Safety and Cardiovascular Efficacy of Hydralazine and Isosorbide Dinitrate in Dialysis-Dependent ESRD (HIDE) Study

A randomized, double-blind, placebo-controlled, single-center pilot study of the safety and effects on cardiac structure and function of hydralazine and isosorbide dinitrate in patients with hemodialysis dependent ESRD

Funding Sponsor:	*National Institutes of Diabetes and Digestive and Kidney Diseases* *National Institutes of Health* *2 Democracy Plaza* *6707 Democracy Boulevard* *Bethesda, MD 20892-5458*
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List of Abbreviations

ACE	angiotensin converting enzyme inhibitor
ADMA	asymmetric dimethyl arginine
AE	adverse event
ALDO	aldosterone
ANCOVA	analysis of covariance
ANG	angiopoietin II
ARB	angiotensin receptor blocker
BID	twice daily
CFR	coronary flow reserve
CKD	chronic kidney disease
CM	centimeter
CRF	case report form
CRP	C-reactive protein
CVD	cardiovascular disease
CVR	coronary vascular resistance
DCC	data coordinating center
DSMB	data safety and monitoring board
END	endostatin
ESRD	end stage renal disease
FTP	file transfer protocol
GFR	glomerular filtration rate
HD	hemodialysis
Hg	mercury
HY	hydralazine
HIPAA	health insurance portability and accounting act
IDS	investigational drug service
IEC	independent ethics committee
IRB	institutional review board
ITT	intention to treat
ISD	isosorbide dinitrate
L	liter
LVEF	left ventricular ejection fraction
LVMI	left ventricular mass index
mEq	milliequivalent
MM	millimeter
MG	milligram
MOP	manual of procedures
MRI	magnetic resonance imaging
MI	myocardial infarction
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NO	nitric oxide
NOS	nitric oxide synthase
OHRP	Office of Human Research Protections
PET	positron emission tomography
PHI	protected health information
PICP	procollagen type I carboxy-terminal peptide
PTH	parathyroid hormone

QD	daily
RCT	randomized controlled trial
SAE	serious adverse event
SAP	statistical analysis plan
SBP	systolic blood pressure
TDI	tissue Doppler index
TSP	thrombospondin II
UAE	unanticipated adverse event
VEGF	vascular endothelial growth factor

Study Summary

Title	**Safety and Cardiovascular Efficacy of Hydralazine and Isosorbide Dinitrate in Dialysis-Dependent ESRD**
Short Title	HIDE Study
Protocol Number	2014P001544/BWH
Phase	Pilot
Methodology	Randomized, double-blind, 2-arm
Study Duration	2 years
Study Center(s)	Brigham and Women's Hospital
Objectives	• To generate pilot data on the safety and tolerability of hydralazine (HY) isosorbide dinitrate (ISD) combination therapy in patients with dialysis-dependent ESRD. • To generate pilot estimates of the effect of HY/ISD combination therapy compared with placebo on cardiovascular efficacy parameters in patients with dialysis-dependent ESRD.
Number of Participants	16
Diagnosis and Main Inclusion Criteria	Dialysis-dependent end-stage renal disease
Study Product, Dose, Route, Regimen	ISD/HY 10 mg/10 mg, 3X/day for 1 week, orally ISD/HY 20 mg/35 mg, 3X/day for 1 week, orally ISD/HY 40 mg/75 mg, 3X/day for 2 weeks, orally
Duration of administration	26 weeks
Major Outcomes	• Safety: hypotension, intra-dialytic cramping • Tolerability: treatment adherence • Efficacy: a) change in mitral annular E' velocity; b) change in CFR • Feasibility: recruitment rate, retention, dosing completeness
Statistical Methodology	Mixed effects linear regression models will be used to assess the direction and time averaged magnitude of change in E' and CFR efficacy parameters, with and without controlling for baseline covariates, to compare the effects of therapy. The incidence of safety events, especially hypotension, in treatment and placebo groups will be compared with Chi-square test or Fisher's exact test.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and the research policies and procedures in effect at the institutions where the study is implemented.

1.1 *Background*

Chronic kidney disease (CKD) affects >13% of the U.S. population[1] and is an important risk factor for cardiovascular disease (CVD)[2]and CV mortality[2-7]. The associations between CKD and CVD are not fully-explained by traditional risk factors[8-11], and for patients with end-stage renal disease (ESRD), standard CV therapies have not reduced mortality[12-15]. Given the growing CKD population[16], the absence of effective therapies represents a critical public health challenge, and better understanding of the underlying mechanisms and identification of effective treatments for CVD in ESRD are needed. Sudden cardiac death is the major cause of CV death in ESRD with only 5.3% of sudden cardiac death due to myocardial infarction (MI) compared with 26.1% from arrhythmias[17]. A high risk of sudden cardiac death is detectable early in CKD[5], and the risk increases dramatically in advanced CKD. For example, in one study sudden cardiac death incidence was 6-fold higher in dialysis patients compared with individuals without CKD[18]. The disproportionate risk of sudden cardiac death relative to MI suggests that plaque rupture and subsequent MI are unlikely to fully explain the high incidence of sudden cardiac death and that myocardial remodeling in ESRD lowers the threshold for arrhythmia generation and increases the risk of CV death independently of coronary plaque rupture. Targeting these myocardial processes could provide a powerful approach for improving CV outcomes in the setting of advanced CKD. The HIDE trial is designed to provide pilot data on the efficacy and safety of a novel, ESRD-targeted therapeutic approach to reducing CV morbidity.

