

In all cases, up-titration will occur only if:

(1) Resting supine systolic blood pressure is ≥95 mmHg;
(2) There is no evidence of orthostatic hypotension (decrease ≥20 mmHg in systolic or ≥10 mmHg in diastolic blood pressure within 3 minutes of standing);[63]
(3) The subject is not experiencing dizziness, vertigo, or other symptoms that, in the opinion of the investigator, may be due to the vasoactive effect of the drugs.

If the drug is not tolerated, subjects will be discontinued from the study. After completion of the run-in phase, subjects will be assigned to different doses of SR-ISMN (60 and 120 mg of SR ISMN daily) for additional 21 weeks (10 subjects per dose). Patients will continue taking ascorbic acid according to their initial allocation to receive or to not receive vitamin C. The design will therefore be a 2x2 factorial, such that 5 subjects will be allocated to ISMN SR 60- mg without vitamin C, 5 subjects will be allocated to ISMN SR 60- mg with vitamin C, 5 subjects will be allocated to ISMN SR 120- mg without vitamin C, and 5 subjects will be allocated to ISMN SR 120- mg with vitamin C. The intervention will be open label, but endpoint assessments will be blinded.

C.6. 12-week visit

The 12-week visit will take place in which we will perform:

- An office BP measurement
- Arterial tonometry and VaSera measurements
- A 2d Echocardiogram
- 24 hour ambulatory BP monitoring and pulse wave analysis monitoring
- A blood draw and urine sample

C.7. Final (24-week) assessments:

After the end of the 24 weeks therapy, arterial tonometry, VaSera measurements, Doppler echocardiography, cardiac MRI, 24-hour BP / pulse wave analysis monitoring, and blood draws will be repeated after the end of 21 weeks of fixed dose ISMN SR therapy (with or without vitamin C), to assess for changes in response to the study intervention. Procedures will be identical to those performed during the baseline assessment.

C8. Adverse Event Reporting

All adverse events will be reported following institutional guidelines. The research team will keep a log of all adverse events that occur in the trial, and any reportable events will be handled accordingly. The study team in charge of the conduct of the trial is up to date on all trainings pertaining to safety guidelines and adverse event reporting. All Serious Adverse Events will be reported to the site review boards, the Data Safety Monitoring Board, as well as the NIH in a timely fashion.

C.7. Data Management

The data for this trial will be collected by the research staff during each visit that the subject attends. Specific forms have been developed for the questions in this trial and will be filled out via pen and paper. Later, this data will be entered into a database developed in RedCap.

All data collected in this trial will be housed inside of a locked cabinet in the office space of the research staff, located at Ravdin 2 (Cardiovascular Institute).

C.8. Early termination

Subjects who withdraw due to intolerable adverse events or other safety concerns, or simply because they no longer wish to participate in the trial, will undergo a termination visit in which we will document: (1) vital signs; (2) compliance with the medications, including pill count; (3) Adverse effects. (4) specific reason for withdrawal.

D. Statistical Considerations

All study outcomes are continuous measures, thus, least-square regression models will be used to estimate effect sizes associated with each intervention. For the run-in phase, we will used mixed models to assess within subject changes to 30- 60 and 120 mg, and also to assess the effect of ascorbic acid on this response.
Analysis of the changes at 24 weeks between the study arms will also be assessed with mixed models to assess the following: (1) whether there are any significant within group changes in the outcome measures (from baseline to the final visit) in response to nitrate therapy at 30, 60 or 120 mg; (2) To test whether the outcome improvement in response to combination therapy is different between the 2 doses; (3) Whether vitamin C appears to have any effect on the responses. We recognize that this is a pilot study and therefore we will pay particular attention to the variability in the response and will interpret trends conservatively, particularly when confidence intervals are wide enough to be inconclusive. We note that, even through there is limited power for the inferential analyses, the data from this study will be critical to perform power calculations and propose a definitive trial, as per the aims of our R56 award. MRI endpoints will involve only 2 measures (baseline

and 24-weeks). However, echocardiographic and tonometry measures will involve three measurements per subject during the long-term phase (baseline, 12-week and 24-week visit). These will be modeled for intervention effects using general linear modeling on the intent-to-treat population.[64, 65] Subject-specific intercepts and slopes will be included as random effects; variability in the actual timing of assessments will be explicitly included in the mixed model by analyzing time as a random effect. Individual growth parameters will be modeled as a function of group, time, and the interaction of group and time. Differences in reflection magnitude over time will be examined via inferential testing of the "group by time" interaction term. Restricted maximum likelihood estimation will be used, and an appropriate covariance matrix will be specified. Linear and quadratic mixed models will be examined. The Akaike information criterion[66] will be used to evaluate overall model fit and to select the best-fitting longitudinal change pattern. For dropouts, the mechanism for missingness will be evaluated. If the number of subjects lost to follow-up is small and the missing observations can be documented as being missing at random (MAR) or missing completely at random (MCAR), analyses will be performed using the complete observed data. More complex approaches will be considered, such as random pattern-mixture models if necessary.[67, 68] Sensitivity analyses for these models will be performed.[69] We will use parsimonious linear models to assess whether the reduction in reflection magnitude predicts the improvement in individual outcomes (LV mass, LV collagen volume fraction, LV diastolic relaxation velocity). Bootstrapping procedures will be used to obtain estimates of the effects and to test their significance via confidence intervals.[70] Analyses will be 1-tailed since we hypothesize specific directions to the relationships or changes in measured parameters between baseline and 24 weeks post-initiation of long-term therapy.

F. PROTECTION OF HUMAN SUBJECTS

F.1. RISKS TO THE SUBJECTS

Human Subjects Involvement and Characteristics

The conduct of these studies will involve 20 human subjects. All subjects will be adults able to give informed consent. Subjects will be enrolled without regard to gender or race. However, an effort will be made for an enrolled population composed of at least 50% females and ~50% members of an ethnic minority according to the demographic characteristics of the Philadelphia area. Children will not be enrolled. All patients will be recruited from the Hospital of the University of Pennsylvania or the Penn Presbyterian Medical Center. The study involves various tests (cardiac MRI with gadolinium, arterial tonometry, ambulatory blood pressure / pulse wave analysis measurements, a blood draw) and the administration of ISMN and ascorbic acid therapy.

Potential Risks: There is minimal risk to the subjects. Potential risks are associated with

the study tests, the study interventions (pharmacologic therapy) and potential breaches in confidentiality.