Interstitial Fibrosis and Microvascular Rarefaction—Uremia inhibits ischemia-driven angiogenesis[19], and experimental CKD is characterized by myocardial microvascular loss, reduced tolerability of ischemia, increased infarct size after coronary ligation, left ventricular hypertrophy (LVH), increased interstitial collagen, and severe myocardial fibrosis[19-25]. Increased fibrosis and decreased myocardial capillary number have also been observed in a few small studies of ESRD patients[26]. Myocardial coronary flow reserve—a measure of microvascular supply and function—also declines in CKD[27-30], and coronary collateral vessels are 41% less abundant in individuals with mild-moderate CKD[31]. LV mass, diastolic function and late gadolinium enhancement (a marker of myocardial scar) also increase dramatically and predict an increased risk of death[32-39]. In combination, LVH, increased interstitial collagen, and microvascular rarefaction increase capillary to cardiomyocyte distances[22, 26], impair myocardial oxygen delivery, and cause chronic cardiomyocyte hypo-perfusion which reduces ischemia tolerance, increases myocyte necrosis after minor decrements in coronary flow, and disrupts myocardial electrical circuits (already impacted by increased interstitial scarring). This likely lowers thresholds for propagation of fatal arrhythmias, and underlies the high incidence of sudden cardiac death in ESRD[5, 40].

Nitric Oxide (NO)—NO concentrations decrease in CKD[41-45]. This likely results from a rise in asymmetric di-methyl arginine (ADMA)[46-49], a potent inhibitor of NO-synthase (NOS) which lowers NO bioavailability and is associated with atherosclerosis, LVH, and high risks of CV mortality in advanced CKD[46-52], and with endothelial dysfunction, LVH, diastolic dysfunction and mortality in ESRD[49, 51, 53-55]. The effect of NO on ESRD- and CKD-associated CV mortality is likely mediated through its role in the progression of myocardial fibrosis and microvascular rarefaction since ADMA and NO deficiency induce expression of TGF-β[56, 57] (a potent cytokine that stimulates fibrosis[58]) and endothelial to mesenchymal transformation which results in microvascular dropout and fibrosis[59-61]. Chronic NOS inhibition has been shown in animal models to cause microvascular rarefaction, endothelial to mesenchymal transformation, and increased collagen synthesis[62-64], and in the heart, over-expression of ADMA and LNAME (a potent NOS inhibitor) or knock out of endothelial NOS produces dramatic increases in perivascular fibrosis[65], LVH, myocardial fibrosis and capillary rarefaction[66-70 71]. Conversely, NOS up-regulation improves myocardial vascular supply[72, 73]. Taken together, these findings support a hypothesis that ADMA and reduced NO bioavailability in ESRD are key contributors to myocardial fibrosis and capillary rarefaction.

Isordil (ISD) and Hydralazine (HY)—The administration of ISD, a long-acting nitrate, increases NO synthesis and NO release[74-78] through its direct conversion into NO by mitochondrial aldehyde dehydrogenase [79, 80]. Long-acting nitrates increase blood NO[81], and in experimental models can reduce myocardial fibrosis[82, 83] and improve post-MI remodeling[84]. Therapeutic efficacy in humans was demonstrated in the AHEFT trial in which combined ISD/HY reduced mortality, hospitalizations and LVH in 1050 African American patients with congestive heart failure[85, 86]. These effects were modified by NOS genotype[87] suggesting that effects were mediated through changes in NO bioavailability. Unfortunately, the efficacy of nitrate therapy is limited by the development of nitrate tolerance which is mediated by counter-regulatory, vaso-constrictive hormones and by NO-induced production of superoxide with deactivation of aldehyde dehydrogenase, and interruption of calcium signaling pathways [80, 88, 89]. HY, a direct vasodilator, counteracts these neuro-humoral changes and inhibits superoxide and peroxynitrate production thereby preventing inactivation of aldehyde dehydrogenase and the decoupling of NO and superoxide synthesis by NOS[23, 90-93]. Clinical studies demonstrating the ability of combination ISD-HY therapy to improve nitrate tolerance provided a primary motivation for the AHEFT trial[94, 95], and suggest that combined ISD-HY is likely to maximize NO bioavailability compared with mono-ISD therapy in ESRD. Additionally, HY may provide some independent benefits. In experimental myocardial infarction, right ventricular accumulation of collagen is reduced by HY therapy[96]. HY also reduces LV stiffness and improves collagen solubility in the spontaneously hypertensive rat[97], and it antagonizes angiotensin induced myocardial fibrosis[98]. Lastly, HY prevents hypertensive arteriolar remodeling, and it induces VEGF synthesis and angiogenesis[99-101]. Thus, HY may have benefits in ESRD independent of its impact on ISD tolerance.

Safety—ISD and HY have been widely used for decades in ESRD, but there are only limited research reports. Safety of ISD is suggested by the lack of a significant renal or dialytic clearance[102-104] and by the tolerability of a similar agent, isosorbide mono-nitrate in one report[105]. The kidney has a limited role in HY metabolism[106], and side effects were infrequent and transient in one report of 18 dialysis patients[107]. However, combination ISD/HY therapy has not been studied in ESRD; hence the need for pilot studies.

Conclusions—ESRD is characterized by a vicious cycle in which ADMA reduces NO bioavailability leading to myocardial fibrosis and capillary loss while increasing synthesis of angiogenesis inhibitors that potentiate the effects of decreased NO and further lower its availability. These effects of ADMA provide a compelling rationale for pilot testing of an NO-donating strategy with proven efficacy in other settings, as a targeted therapy to reduce CV events in ESRD.

1.2 *Investigational Agents*

Isosorbide Dinitrate

Isosorbide dinitrate (ISD) is described chemically as 1,4:3,6-dianhydro-d-glucitol-2,5-dinitrate 9 and its structural formula is shown in Figure 1.

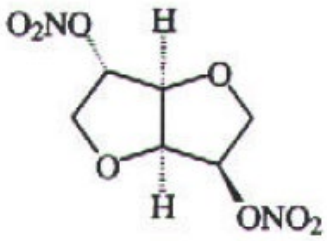


Figure 1

ISD is a white to off-white, crystalline powder with the empirical formula C6H8N2O8 and a molecular weight of 236.14. It is freely soluble in organic solvents such as alcohol, chloroform and ether, but is only sparingly soluble in water.