(1) **Cardiac MRI:** The non-invasive nature and the lack of ionizing radiation make the risks associated with MRI small. A MRI scan requires the participant to be in a partially enclosed space inside the scanner. Some people may find this to be uncomfortable. Claustrophobia is an exclusion criterion for the study. The MRI scanner produces different types of noises during a scan. Participants are special earplugs to reduce the noise. A MRI scanner has a strong magnet which attracts certain metals. If anyone has these types of metal in their body, the MRI's strong magnetic field can cause them to move which may cause injury. Subjects with be thoroughly screened for the presence of body metal and excluded from the study if they are unable to safely undergo a cardiac MRI.

(2) **Administration of gadolinium contrast:** Gadolinium-based contrast agents may cause allergic reactions. In patients with advanced chronic kidney disease, these compounds may cause an extremely rare potentially fatal skin disease called nephrogenic systemic fibrosis. We will exclude subjects with advanced chronic kidney disease in this study (estimated glomerular filtration rate <30 mL/min/1.73 m^2). There have only been two case reports of gadolinium-related nephrogenic systemic fibrosis in the 30-60 mL/min/1.73 m^2 range (UpToDate accessed February 8, 2012). The nephrologic investigator (Townsend) has reviewed and approved this aspect; Townsend has also conducted studies of gadolinium in CKD (Townsend RR et al. Safety of intravenous gadolinium (gd-bopta) infusion in patients with renal insufficiency. *Am J Kidney Dis*. 2000; 36:1207-1212). Chirinos routinely performs contrast-enhanced cardiac MRIs and has also approved this aspect, which is consistent with the routine clinical use of gadolinium-based compounds.

(3) **Phlebotomy** carries minimal risk of infection and temporary pain.

(4) **Arterial tonometry (Sphygmocor) and cuff-based arterial stiffness measurements (VaSera)** is non-invasive and does not have any known risks.

(5) **Study medications:**

a. ISMN at the doses that will be used in this study has a long track record of safety. There are possible risks or discomforts related to the study medication. The most common side effects of ISN are headache and mild dizziness, which tend to resolve or improve spontaneously even with continued intake. Side effects may also include warmth, redness, or tingly feeling under the skin. Some people have allergic reactions to this medication but this is very rare.

- Ascorbid acid: Vitamin C, also known as ascorbic acid, is available over the counter. Some studies have shown that higher blood levels of vitamin C are associated with lower risk of death from all-causes, cancer, and cardiovascular disease.[54, 55, 71] In patients with diabetes, some studies suggest that vitamin C supplementation reduces the risk, whereas others suggest that it increases the risk, of cardiovascular disease.[54, 55, 71] Despite the multiple studies, there is no evidence that vitamin C supplements exert benefit in this patient population. However, there is no evidence of harm, with excellent tolerability with doses up to 2-10 grams/day in adults) exert any adverse or toxic effects. An upper level of 2 grams/day is recommended in order to prevent some adults from experiencing diarrhea and other gastrointestinal symptoms. In this study, we will use a dose of 500 mg three times daily, which is below this dose. Potential risks of discomforts include diarrhea, nausea, vomiting, heartburn, stomach cramps and headache. Such symptoms are not generally serious. Vitamin C may interact with warfarin, may increase the risk of oxalate kidney stone recurrence and may precipitate hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.[72] Patients with either of these conditions will be excluded from the study. If patients experience any gastrointestinal symptoms or intolerance to vitamin C, we will reduce the dose to 500 mg bid, or 500 mg once daily. If vitamin C is still not tolerated, we will discontinue vitamin C, but subjects may remain in the trial taking only the assigned dose of ISMN-SR.

(6) **Ambulatory blood pressure monitoring** may cause discomfort in the arm during cuff inflations but no serious risks are associated with this procedures. Ambulatory pulse wave analysis (Aurora device) may cause mild discomfort in the wrist.

(7) During various procedures (cardiac MRI, echocardiography), we will **use adhesive electrodes** attached to the participant's skin to record the electrical signal from the heart. These may occasionally cause skin itching and irritation.

(8) As with any clinical research study, there is a potential for breach of confidentiality. Adequate measures will be taken to minimize this risk (below).

These potential risks have been thoroughly considered and adequate protective measures will be implemented in the study (please see below).

F. 2. ADEQUACY OF PROTECTION AGAINST RISKS

a. Recruitment and Informed Consent: Written informed consent will be obtained from the subjects by the investigators prior to entry into the research study. This will be performed in accordance with the guidelines and under the supervision of the University

of Pennsylvania Institutional Review Boards. The study procedures and interventions and the associated risks will be explained to the subjects during the informed consent process. Only IRB-approved consent forms and related materials will be used.

b. Protection against risks associated with the cardiac MRI: Subjects with be thoroughly screened for the presence of body metal and excluded from the study if they are unable to safely undergo a cardiac MRI. We will exclude patients who are not suitable candidates for a cardiac MRI by virtue of having the following absolute or relative contraindications: (i) Central nervous system aneurysm clips; (ii) Implanted neural stimulators; (iii) Implanted cardiac pacemaker or defibrillator; (iv) Cochlear implant; (v) Ocular foreign body (e.g. metal shavings); (vi) Other implanted medical devices: (e.g. drug infusion ports); (vii) Insulin pump; (viii) Metal shrapnel or bullet; (ix) Claustrophobia; (x) Extreme obesity rendering the patient unable to fit into narrow-bore scanners; (xi) Unwillingness of the patient to undergo a cardiac MRI. All patients with metallic implants will be individually evaluated prior to MRI. Women with child-bearing potential will have a serum pregnancy test to exclude pregnancy immediately before study participation. Pregnant women will be excluded. Screening for MRI-related exclusion criteria will done during a phone interview (pre-screen) and with an on-site questionnaire at the time of the scan.

Once subjects are deemed able to safely undergo a cardiac MRI, these will be performed according to standard safety practices as per our institutional standards. Voice contact with patients will be maintained throughout the scan. A crash cart is available and a code team can be deployed immediately should serious allergic reactions to gadolinium contrast occur.