ISD is a vasodilator affecting both arteries and veins. Its dilator properties result from the release of nitric oxide and the subsequent activation of guanylyl cyclase, and ultimate relaxation of vascular smooth muscle.

Absorption and Distribution

Absorption of ISD from tablets after oral dosing is nearly complete. The average bioavailability of ISD is about 25%, but is highly variable (10%-90%) due to first-pass metabolism and increases progressively during chronic therapy. Serum concentrations reach their maximum about one hour after ingestion. The volume of distribution of ISD is 2 to 4 L/kg. About 28% of circulating ISD is protein bound. Under steady-state conditions, ISD accumulates significantly in muscle (pectoral) and vein (saphenous) wall relative to simultaneous plasma concentrations.

Metabolism and Elimination

ISD undergoes extensive first-pass metabolism in the liver and is cleared at a rate of 2 to 4 L/minute with a serum half- life of about 1 hour. The clearance of ISD is primarily by de-nitration to the 2-mononitrate (15 to 25%) and the 5-mononitrate (75 to 85%). Both metabolites have biological activity**,** especially the 5-mononitrate which has an overall half- life of about 5 hours. The 5-mononitrate is cleared by de-nitration to isosorbide, glucuronidation to the 5-mononitrate glucuronides, and by de-

nitration/hydration to sorbitol. The 2-mononitrate appears to participate in the same metabolic pathways with a half- life of about 2 hours. Most ISD is eliminated by the kidneys as conjugated metabolites.

Hydralazine

Hydralazine hydrochloride (HY) is described chemically as 1-hydrazinophthalazine monohydrochloride and its structural formula are shown in Figure 2.

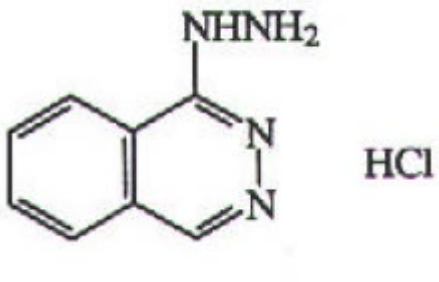


Figure 2

HY is a white to off-white, crystalline powder with the empirical formula C8H8N4·HCl and a molecular weight of 196.64. It is soluble in water, slightly soluble in alcohol, and very slightly soluble in ether.

Absorption and Distribution

About 2/3rd of a 50-mg dose of ^{14}C-hydralazine HCl given in gelatin capsules was absorbed in hypertensive participants. In patients with heart failure, mean absolute bioavailability of a single oral dose of HY 75 mg varies from 10 to 26%, with the higher percentages in slow acetylators (See *Metabolism and Elimination*).

Administration of doses escalating from 75 mg to 1000 mg thrice daily to patients with congestive heart failure resulted in an up to 9-fold increase in the dose normalized AUC, indicating nonlinear kinetics of HY, probably reflecting saturable first pass metabolism. After intravenous administration of hydralazine in a dose of 0.3 mg/kg, the steady-state volume of distribution in patients with congestive heart failure was 2.2 L/kg.

Metabolism and Elimination

Metabolism is the main route for the elimination of hydralazine. Negligible amounts of unchanged hydralazine are excreted in urine. Hydralazine is metabolized by acetylation, ring oxidation and conjugation with endogenous compounds including pyruvic acid. Acetylation occurs predominantly during the first pass after oral administration which explains the dependence of the absolute bioavailability on the acetylator phenotype. About 50% of patients are fast acetylators and have lower exposure. After oral administration of hydralazine, the major circulating metabolites are hydralazine pyruvate hydrazone and methyltriazolophthalazine. Hydralazine is the main pharmacologically active entity; hydralazine pyruvate hydrazone has only minimal hypotensive and tachycardic activity. The

pharmacological activity of methyltriazolophthalazine has not been determined. The major identified metabolite of hydralazine excreted in urine is acetylhydrazinophthalazinone.

Pharmacokinetic Drug-Drug Interactions

Administration of hydralazine can increase the exposure to a number of drugs including beta blockers.

1.3 *Clinical Data to Date*

Heart Failure: The AHEFT trial was a multi-center trial that enrolled 1050 African American patients with NYHA class 3 or 4 heart failure to ISD/HY 120/225 mg/day in 3 divided doses or placebo. There was a significant reduction in mortality (10.2 vs. 6.2%, P=0.02) with active therapy and a 33% reduction in rate of hospitalization for heart failure, P<0.001[85].

ESRD: ISD and HY have been widely used for decades in ESRD, but there are only limited research reports. Safety of ISD is suggested by the lack of a significant renal or dialytic clearance[102-104] and because a similar agent, isosorbide mono-nitrate, was well tolerated in one report[105]. In addition, renal function has a limited role in HY metabolism[106], and side effects were infrequent and transient in one report of 18 dialysis patients[107]. However, combination therapy has not been studied in ESRD highlighting the need for pilot studies.

1.4 *Dose Rationale and Risks*

As noted above (section 1.3), despite the clinical use of the investigational agents individually or in combination in patients with ESRD, ISD/HY combination therapy has not been previously tested in a clinical trial of patients on chronic hemodialysis. Given the absence of dialysis-specific data and the pilot nature of this trial, the starting and target doses were selected on the basis of the doses used in the AHEFT trial[85] in which combination therapy was associated with significant survival benefits (1.3). Allowances have been made to allow for scheduled dose reductions to the minimum clinical dose of ISD/HY in the event of intolerance. The proposed doses of ISD are equivalent to the doses of isosorbide mono-nitrate that were well tolerated in a 6 month trial of 144 chronic hemodialysis patients[105].

HY is routinely used for clinical purposes in chronic dialysis patients. Although, there is minimal clinical trial data on its use in this setting, HY was well tolerated in a single clinical trial enrolling 18 maintenance dialysis patients[107].