All cardiac MRIs will be interpreted clinically by Dr. Chirinos and by a board-certified radiologist for incidental findings. Any incidental findings will be reported to the patient and at their request/approval, to their primary care physician.

c. Protection against risks associated with gadolinium contrast: Serum creatinine will be measured prior to each scan to assure adequate renal function for the administration of gadolinium-based contrast as per our inclusion/exclusion criteria. Pregnancy status will be determined for all women of childbearing age before enrollment and before the final MRI scan. Pregnant women will be excluded and/or discontinued from the study. Subjects will not receive two injections of gadolinium within one week of each other as frequent use of gadolinium can cause nephrogenic systemic fibrosis (NSF). If a subject has recently received an MRI for clinical purposes, the research MRI will be delayed to avoid the second injection of gadolinium within the one week-timeframe.

d. Protection against risks of study medications: To reduce the risk of hypotension and related side effect, participants will be instructed to get up slowly when rising from a sitting or lying position. The study team will specifically assess for the presence of

hypotension and orthostatic hypotension, as well as symptoms such as dizziness after the medication is initiated or after the dose in increased, in order to determine whether it is safe for participants to continue taking these medications, or to uptitrate the dose. If participants find these side effects too uncomfortable, they will be deemed intolerant to the drugs and discontinued from the study. Study subjects can always withdraw from the study at any time and this will be made clear to them. We will specifically exclude individuals who are taking sildenafil (Viagra®), tadalafil (Cialis®), or vardenafil (Levitra®) and we will instruct them not to take these medications while participating in the study, as well as the risks associated with this combination. If subjects express the wish to take these medications within the 24 weeks following consent, they will not be included in the study. We will exclude subjects with a history of kidney stones, since vitamin C may precipitate the recurrence of oxalate kidney stones. We will also exclude patients with G6PD deficiency, since vitamin C may precipitate hemolysis in this condition.[72, 73]

e. Measures to minimize the risk of breach in confidentiality: All records will be treated with strict confidentiality according to HIPAA guidelines (all study personnel are trained on HIPAA regulations). Blood samples obtained from subjects will be used only for research purposes. Records will be treated with strict confidentiality and stored in a secured, limited access area. A subject ID number rather than name will identify all collected samples and images. A secure database of patient information will be maintained. Only the investigators will have access to research information and will follow IRB and institutional HIPAA guidelines. Paper files will be saved under lock in a secure IRB-approved area.

f. Other measures to minimize risk: Phlebotomy, arterial tonometry and Doppler echocardiographic examinations will only be performed by appropriately trained personnel as per our institutional standards. These procedures will be performs in CTRC-based cardiovascular phenotyping unit at either HUP or Penn Presbyterian medical center.

F.3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

There are no anticipated direct benefits to the subjects as a result of their participation in this study nor will this be implied when obtaining consent. However, if our hypothesis is correct, subjects in the active medication groups may experience improvements in their f LV hypertrophy and fibrosis, which are associated with the risk of heart failure. However, any potential benefit will not be implied during informed consent or enrollment. The results of this study may ultimately lead to an effective strategy to prevent HF in CKD, which is urgently needed. Since there is minimal risk and potential benefits to medical knowledge and society, the risk / benefit ratio is acceptable.

Overall, the minimal risk to human subjects for participation in this study is greatly

outweighed by potential scientific knowledge that may result from this work.

F. 4. IMPORTANCE OF THE KNOWLEDGE GAINED

If our hypothesis of a causal role is correct, this study may identify a potential intervention to induce reserve remodeling and prevent incident heart failure in CKD patients. Furthermore, if targeting wave reflections improves diastolic function, LV remodeling and the clinical status of CKD patients, this would lead to a better understanding of the mechanisms that lead to, or contribute to, cardiac dysfunction in CKD, which will accelerate the discovery of new treatments for this condition.

G. DATA AND SAFETY MONITORING PLAN

1. Adverse events

The Principal Investigator will be responsible for ensuring that all adverse events are noted, followed and reported to the IRB and DSMB as appropriate. The PI will continuously supervise all aspects of the study and review the records of the study subjects following each visit and at the end of their participation. Any adverse events (AE) will be rated on a 0-5 scale (S0-no AE; 1-mild AE, no treatment required; 2-moderate AE that responds to treatment; 3-severe AE that would limit normal activities and require a prolongation of hospitalization; 4-life-threatening or disabling AE; 5-Fatal AE). Any AE rated >=3 will be immediately reported to the IRB. All AEs will be reported to the IRB on an annual basis. In the case of a serious AE, the IRB will makes a determination about the necessity to modify the protocol, include additional information in the consent form, inform previous participants, temporarily hold enrollment of patients, or terminate the study. All study procedures and cumulative adverse events are subject to full committee review at least yearly.

Adverse events known to be associated with ISMN include:

- Headache
- Mild dizziness
- Warmth, redness, or tingly feeling under skin
- Pounding heart beat
- Dry mouth
- Nausea/Vomiting
- Sweating
- Feelings of passing out
- An allergic reaction to this drug is very rare, but could manifest as hives, difficulty breathing, or swelling of the face, lips, tongue, or throat.
- Symptomatic hypotension.

Adverse effects that may be associated with vitamin C include:[54, 55]

- Diarrhea
- Nausea
- Vomiting
- Heartburn
- Stomach cramps
- Headache

These will be specifically monitored during the study visits and calls. Any additional adverse events will be assessed by the PI (Dr. Chirinos, cardiologist) in consultation with Dr. Townsend (nephrologist and hypertension expert) to determine the relatedness to the study drug, the best clinical action to ensure patient safety, and the appropriate classification of the events for reporting purposes.

2. Data and Safety Monitoring Board (DSMB)

We will establish a Data Safety Monitoring Board to oversee the study. The initial DSMB meeting will be held as soon as possible after a notice of grant award and before initiating enrollment. Thereafter, the DSMB will meet via teleconference (when 40% of patients have been enrolled; when 80% of patients have been enrolled, and when all patients have closed out of the study). The meetings will consist of an open format meeting, since there is no blinding in the R56 phase of the study. All SAE's will be reported to the DSMB in a timely manner as per NIH guidelines.

DSMB meetings will generally be conducted by teleconference and coordinated by the Research Staff Team at the University of Pennsylvania.

A quorum, defined as the chairperson and the DSMB statistician will be required to hold a DSMB meeting. Critical decisions of the DSMB should be made by unanimous vote. However, if this is not possible, majority vote will decide. In addition to the PI, a facilitator (a member of the research staff) will attend the DSMB meetings as a non-voting member in order to facilitate data presentation and follow-up reporting, unless deemed not necessary by the DSMB.