Potential Risks

Both ISD and HY may cause gastrointestinal symptoms, allergic reactions, or decreased blood pressure which could result in light-headedness or symptomatic hypotension, particularly during dialysis. In addition, both ISD and HY (similar to most vasoactive agents) can cause severe hypotension or syncope when given to patients with cardiac outflow obstruction. ISD cannot be given in combination with phosphodiesterase inhibitors used for treatment of erectile dysfunction due to significant hypotensive effects of the combination. Finally, HY can rarely cause a drug associated lupus-like syndrome or peripheral neuropathy when used for prolonged periods. Each of these potential adverse reactions is important, but they should be interpreted within the context of the widespread clinical use of both agents in dialysis patients for many years.

No specific metabolic or hematologic complications of therapy are anticipated. However, to provide an additional margin of safety, participant dialysis laboratory studies will be monitored throughout the trial by coordinating with the outpatient dialysis units and by reviewing labs drawn according to standard clinical schedules.

1.4.1 Benefits

Benefits of ISD/HY therapy in the dialysis population remain unproven at this time. Hypothesized benefits of therapy include improved blood pressure control, improvement in cardiovascular function and structure, and a decrease in arrhythmias.

2 Study Objectives

The primary objective of this study is to characterize the safety, tolerability, and cardiovascular impact of combination ISD/HY therapy compared with placebo in maintenance hemodialysis patients, and to assess the feasibility of larger trials. The primary efficacy parameters are the change in E' on tissue Doppler echocardiography (TDI) as an index of diastolic function and a surrogate for myocardial fibrosis, and the change in coronary flow reserve (CFR) on myocardial PET imaging as an index of microvascular supply and function. These parameters are designed to broaden insight into the potential effects of therapy on cardiac structure and function in individuals with dialysis-dependent ESRD and to assess the feasibility of conducting a full-scale trial.

2.1 *Primary Objectives*

Safety

- To evaluate the safety of ISD/HY therapy in individuals with ESRD on HD.
    - Safety events include the following:
        - Serious hypotension is defined as hypotension requiring hospitalization or treatment in an emergency room and not attributable to overt sepsis, acute myocardial infarction, or other cardiovascular event (e.g. aortic dissection)
        - Inter-dialytic hypotension
        - Intra-dialytic hypotension and symptoms of hypotension (e.g., muscle cramping)
        - Treatment-emergent events defined as the combined incidence of death, myocardial infarction, stroke, hospitalizations, , and serious hypotension (hypotension requiring hospitalization or treatment in an emergency room and not attributable to overt sepsis, acute myocardial infarction, or other cardiovascular event)
        - The individual components of the treatment-emergent endpoint
        - Cardiovascular death
        - Gastrointestinal events or headache requiring dose adjustment or discontinuation

Tolerability

- To evaluate the tolerability of assigned therapy in individuals with ESRD on maintenance hemodialysis. Tolerability events will include reduction in dose of study medication during the course of the treatment period or discontinuation of study drug.

Efficacy

- To test the hypothesis that ISD/HY combination therapy improves diastolic function in individuals with ESRD on HD compared with placebo. The change in E' on TDI echocardiography of the left ventricle from baseline to end of study will be used in the primary endpoint assessment of diastolic function.
- To test the hypothesis that ISD/HY combination therapy improves microvascular function in individuals with ESRD on HD compared with placebo. The change in CFR on myocardial PET from baseline to end of study will be used for the primary endpoint assessment of diastolic function.

Feasibility

- To assess feasibility of conducting a full-scale trial.
    - Feasibility will be assessed based on recruitment rate and dropout rates.

2.2 *Secondary Objectives*

Secondary objectives are designed to expand understanding of cardiovascular structure and function and their association with ISD/HY therapy in ESRD by analysis of the following parameters:

- Change between baseline and 26 weeks in markers of fibrosis and circulating inhibitors of angiogenesis including procollagen type I carboxy terminal peptide (PICP), asymmetric dimethylarginine (ADMA), endostatin (END), angiopoietin II (ANG) and thrombospondin II (TSP)
- Change between baseline and 26 weeks in left ventricular mass index (LVMI). Change between baseline and 26 weeks in ejection fraction. Change between baseline and 26 weeks in myocardial strain and strain rate
- Change between baseline and 26 weeks in stress and resting myocardial blood flow
- Association between baseline CFR and E'
- Association between change in CFR and change in E'
- Association between baseline ADMA, ANG, END, TSP or PICP and baseline CFR or E'

3 Study Design

3.1 *General Design*

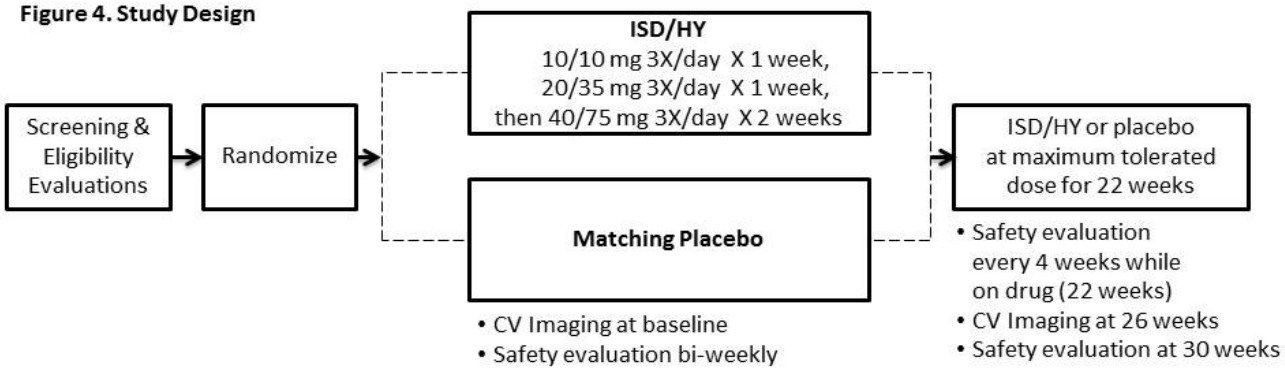
This is a randomized, placebo-controlled 2-arm trial that will compare placebo with ISD/HY combination therapy. Participants will be randomized in a 1:1 fashion to ISD/HY or placebo. As shown in Figure 4, the study will be conducted in 2 phases: a 4-week dose escalation phase and a 22-week treatment phase.