Our DMSB will have 3 members, including an expert in hypertension, a nephrologist and one biostatistician. As per NIH guidelines for locally appointed boards, our local IRB will be responsible for ensuring adequate / appropriate membership. We will follow our institutions' conflict of interest policy. No member of the DSMB will have direct involvement in the conduct of the study. Furthermore, no member will have financial, proprietary, professional, or other interests that may affect impartial, independent decision-making by the DSMB. All DSMB and ad hoc members will sign a Conflict of Interest certification to that effect at the time they are asked to participate. Interests that may create a potential conflict of interest should be disclosed to the DSMB prior to any further discussion. The

DSMB will determine how to handle such potential conflict. The DSMB can require that a member with a potential conflict not vote or take other means deemed appropriate. Our proposed DSMB member list is attached to this document.

The primary responsibilities of the DSMB will be to 1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and 2) make determinations concerning the continuation, modification, or termination of the study. The DSMB will review the study protocol for prior to implementation of the study and may request changes to adverse event monitoring procedures.

Initial meeting: The initial DSMB meeting is planned for October 2015. At this meeting the DSMB should discuss the protocol and the DSMB charter, which includes triggers set for data review or analyses, definition of a quorum, and guidelines for monitoring the study. Guidelines will also address stopping the study for safety concerns. At this meeting, the DSMB will also develop procedures for conducting business (e.g., voting rules, attendance, etc.).

Recurring reports and meetings: The DSMB will convene via conference call at predetermined milestones (when 40% of patients have been enrolled; when 80% of patients have been enrolled; when all patients have closed out of the study) and review the incidence of adverse events. The DSMB will have the power to recommend termination of the study based on the evaluation of these results, due to safety concerns. The Principal Investigator, along with the study biostatistician, will complete reports, detailing the study progress status, cumulative data for adverse events any protocol deviations. All data in the open report, including adverse event data, will not be grouped by treatment period, to preserve blinding to the investigators. No interim efficacy analyses are planned unless requested by the DSMB. Any factors external to the study such as scientific or therapeutic developments that may impact participant safety or the ethics of the study will also be discussed. Confidentiality will always be maintained during all phases of DSMB review and deliberations.

The DSMB will conclude each review with their recommendations to the NIH program officer as to whether the study should continue without change, be modified, or terminated. After each meeting, monitoring board meeting minutes will summarize the topics discussed and list the all recommendations. After each board meeting, throughout the active phase of a study, the lead investigators will arrange for a summary of board recommendations to be sent to each participating IRB.

A final report after each meeting will be prepared and submitted to the NIH Program Officer and the Awardee's Institution IRB. This report will include all adverse events.

Expedited reporting will occur for all deaths as per the following guidelines (http://www.nia.nih.gov/research/dea/implementation-policies-human-intervention-studies), usually within 24 hours of study's knowledge of death. The report of death will be submitted to NIA Program Officer and to the DSMB Chair. When SAEs occur that are unanticipated (i.e., not listed in the Data and Safety Monitoring Plan) and that are related to the intervention, they will be reported to NIH Program Officer and to the DSMB Chair, within 48 hours of study's knowledge of SAE.

A summary of all other SAEs will be reported to NIH Program Officer and to the DSMB during each report.

APPENDIX I

ISMN prescribing information

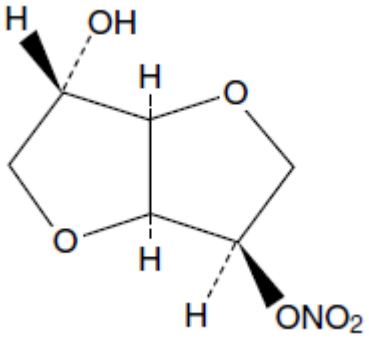
IMDUR®
(isosorbide mononitrate)
Extended Release Tablets

DESCRIPTION

Isosorbide mononitrate (ISMN), an organic nitrate and the major biologically active metabolite of isosorbide dinitrate (ISDN), is a vasodilator with effects on both arteries and veins.

IMDUR® Tablets, for oral administration, contain either 30 mg, 60mg or 120 mg of isosorbide mononitrate in an extended-release formulation. In addition, each tablet contains the following inactive ingredients: col loidal silicon dioxide, hydrogenated castor oil, hypromellose, lactose monohydrate, magnesium stearate, microcrystalline cellulose and talc.

The molecular formula of ISMN is C6H9NO6 and the molecular weight is 191.14. The chemical name for ISMN is 1,4:3,6-dianhydro-,Dglucitol 5-nitrate; the compound has the following structural formula:



ISMN is a white, crystalline, odorless compound which is stable in air and in solution, has a melting point of about 90°C, and an optical rotation of +144° (2% in water, 20°C). Isosorbide mononitrate is freely soluble in water, ethanol, methanol, chloroform, ethyl acetate, and dichloromethane.

CLINICAL PHARMACOLOGY

Mechanism of Action

The IMDUR product is an oral extended-release formulation of ISMN, the major active metabolite of isosorbide dinitrate; most of the clinical activity of the dinitrate is attributable to the mononitrate. The principal pharmacological action of ISMN and all organic nitrates in general is relaxation of vascular smooth muscle, producing dilatation of peripheral arteries and veins, especially the latter. Dilatation of the veins promotes peripheral pooling of blood, decreases venous return to the heart, thereby reducing left ventricular end-diastolic pressure and pulmonary capillary wedge pressure (preload). Arteriolar relaxation reduces systemic vascular resistance, systolic arterial pressure and mean arterial pressure (afterload). Dilatation of the coronary arteries also occurs. The relative importance of preload reduction, afterload reduction, and coronary dilatation remains undefined.

Pharmacodynamics

Dosing regimens for most chronically used drugs are designed to provide plasma concentrations that are continuously greater than a minimally effective concentration. This strategy is inappropriate for organic nitrates. Several well-controlled clinical trials have used exercise testing to assess the antianginal efficacy of continuously delivered nitrates. In the large majority of these trials, active agents were indistinguishable from placebo after 24 hours (or less) of continuous therapy. Attempts to overcome tolerance by dose escalation, even to doses far in excess of those used acutely, have consistently failed. Only after nitrates have been absent from the body for several hours has their antianginal efficacy been restored. IMDUR Tablets, during long-term use over 42 days dosed at 120 mg once daily, continued to improve exercise performance at 4 hours and at 12 hours after dosing but its effects (although better than placebo) are less than or at best equal to the effects of the first dose of 60 mg.

Pharmacokinetics and Metabolism

After oral administration of ISMN as a solution or immediate-release tablets, maximum plasma concentrations of ISMN are achieved in 30 to 60 minutes, with an absolute bioavailability of approximately 100%. After intravenous administration, ISMN is distributed into total body water in about 9 minutes with a

volume of distribution of approximate ly 0.6-0.7 L/kg. Isosorbide mononitrate is approximately 5% bound to human plasma proteins and is distributed into blood cells and saliva. Isosorbide mononitrate is primarily metabolized by the liver, but unlike oral isosorbide dinitrate, it is not subject to first pass metabolism. Isosorbide mononitrate is cleared by denitration to isosorbide and glucuronidation as the mononitrate, with 96% of the administered dose excreted in the urine within 5 days and only about 1% eliminated in the feces. At least six different compounds have been detected in urine, with about 2% of the dose excreted as
the unchanged drug and at least five metabolites. The metabolites are not pharmacologically active. Renal clearance accounts for only about 4% of total body clearance. The mean plasma elimination half-life of ISMN is approximately 5 hours. The disposition of ISMN in patients with various degrees of renal insufficiency, liver cirrhosis, or cardiac dysfunction was evaluated and found to be similar to that observed in healthy subjects. The elimination half-life of ISMN was not prolonged, and there was no drug accumulation in patients with chronic renal failure after multiple oral dosing. The pharmacokinetics and/or bioavailability of IMDUR Tablets have been studied in both normal volunteers and patients following single and multiple-dose administration. Data from these studies suggest that the pharmacokinetics of ISMN administered as IMDUR Tablets are similar between normal healthy volunteers and patients with angina pectoris. In single- and multiple-dose studies, the pharmacokinetics of ISMN were dose proportional between 30 mg and 240 mg. In a multiple-dose study, the effect of age on the pharmacokinetic profile of IMDUR 60 mg and 120 mg (2 x 60 mg) was evaluated in subjects ≥45 years. The results of that study indicate that there are no significant differences in any of the pharmacokinetic variables of ISMN between elder ly (≥65 years) and younger individuals (45-64 years) for the IMDUR 60 mg dose. The administration of IMDUR Tablets 120 mg (2 x 60 mg tablets every 24 hours for 7 days) produced a dose-proportional increase in Cmax and AUC, without changes in Tmax or the terminal half-life. The older group (65-74 years) showed 30% lower apparent oral clearance (Cl/F) following the higher dose, i.e., 120 mg, compared to the younger group (45-64 years); Cl/F was not different between the two groups following the 60 mg regimen. While Cl/F was independent of dose in the younger group, the older group showed slightly lower Cl/F following the 120 mg regimen compared to the 60 mg regimen. Differences between the two age groups, however, were not statistically significant. In the same study, females showed a slight (15%) reduction in clearance when the dose was increased. Females showed higher AUCs and Cmax compared to males, but these differences were accounted for by differences in body weight between the two groups. When the data were analyzed using age as a variable, the results indicated that there were no significant differences in any of the pharmacokinetic variables of ISMN between older (≥65 years) and younger individuals (45-64 years). The results of this study,

however, should be viewed with caution due to the small number of subjects in each age subgroup and consequently the lack of sufficient statistical power.
The following table summarizes key pharmacokinetic parameters of ISMN after single- and multiple-dose administration of ISMN as an oral solution or IMDUR Tablets:

	SINGLE-DOSE STUDIES		MULTIPLE-DOSE STUDIES	
PARAMETER	ISMN 60 mg	IMDUR 60 mg	IMDUR 60 mg	IMDUR 120 mg
C_{max}(ng/mL)	1242-1534	424-541	557-572	1151-1180
T_{max}(hr)	0.6-0.7	3.1-4.5	2.9-4.2	3.1-3.2
AUC (ng•hr/mL)	8189-8313	5990-7452	6625-7555	14241-16800
$t_{1/2}$ (hr)	4.8-5.1	6.3-6.6	6.2-6.3	6.2-6.4
Cl/F (mL/min)	120-122	151-187	132-151	119-140

Food Effects
The influence of food on the bioavailability of ISMN after single-dose administration of IMDUR Tablets 60 mg was evaluated in three different studies involving either a “light” breakfast or a high-calorie, high-fat breakfast. Results of these studies indicate that concomitant food intake may decrease the rate (increase in Tmax) but not the extent (AUC) of absorption of ISMN.

Clinical Trials
Controlled trials with IMDUR Tablets have demonstrated antianginal activity following acute and chronic dosing. Administration of IMDUR Tablets once daily, taken early in the morning on arising, provided at least 12 hours of antianginal activity. In a placebo-controlled parallel study, 30, 60, 120 and 240 mg of IMDUR Tablets were administered once daily for up to 6 weeks. Prior to randomization, all patients completed a 1- to 3-week single-blind placebo phase to demonstrate nitrate responsiveness and total exercise treadmill time reproducibility. Exercise tolerance tests using the Bruce Protocol were conducted prior to and at 4 and 12 hours after the morning dose on days 1, 7, 14, 28 and 42 of the doubleblind period. IMDUR Tablets 30 and 60 mg (only doses evaluated acutely) demonstrated a significant increase from baseline in total treadmill time relative to placebo at 4 and 12 hours after the administration of the first dose. At day 42, the 120 and 240 mg

dose of IMDUR Tablets demonstrated a significant increase in total treadmill time at 4 and 12 hours post dosing, but by day 42, the 30 and 60 mg doses no longer were differentiable from placebo. Throughout chronic dosing, rebound was not observed in any IMDUR treatment group. Pooled data from two other trials, comparing IMDUR Tablets 60 mg once daily, ISDN 30 mg QID, and placebo QID in patients with chronic stable angina using a randomized, double-blind, three-way crossover design found statistically significant increases in exercise tolerance times for IMDUR Tablets compared to placebo at hours 4,
8 and 12 and to ISDN at hour 4. The increases in exercise tolerance on day 14, although statistically significant compared to placebo, were about half of that seen on day 1 of the trial.