All randomized participants will initiate treatment with therapy three times daily. The active therapy group will receive a one-week supply of ISD/HY 10/10 followed by a one-week supply of ISD/HY 20/35 3x/day. The placebo control group will receive an identical supply of placebo and will follow the same

schedule as the active therapy group. At the 1 week and the 2 week visits, tolerability and hypotension will be assessed. If the starting dose and first dose titration is tolerated, participants will be increased to ISD/HY 40/75 mg 3x/day (active therapy) or placebo and they will receive an additional 2 weeks drug supply. Tolerability will be re-evaluated after an additional 2 weeks and at 4 week intervals thereafter. At 4 weeks, participants tolerating the full dose will receive a 12-week supply of study medication at the appropriate dose. At 16 weeks, an additional 10-week supply will be provided. Participants not tolerating the full dose at this point may reduce to the starting doses of active therapy at this visit. In the event of poor tolerance or drug related adverse events (e.g. hypotension, intra-dialytic cramping), the dose of study medication may be held or reduced to a minimum dose of ISD/HY 10/10 mg 3x/day (active therapy) or matched placebo 3x/day. Except where un-blinding is necessary for safety purposes, participants not tolerating the lowest dose of active therapy will be reduced to double placebo therapy in order to maintain unbiased assessments of study endpoints.

In order to maintain the blind, all study participants, regardless of randomized assignment, will receive new dosing kits within 1 day (with a window of up to 3 days) of the 1, 2, 4, and 16 week visits, and will be evaluated bi-weekly during the dose escalation phase.

Figure 4. Study Design



3.2 *Study Endpoints*

3.2.1 Safety Endpoints

The safety endpoints are:

1) Adverse events
2) Adverse events that preclude further treatment with the study agent
3) Serious hypotension defined as hypotension requiring hospitalization or treatment in an emergency room and not attributable to overt sepsis, acute myocardial infarction, or other cardiovascular event (e.g. aortic dissection
4) Recurrent intra-dialytic hypotension is defined as systolic blood pressure <80 mm Hg during ≥3 dialysis sessions per rolling 30-day period or treatment for either hypotension or symptoms of hypotension if systolic blood pressure is <100 mm Hg during ≥3 dialysis sessions per rolling 30-day period. Treatment includes saline infusion, lowering of the ultrafiltration rate or other

interventions directed at hypotension such as vasopressor agents. Symptoms include but are not limited to muscle cramping, lightheadedness, and nausea.

5) Inter-dialytic hypotension defined as systolic blood pressure <90 mm Hg or hypotension requiring adjustment in blood pressure medications or treatment in an emergency or hospitalized setting.
6) Mean pre-dialysis blood pressure
7) Mean lowest intra-dialytic blood pressure
8) Cardiovascular hospitalization
9) Stroke
10) Death

3.2.2 Tolerability Endpoints

Tolerability will be assessed on the basis of whether participants can continue the assigned dose throughout the entire treatment period. Failure of overall tolerability will be defined by discontinuation or reduction in dose of study medication. Tolerability events will be sub-classified according to whether discontinuation is due to gastrointestinal symptoms or headache.

3.2.3 Efficacy Endpoints

The primary efficacy parameters will be measured between baseline and 26 weeks:

1) Change in mitral annular E' velocity will be measured using Tissue Doppler Index (TDI) echocardiography of the left ventricle as a measure of diastolic function and as surrogate measure of left ventricular fibrosis.
2) Change in coronary flow reserve (CFR) will be measured using ^{13}NH3 myocardial PET scanning as a surrogate measure of microvascular structure and function. CFR will be calculated as the ratio of absolute myocardial blood flow at stress over rest for the entire left ventricle. In the case of flow defects the primary measure of CFR used will be the CFR over flow defect-free portion of the LV.

Preliminary estimates of the impact of ISD/HY on microvascular function can only be evaluated via PET scanning, and will provide a critical metric necessary for understanding the potential of ISD/HY to impact cardiovascular health. Results of PET scanning are thus expected, to provide direct insights into the underlying scientific hypotheses and potential for therapeutic efficacy that serve as a critical complement to the assessment of LV mass and function by echocardiography.

E’ has been chosen to be the primary echocardiographic endpoint because the intra-observer coefficient of variation is low[113] and multiple studies demonstrate a high correlation with myocardial fibrosis[114-116]--a primary biologic endpoint of interest. E’, for example, parallels the accumulation of myocardial collagen and fibrosis in the senescence prone mouse[117], and correlates with endomyocardial fibrosis after human cardiac transplant[118] as well as the presence of late gadolinium enhancement[119]—a highly-validated measure of myocardial scar—across multiple disease states. These and other data demonstrating the strong correlation of TDI with tissue fibrosis across different disease states and

multiple species, strongly establish TDI as a non-invasive marker of myocardial fibrosis well-suited for repeated measure studies. Furthermore, abnormalities in TDI have been strongly linked to mortality in multiple disease states[120-122] demonstrating that TDI is a clinically relevant measure. In addition, ESRD-specific studies show that E' is highly correlated with LV mass and other indices of diastolic function, and that changes in E' predict mortality[39, 123].

In contrast to other echocardiographic measures, E' (or the ratio of E/E') is minimally affected by intravascular volume status[124-126]—a critical consideration in ESRD where volume status can vary widely. The adjunctive information on diastolic and systolic function and filling pressures generated by echocardiography provide additional advantages to the use of TDI while the measurement of SR and strain may allow detection of subtle changes in myocardial function due to fibrotic processes[127]. Finally, these measures are known to respond to therapeutic interventions that exert anti-fibrotic effects[128]. Alternative approaches for assessing fibrosis include myocardial biopsy which is considered the gold standard, and cardiac magnetic resonance imaging with gadolinium. However, both of these have risks of serious complications and thus are not suitable for a research study.