INDICATIONS AND USAGE
IMDUR Tablets are indicated for the prevention of angina pectoris due to coronary artery disease. The onset of action of oral isosorbide mononitrate is not sufficiently rapid for this product to be useful in aborting an acute anginal episode.

CONTRAINDICATIONS
IMDUR Tablets are contraindicated in patients who have shown hyper sensitivity or idiosyncratic reactions to other nitrates or nitrites.

WARNINGS
Amplification of the vasodilatory effects of IMDUR by sildenafil can result in severe hypotension. The time course and dose dependence of this interaction have not been studied. Appropriate supportive care has not been studied, but it seems reasonable to treat this as a nitrate overdose, with elevation of the extremities and with central volume expansion. The benefits of ISMN in patients with acute myocardial infarction or congestive heart failure have not been established; because the effects of isosorbide mononitrate are difficult to terminate rapidly, this drug is not recommended in these settings.
If isosorbide mononitrate is used in these conditions, careful clinical or hemodynamic monitoring must be used to avoid the hazards of hypotension and tachycardia.

PRECAUTIONS
General
Severe hypotension, particularly with upright posture, may occur with even small doses of isosorbide mononitrate. This drug should, therefore, be used with caution in patients who may be volume depleted or who, for whatever reason, are already hypotensive.
Hypotension induced by isosorbide mononitrate may be accompanied by paradoxical bradycardia and increased angina pectoris. Nitrate therapy may

aggravate the angina caused by hypertrophic cardiomyopathy. In industrial workers who have had long-term exposure to unknown (presumably high) doses of organic nitrates, tolerance clearly occurs. Chest pain, acute myocardial infarction, and even sudden death have occurred during temporary withdrawal of nitrates from these workers, demonstrating the existence of true physical dependence. The importance of these observations to the routine, clinical use of oral isosorbide mononitrate is not known.

Information for Patients

Patients should be told that the antianginal efficacy of IMDUR Tablets can be maintained by carefully following the prescribed schedule of dosing. For most patients, this can be accomplished by taking the dose on arising. As with other nitrates, daily headaches sometimes accompany treatment with isosorbide mononitrate. In patients who get these headaches, the headaches are a marker of the activity of the drug.

Patients should resist the temptation to avoid headaches by altering the schedule of their treatment with isosorbide mononitrate, since loss of headache may be associated with simultaneous loss of antianginal efficacy. Aspirin or acetaminophen often successfully relieves isosorbide mononitrate-induced headaches with no deleterious effect on isosorbide mononitrate's antianginal efficacy.

Treatment with isosorbide mononitrate may be associated with lightheadedness on standing, especially just after rising from a recumbent or seated position. This effect may be more frequent in patients who have also consumed alcohol.

Drug Interactions

The vasodilating effects of isosorbide mononitrate may be additive with those of other vasodilators. Alcohol, in particular, has been found to exhibit additive effects of this variety. Marked symptomatic orthostatic hypotension has been reported when calcium channel blockers and organic nitrates were used in combination. Dose adjustments of either class of agents may be necessary.

Drug/Laboratory Test Interactions

Nitrates and nitrites may interfere with the Zlatkis-Zak color reaction, causing falsely low readings in serum cholesterol determinations.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No evidence of carcinogenicity was observed in rats exposed to isosorbide mononitrate in their diets at doses of up to 900 mg/kg/day for the first 6 months and 500 mg/kg/day for the remaining duration of a study in which males were dosed for up to 121 weeks and females were dosed for up to 137 weeks. No

evidence of carcino genicity was observed in mice exposed to isosorbide mononitrate in their diets for up to 104 weeks at doses of up to 900 mg/kg/day.
Isosorbide mononitrate did not produce gene mutations (Ames test, mouse lymphoma test) or chromosome aberrations (human lymphocyte and mouse micronucleus tests) at biologically relevant concentrations. No effects on fertility were observed in a study in which male and female rats were administered doses of up to 750 mg/kg/day beginning, in males, 9 weeks prior to mating, and in females, 2 weeks prior to mating.

Pregnancy

Teratogenic Effects
Pregnancy Category B. In studies designed to detect effects of isosorbide mononitrate on embryo-fetal development, doses of up to 240 or 248 mg/kg/day, administered to pregnant rats and rabbits, were unassociated with evidence of such effects. These animal doses are about 100 times the maximum recommended human dose (120 mg in a 50 kg woman) when comparison is based on body weight; when comparison is based on body surface area, the rat dose is about 17 times the human dose and the rabbit dose is about 38 times the human dose. There are, however, no adequate and well-controlled studies in
pregnant women. Because animal reproduction studies are not always predictive of human response, IMDUR Tablets should be used during pregnancy only if clearly needed.

Nonteratogenic Effects
Neonatal survival and development and incidence of stillbirths were adversely affected when pregnant rats were administered oral doses of 750 (but not 300) mg isosorbide mononitrate/kg/day during late gestation and lactation. This dose (about 312 times the human dose when comparison is based on body weight and 54 times the human dose when comparison is based on body surface area) was associated with decreases in maternal weight gain and motor activity and evidence of impaired lactation.

Nursing Mothers
It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when ISMN is administered to a nursing mother.

Pediatric Use
The safety and effectiveness of ISMN in pediatric patients have not been established.

Geriatric Use

Clinical studies of IMDUR Tablets did not include sufficient information on patients age 65 and over to determine whether they respond differently from younger patients. Other reported clinical experience for IMDUR has not identified differences in response between elderly and younger patients. Clinical experience for organic nitrates reported in the literature identified a potential for severe hypotension and increased sensitivity to nitrates in the elderly. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy. Elderly patients may have reduced baroreceptor function and may develop severe orthostatic hypotension when vasodilators are used. IMDUR should therefore be used with caution in elderly patients who may be volume depleted, on multiple medications or who, for whatever reason, are already hypotensive. Hypotension induced by isosorbide mononitrate may be accompanied by paradoxical bradycardia and increased angina pectoris.

Elderly patients may be more susceptible to hypotension and may be at a greater risk of falling at therapeutic doses of nitroglycerin. Nitrate therapy may aggravate the angina caused by hypertrophic car diomyopathy, particularly in the elderly.