CFR has been chosen as the co-primary efficacy endpoint because it is the best available tool for evaluation of microvascular supply and function in the study population. The correlation coefficient for CFR among 4 readers in the proposed study lab was 0.94 indicating excellent reproducibility. In the absence of critical coronary stenosis, hyperemic MBF is determined primarily by capillary density and diameter since capillaries cannot vasodilate in response to adenosine. CFR thus primarily measures microvascular supply, rather than coronary atherosclerosis[129-131], and it correlates well with human myocardial microvascular histology[132, 133]. It is strongly associated with mortality[134], and values are low in CKD and predict a poor prognosis[135]. PET CFR is thus non-invasive, reproducible, well-correlated with myocardial histology, and predictive of outcomes. The gold standard, myocardial biopsy, would be prohibitively risky in this population. Thus, CFR is the best non-invasive endpoint available for assessment of the cardiac microvasculature.

Secondary efficacy endpoints are:

1) Change between baseline and 26 weeks in markers of fibrosis and circulating inhibitors of angiogenesis including procollagen type I carboxy terminal peptide (PICP), asymmetric dimethylarginine (ADMA), endostatin (END), angiopoietin II (ANG) and thrombospondin II (TSP)
2) Change between baseline and 26 weeks in left ventricular mass index (LVMI). Change between baseline and 26 weeks in ejection fraction. Change between baseline and 26 weeks in myocardial strain and strain rate.
3) Change between baseline and 26 weeks in stress and resting myocardial blood flow
4) Association between baseline CFR and E'
5) Association between change in CFR and change in E'
6) Association between baseline ADMA, ANG, END, TSP or PICP and baseline CFR or E'

3.2.4 Feasibility Endpoints

An objective of this study is to assess the feasibility of conducting a large-scale trial powered for clinical outcomes. Feasibility will be assessed based on rates of recruitment, withdrawal, and loss-to-follow-up, reasons for ineligibility, and adherence to the study drug administration schedule.

4 Participant Selection and Withdrawal

4.1 *Inclusion Criteria*

a) Maintenance hemodialysis therapy for end-stage renal disease
b) Age 18-85 years
c) ≥90 days since dialysis initiation
d) Ability to provide informed consent
e) All pre-dialysis seated systolic blood pressure measurements must be ≥120 mm Hg in the 2 weeks before the screening visit and systolic bp must be ≥120 mm Hg on the day of randomization.

4.2 *Exclusion Criteria*

a) Serum potassium ≥6.5 mEq/L within 2 months prior to screening
b) Unscheduled dialysis for hyperkalemia within the 3 months prior to screening
c) Pre-dialysis hypotension defined as pre-dialysis SBP <100 mm Hg (seated measurement) within 4 weeks prior to the screening visit
d) Recurrent intra-dialytic hypotension defined as either of the following:
    - systolic blood pressure <80 mm Hg during ≥3 dialysis sessions in the 30 days prior to the screening visit, or
    - treatment for hypotension or symptoms of hypotension during ≥3 dialysis sessions in the 30 days prior to the screening visit. For this criterion, hypotension is defined as systolic blood pressure <100 mm Hg.
e) Mitral valve repair or replacement
f) Severe mitral valve disease by echocardiography, coronary angiography or cardiac magnetic resonance imaging
g) Anticipated kidney transplant, change to peritoneal dialysis, or transfer to another dialysis unit within 6 months
h) Expected survival <6 months
i) Allergy to study medications (ISD, HY, adenosine or dipyridamole)
j) Active use of sildenafil, vardenafil or tadalafil
k) Current severe aortic stenosis or other cause of LV outflow obstruction
l) Pregnancy, anticipated pregnancy, or breastfeeding, confirmed by serum pregnancy test on the day of PET scan
m) Incarceration
n) Participation in another intervention study
o) Use of monoamine oxidase inhibitors
p) Contraindication to adenosine including

- 2nd or 3rd degree heart block, sick sinus syndrome or symptomatic bradycardia (without a functioning pacemaker)
- moderate or severe asthma
- chronic obstructive pulmonary disease

q) Active use of any of the study medications unless participant and physician willing to discontinue prior to enrollment.

4.3 *Participant Recruitment and Screening*

Participants at dialysis units affiliated with investigator and co-investigator practices will be pre-screened for eligibility. In addition to active screening of dialysis unit rosters by study personnel, informational handouts and brochures may be disseminated at affiliated dialysis units in order to allow for interested participants to learn about the study and to contact the study investigator if interested. All study material must be approved by local IRBs before dissemination to potential study participants.

Dialysis unit labs, medical records at the investigator's institution, and treatment or history records at local dialysis units will be reviewed to assess eligibility for enrollment. No study-specific testing is required to confirm eligibility, except a serum pregnancy test (to assess eligibility on the day of PET scan) for women of childbearing potential, and serum potassium if a measurement is not available through clinical measurements within the specified time window. Prior to enrollment, each participant's treating nephrologist will be contacted to assess suitability for enrollment. If a participant is not eligible at the initial screening visit, he/she may be rescreened one additional time, after the appropriate interval has passed.

Once preliminary eligibility is confirmed, informed consent will be obtained by a qualified investigator or study site designee during an in-person visit. This visit may take place either at the local dialysis unit or at the investigator's institution, according to investigator and participant preferences. A randomization/baseline visit will be scheduled within 4 weeks of the screening visit.

4.4 *Early Withdrawal of Participants*

4.4.1 When and How to Withdraw Participants

Early withdrawals will be discouraged and participants who are not willing to continue the intervention will be encouraged to remain in the study and continue study evaluations. However, participants may be withdrawn from the study under the following circumstances that have the potential to compromise patient autonomy or safety:

a) Pregnancy
b) Withdrawal of consent
c) Allergy to or documented intolerance of study medications (e.g. recurrent hypotension on the lowest dose of study medications)
d) Non-compliance with dialysis schedule compromising ability to follow serum potassium on a monthly basis
e) Organ transplantation
f) Change to a different dialysis modality

g) Initiation of sildenafil, vardenafil or tadalafil therapy
h) Transfer to non-participating dialysis unit

4.4.2 Data Collection and Follow-up for Withdrawn Participants

In the case of withdrawal, every attempt will be made to obtain consent to continue to follow patients for the occurrence of mortality, hospitalizations, and other safety signals via telephone or in-person contact with participants, relatives, and dialysis unit staff and records. As a last resort, the social security death index will be queried for mortality events on individuals otherwise lost to follow-up.

Participants will be deemed as lost to follow-up after ≥ 3 attempts have been made to contact the patient via their preferred means of contact (telephone or email), ≥1 attempt has been made to contact the patient via their secondary means of contact, and ≥1 attempt to contact next of kin is unanswered.

For withdrawn participants not withdrawing consent, attempts will be made to obtain endpoint TDI echocardiograms and PET CFR according to the original study in order to preserve the intention to treat analysis. PET scans will not be obtained in the event of withdrawal/termination due to pregnancy.

5 Study Drug

5.1 *Description*

Study drugs will be administered as matched, gel-coated capsules containing ISD (10, 20 or 40 mg), HY (10, 35, or 75 mg), or placebo (methylcellulose powder).

5.2 *Treatment Regimen*

As depicted in Figure 4, 16 participants will be randomized in a 1:1 fashion to either placebo 3x daily or ISD/HY 10 mg/10 mg, 3x daily for 1 week. Doses scheduled to be administered within 2 hours prior to a dialysis session will be held and administered post-dialysis on dialysis days. Participants will be contacted by phone or in person to review tolerability and adverse events at 1 week after drug initiation and will be examined in person at 2 weeks. Dialysis records will be reviewed for hypotension at each visit. The sequence will be repeated following the scheduled dose titration which will occur following the 2-week visit. Adjustment to dosage of concomitant medications, the dialysis prescription, or the study drug on the basis of hypotension will occur as described in Section 6.7.

All randomized participants will receive an initial one-week supply of study medication following the baseline visit, and a one-week supply after the Week 1 visit. Those assigned to ISD/HY will begin treatment at 10/10 mg 3X daily. Participants randomized to placebo will follow the identical schedule. Participants will be contacted at 1 week to check for the occurrence of adverse events and dose-limiting symptoms, and those tolerating the study drug will have the dose increased to ISD/HY 20/35 mg 3X daily or placebo. At Week 2 participants will be examined in-person at their dialysis units. Adverse events will be reviewed and dialysis run sheets will be examined for the occurrence of inter- or intra-dialytic hypotension at each visit. In the absence of dose-limiting hypotension or other adverse events, the dose of ISD/HY will be increased to 40/75 mg 3X/daily following the Week 2 visit.

During the 4-week dose escalation phase, a study drug request form will be entered into the data management system at 1 week, 2 weeks and 4 weeks to request the next supply of study drug. Unless there is a contraindication to a dose increase (e.g. hypotension), the investigator will indicate approval for dose escalation on the request form. The Data Management System will generate a unique kit number based on the randomized treatment assignment and the prior dose. If up-titration to the next dose is contraindicated, the Clinical Center investigator will indicate this on the study drug request form and a kit number corresponding to the current dose of will be generated.

If down-titration of the study drug dose is required during the dose escalation phase or during the treatment phase, the Clinical Center investigator will request a dose reduction on the study drug request form. In this case, a supply of blinded study medication using the next lower dose of ISD/HY or placebo will be provided. In the event that a participant is already receiving the lowest dose, study medication will be discontinued. In the event that a 3rd down titration of dose is required study medications will also be discontinued. Un-blinding to preserve patient safety will be considered via a formal request to the DCC.

If the dose escalation schedule is interrupted (e.g., if a participant is hospitalized at the scheduled time of dose escalation and did not take study drug during the hospitalization), dose escalation will be delayed until the participant has had access to the current dose of study drug for the specified interval (1 week for the first dose level, 2 weeks for the second dose interval). If, due to a considerable delay in dose escalation, a participant has not reached the final dose assignment by their Week 8 visit, the participant will remain at the current dose of study drug for the remainder of the study and the treatment phase will be shortened accordingly such that the total duration of study participation is not increased as a result of the delay. For example, if dose escalation is not completed until week 10, the treatment phase will be 16 weeks rather than 22 weeks.

Participants will be contacted by phone or in person at Weeks 1 and 3 (plus or minus 3-5 days) as described above and will be examined in-person at Weeks 2 and 4. Dialysis records will be reviewed at each time point. Following the Week 4 visit a 12-week supply of the maximal tolerated dose of study medication will be distributed to participants who will be evaluated in-person at the dialysis unit every 4 weeks for the duration of the study. A 10-week supply of study medication will be distributed to participants following the Week 16 visit. At Week 26, participants will have a close-out visit, echocardiography and PET scan at the medical center and will then discontinue study medications.

In the event of dose-limiting symptoms such as headache, hypotension or gastrointestinal distress, dose increases will be deferred until symptoms have resolved and there has been adjustment to concomitant therapies, diet or dialysis prescription as described in Section 6.7, the dose of study medication or matched placebo may be reduced as applicable. During both the dose escalation phase and the treatment phase, the dose may be reduced to the next lower dose level in the event of adverse events as described in Section 6.7. In order to maintain the blind, all participants will receive blinded medication supplies according to the specified dose within 3 days of the Week 1, Week 2 and Week 4 visits, and within 1 week of the Week 16 week visit, regardless of whether the dose is changed.

Method for Assigning Participants to Treatment Groups

The randomization schedule will be stratified by left ventricular ejection fraction (LVEF) at ≤45% vs >45% to ensure balance across treatment arms of patients with preserved versus reduced LVEF. A clinical echocardiogram will be used for LVEF determination if available within 6 months. If no echocardiogram is available within the required timeframe then a study cardiologist will perform a limited reading on the study echocardiogram done during the baseline visit. There may be a delay of up to 72 hours between the baseline visit and randomization to allow for the completion of this limited reading.