ADVERSE REACTIONS

The table below shows the frequencies of the adverse events that occurred in >5% of the subjects in three placebo-controlled North American studies in which patients in the active treatment arm received 30 mg, 60 mg, 120 mg, or 240 mg of isosorbide mononitrate as IMDUR Tablets once daily. In parentheses, the same table shows the frequencies with which these adverse events were associated with the discontinuation of treatment. Overall, 8% of the patients who received 30 mg, 60 mg, 120 mg, or 240 mg of isosorbide mononitrate in the three placebo-controlled North American studies discontinued treatment because of adverse events.

Most of these discontinued because of headache. Dizziness was rarely associated with withdrawal from these studies. Since headache appears to be a dose-related adverse effect and tends to disappear with continued treatment, it is recommended that IMDUR treatment be initiated at low doses for several days before being increased to desired levels.

FREQUENCY AND ADVERSE EVENTS (DISCONTINUED)*

Three Controlled North American Studies					
Dose	Placebo	30 mg	60 mg	120 mg**	240 mg**
Patients	96	60	102	65	65
Headache	15% (0%)	38% (5%)	51% (8%)	42% (5%)	57% (8%)
Dizziness	4% (0%)	8% (0%)	11% (1%)	9% (2%)	9% (2%)

*Some individuals discontinued for multiple reasons.
**Patients were started on 60 mg and titrated to their final dose.

In addition, the three North American trials were pooled with 11 controlled trials conducted in Europe. Among the 14 controlled trials, a total of 711 patients were randomized to IMDUR Tablets. When the pooled data were reviewed, headache and dizziness were the only adverse events that were reported by >5% of patients. Other adverse events, each reported by ≤5% of exposed patients, and in many cases of uncertain relation to drug treatment, were:
Autonomic Nervous System Disorders: Dry mouth, hot flushes.
Body as a Whole: Asthenia, back pain, chest pain, edema, fatigue, fever, flu-like symptoms, malaise, rigors. *Cardiovascular Disorders, General:* Cardiac failure, hypertension, hypotension. *Central and Peripheral Nervous System Disorders:* Dizziness, headache, hypoesthesia, migraine, neuritis, paresis, paresthesia, ptosis, tremor, vertigo. *Gastrointestinal System Disorders:* Abdominal pain, constipation, diarrhea, dyspepsia, flatulence, gastric ulcer, gastritis, glossitis, hemorrhagic gastric ulcer, hemorrhoids, loose stools, melena, nausea, vomiting. *Hearing and Vestibular Disorders:* Earache, tinnitus, tympanic membrane perforation. *Heart Rate and Rhythm Disorders:* Arrhythmia, arrhythmia atrial, atrial fibrillation, bradycardia, bundle branch block, extrasystole, palpitation, tachycardia, ventricular tachycardia. *Liver and Biliary System Disorders:* SGOT increase, SGPT increase. *Metabolic and Nutritional Disorders:* Hyperuricemia, hypokalemia. *Musculoskeletal System Disorders:* Arthralgia, frozen shoulder, muscle weakness, musculoskeletal pain, myalgia, myositis, tendon disorder, torticollis. *Myo-, Endo-, Pericardial and Valve Disorders:* Angina pectoris aggravated, heart murmur, heart sound abnormal, myocardial infarction, Q wave abnormality. *Platelet, Bleeding and Clotting Disorders:* Purpura, thrombocytopenia. *Psychiatric Disorders:* Anxiety, concentration impaired, confusion, decreased libido, depression, impotence, insomnia, nervousness, paroniria, somnolence. *Red Blood Cell Disorder:* Hypochromic anemia. R*eproductive Disorders, Female:* Atrophic vaginitis, breast pain.
Resistance Mechanism Disorders: Bacterial infection, moniliasis, viral infection.

Respiratory System Disorders: Bronchitis, bronchospasm, coughing, dyspnea, increased sputum, nasal congestion, pharyngitis, pneumonia, pulmonary infiltration, rales, rhinitis, sinusitis. *Skin and Appendages Disorders:* Acne, hair texture abnormal, increased sweating, pruritus, rash, skin nodule. *Urinary System Disorders:* Polyuria, renal calculus, urinary tract infection. *Vascular (Extracardiac) Disorders:* Flushing, intermittent claudication, leg ulcer, varicose vein. *Vision Disorders:* Conjunctivitis, photophobia, vision abnormal. In addition, the following spontaneous adverse event has been reported during the marketing of isosorbide mononitrate: syncope.

OVERDOSAGE

Hemodynamic Effects

The ill effects of isosorbide mononitrate overdose are generally the result of isosorbide mononitrate's capacity to induce vasodilatation, venous pooling, reduced cardiac output, and hypotension. These hemodynamic changes may have protean manifestations, including increased intracranial pressure, with any or all of persistent throbbing headache, confusion, and moderate fever; vertigo,
palpitations; visual disturbances; nausea and vomiting (possibly with colic and even bloody diarrhea); syncope (especially in the upright posture); air hunger and dyspnea, later followed by reduced ventilatory effort; diaphoresis, with the skin either flushed or cold and clammy; heart block and bradycar dia; paralysis; coma; seizures and death.

Laboratory determinations of serum levels of isosorbide mononitrate and its metabolites are not widely available, and such determinations have, in any event, no established role in the management of isosorbide mononitrate overdose. There are no data suggesting what dose of isosorbide mononitrate is likely to be life threatening in humans. In rats and mice, there is significant lethality at doses of 2000 mg/kg and 3000 mg/kg, respectively.

No data are available to suggest physiological maneuvers (eg, maneuvers to change the pH of the urine) that might accelerate elimination of isosorbide mononitrate. In particular, dialysis is known to be ineffective in removing isosorbide mononitrate from the body. No specific antagonist to the vasodilator effects of isosorbide mononitrate is known, and no intervention has been subject to controlled study as a therapy of isosorbide mononitrate overdose. Because the hypotension associated with isosorbide mononitrate overdose is the result of venodilatation and arterial hypovolemia, prudent therapy in this situation should be directed toward an increase in central fluid volume. Passive elevation of the patient's legs may be sufficient, but intravenous infusion of normal saline or similar fluid may also be necessary. The use of epinephrine or other arterial vasoconstrictors in this setting is likely to do more harm than good. In patients with renal disease or congestive heart failure, therapy resulting in central volume

expansion is not without hazard. Treatment of isosorbide mononitrate overdose in these patients may be subtle and difficult, and invasive monitoring may be required.

Methemoglobinemia

Methemoglobinemia has been reported in patients receiving other organic nitrates, and it probably could also occur as a side effect of isosorbide mononitrate. Certainly nitrate ions liberated during metabolism of isosorbide mononitrate can oxidize hemoglobin into methemo globin. Even in patients totally without cytochrome b5 reductase activity, however, and even assuming that the nitrate moiety of isosorbide mononitrate is quantitatively applied to oxidation of hemoglobin, about 2 mg/kg of isosorbide mononitrate should be required before any of these patients manifest clinically significant (≥10%) methemoglobine mia. In patients with normal reductase function, significant production of methemoglobin should require even larger doses of isosorbide mononitrate. In one study in which 36 patients received 2-4 weeks of continuous nitroglycerin therapy at 3.1 to 4.4 mg/hr (equivalent, in total administered dose of nitrate ions, to 7.8-11.1 mg of isosorbide mono-nitrate per hour), the average methemoglobin level measured was 0.2%; this was comparable to that observed in parallel patients who received placebo. Notwithstanding these observations, there are case reports of significant methemoglobinemia in association with moderate overdoses of organic nitrates. None of the affected patients had been thought to be unusually susceptible.

Methemoglobin levels are available from most clinical laboratories. The diagnosis should be suspected in patients who exhibit signs of impaired oxygen delivery despite adequate cardiac output and adequate arterial pO2. Classically, methemoglobinemic blood is described as chocolate brown without color change on exposure to air. When methemoglobinemia is diag nosed, the treatment of choice is methylene blue, 1-2 mg/kg intravenously.

DOSAGE AND ADMINISTRATION

The recommended starting dose of IMDUR Tablets is 30 mg (given as a single 30 mg tablet or as 1/2 of a 60 mg tablet) or 60 mg (given as a single tablet) once daily. After several days, the dosage may be increased to 120 mg (given as a single 120 mg tablet or as two 60 mg tablets) once daily. Rarely, 240 mg may be required. The daily dose of IMDUR Tablets should be taken in the morning on arising. IMDUR Extended Release Tablets should not be chewed or crushed and should be swallowed together with a half-glassful of fluid. Do not break the 30 mg tablet.

HOW SUPPLIED

IMDUR Extended Release Tablets 30 mg are white, capsule-shaped tablets scored on one side and engraved “IMDUR” on the unscored side. They are supplied as follows:
Bottles of 100 NDC 0085-1374-01
IMDUR Extended Release Tablets 60 mg are white, capsule-shaped tablets scored on one side with “60-60” and engraved “IMDUR” on the unscored side. They are supplied as follows:
Bottles of 100 NDC 0085-2028-01
IMDUR Extended Release Tablets 120 mg are white, capsule-shaped tablets engraved “IMDUR” on one side and “120” on the other side.
They are supplied as follows:
Bottles of 100 NDC 0085-0091-01
Store at controlled room temperature 20°-30°C (68°-86°F) [See USP].
IMDUR®
(isosorbide mononitrate)
Extended Release Tablets
Manufactured by: Kremers Urban Pharmaceuticals Inc.,
Seymour, IN 47274, USA
Distributed by: Schering Corporation, a subsidiary of Whitehouse Station, NJ 08889, USA

APPENDIX II

List of procedures / visits and schedule of events

A. List of procedures per visit

Baseline Visit	• Read and sign this informed consent form (ICF). • If you are a pre-menopausal woman, confirm that you are not pregnant by a urine pregnancy test. • Have a medical history assessment, a physical, and record vital signs. • Have your blood drawn and collect urine. • Arterial Tonometry / VaSera test. • Have a Doppler Echocardiogram. • Complete the MRI of your Heart. • Setup the 24 Hour BP monitoring and pulse wave analysis watch. • Medication given out. • Bicycle Exercise Test • Activity Monitor
Week 1 Visit	• Collect Vital Signs. • Review Side Effects • Increase medication dose to 60 mg daily. • Collect medication of the previous dose. • Arterial Tonometry / VaSera test. • Download and replacement of pulse wave analysis watch • Blood draw and urine collection • 24 Hour BP Monitoring • Activity Monitor
Week 2 Visit	• Collect Vital Signs. • Review Side Effects • Increase medication dose to 120 mg daily. • Collect medication of the previous dose. • Download and replacement of pulse wave analysis watch • Collect information on potential side effects. • Arterial Tonometry / VaSera test. • Replace pulse wave analysis watch.

	• Medication given out. • Arterial Tonometry and VaSera • 24 Hour BP Monitoring • Activity Monitor
Week 3 Visit	• Collect Vital Signs. • Review Side Effects • 24 Hour BP Monitoring and pulse wave analysis watch Arterial Tonometry / VaSera test. • Have a Doppler Echocardiogram. • Bicycle Exercise Test • Blood Draw and urine collection • Activity Monitor: 2 weeks prior visit
3 Month Visit	• The Arterial Tonometry and VaSera test. • Have a Doppler Echocardiogram. • Setup the 24 Hour BP monitoring and pulse wave analysis watch. • Medication given out. • Activity Monitor: 2 weeks prior to the visit • Review Side Effects • Blood Draw and urine collection
6 Month Visit	• If you are a pre-menopausal woman, confirm that you are not pregnant by a urine pregnancy test • Have a medical history assessment, a physical, and record vital signs. • Review Side Effects • Have your blood drawn and urine collection. • The Arterial Tonometry and VaSera test. • Have a Doppler Echocardiogram. • Complete the MRI of your Heart. • Setup the 24 Hour BP monitoring and pulse wave analysis watch. • After 2 days of wearing pulse wave analysis watch, discontinue the medication. • Bicycle Exercise Test • Activity Monitor: 2 weeks prior to the visit

B. Schedule of events

Test	Baseline visit	Week 1	Week 2	Week 3	Week 12	Week 24
Medical History	X					X
Physical	X					X
Vital Signs	X	X	X	X	X	X
Potential change in study medication		X	X	X		
ArterialTonometry and VaSera	X	X	X	X	X	X
Doppler Echocardiogram	X			X	X	X
• *24 Hour BP Monitoring*	X			X	X	X
• *Pulse wave analysis watch*	X	X	X	X	X	X
• *Cardiac MRI*	X					X
• *Blood draw and urine collection*	X	X	X	X	X	X
Review Side Effects		X	X	X	X	X
Activity Monitor	X	X	X	X	2 weeks prior to the visit	2 weeks prior to the visit
Bicycle Exercise Test	X			X		x

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