Randomly permuted blocks of random sizes will be used to control the balance of participants assigned to each treatment regimen within each stratum. This method guarantees that at no time during randomization will the number of participants in any arm be grossly imbalanced, and ensures that the investigator will be unable to predict assignments. All randomization schedules will remain confidential, and known only to authorized members of the central pharmacy.

All relevant screening and eligibility confirmation data must be entered into the data management system in advance of randomization, in order for an eligible participant to be randomized. When the study team agrees that all eligibility criteria have been met, the patient will be randomized according to the pre-prepared, stratum specific randomization schedule. This randomization process will provide a randomized treatment assignment for that participant within the relevant stratum, linked with a specific drug kit/box prepared at the study pharmacy. The kit will then be provided to the investigative team for distribution at the randomization/baseline visit.

5.3 *Preparation and Administration of Study Drug and Maintenance of Blind*

Study drug and matching controls will be prepared by the Investigational Drug Service (IDS). The IDS will distribute study drug kits, containing a 1-week supply at baseline and Week 1, a 2-week supply at Week 2, a 12-week supply at Week 4, and a 10-week supply at Week 16. Drug supplies will be packaged to contain a 3-day or 5-day supply of extra medication depending on the visit interval.

All doses of ISD, HY, and placebo will be manufactured to have identical appearances using gelatin encapsulation or equivalent methods. IDS personnel responsible for preparation of study drug will not have access to study data or results and will not participate in analyses of study data or publications. However, appropriate IDS personnel will have access to the randomization scheme and code in order to prepare study drug kits containing the correct dose of active medication or placebo for each group and study participant.

During the dose titration phase, IDS personnel will prepare kits containing a 10-day supply of the specified dose of ISD/HY or matched placebo for each group at baseline and Week 1. These packages will be replaced with kits containing a 2-week + 5 day supply of the next dose of study drug or placebo following the Week 2 visit, a 12-week + 5 day supply following the Week 4 visit, and a 10-week + 5 day supply following the Week 16 visit. If up-titration to the next dose is not indicated (e.g. due to hypotension or gastrointestinal symptoms), the investigator will notify the IDS, and a package containing

the last dose of ISD/HY or placebo will be substituted. If at any time, the participant experiences dose-limiting symptoms requiring down-titration of study drug, the IDS will be notified to prepare a new supply of study drug at the next lowest dose for the appropriate number of days remaining until the next scheduled drug distribution. The participants will be instructed to discontinue study medication until they receive the new package.

The investigator will notify the IDS when down-titration of dose is required for a particular participant. In this case, the IDS will prepare a supply of blinded study medication using the next lower dose. In the event that a participant is already receiving the lowest dose, placebo will be substituted. As only 3 dose-level of medications are specified, study medications will be discontinued in the event that a down titration below the lowest dose of study medications is required. Un-blinding to preserve patient safety will be allowed after formal documentation of the need by the investigator and a formal request to the IDS.

5.4 *Participant Adherence Monitoring*

Empty pill containers will be returned to site investigator at the conclusion of each 2-week period during the dose escalation phase, and at 16 weeks and the end of the study during the treatment phase. Pill counts will be performed to assess participant adherence with prescribed study medications.

5.5 *Prior and Concomitant Therapy*

Baseline medication use (both oral and intravenous) and history of prior coronary revascularization procedures will be collected at baseline. A participant's standard of care medication may be changed prior to study participation if considered clinically appropriate. No changes in drug dose or medications will be made without the approval of the prescribing physician. Concomitant medical therapies and coronary revascularizations will be collected throughout the course of the study. Use of sildenafil, vardenfil or tadalafil is listed as an exclusion criterion. Participants requiring the initiation of therapy with these agents during the study will have study medications withdrawn, but will continue to be followed for adverse events and efficacy assessments. No other concomitant therapies are prohibited.

5.6 *Packaging*

Study drug will be distributed in participant-specific containers. Labeling will include patient ID, protocol number, study-center, expiration date, instructions, and "prescribing" physician (study investigator).

5.7 *Blinding of Study Drug*

Generic ISD, HY will be purchased, and matching placebos will be prepared by the IDS. The identity of matched placebos will be concealed from both investigator and participants. Only the IDS will remain unblinded. See Section 8.4 for further information on un-blinding procedures, if needed.

5.8 *Receiving, Storage, Dispensing and Return*

5.8.1 Receipt of Drug Supplies

The IDS pharmacist will distribute study agent to the participant during their study visits. Alternatively, investigator may deliver drug supply to the patients during the study visits.

General Study Product Accountability, Patient Specific Study Product Accountability, and if necessary, Shipment Tracking Accountability Logs will be created by the IDS staff and study product use documented on respective logs. Documentation includes study product receipt, storage, dispensing, and final disposition. Study product must be inventoried at least once per month within 30 days of the count for the previous month.

5.8.2 Storage

ISD, HY, and matched placebo should be stored at <25ºC. Study supplies will be stored in the IDS pharmacy until the date of distribution to study participants.

5.8.3 Dispensing of Study Drug

The pharmacist or trained site designee will dispense study drug to the patient and complete a dispensing/accountability log for each participant. Documentation of training will be held in the site's regulatory binder.

5.8.4 Return or Destruction of Study Drug

Medication containers will be returned by patients at the end of each 2-week or 90-day period and remaining pills counted to assess adherence. Unused supplies of study drug will be destroyed by study staff once the pill count is completed and documented.

6 Study Procedures

Study procedures and testing are provided in the visit schedule in **Section 15**. Medical history (hypertension, coronary artery disease, coronary revascularization, diabetes, hypercholesterolemia, cerebral or peripheral vascular disease, peptic ulcer disease, cancer, smoking, arrhythmia, cause of ESRD, dialysis vintage, prior transplant), baseline medications, dialysis unit labs (chemistries, PTH, complete blood count), vital signs, and dialysis prescription will be collected at baseline.

6.1 *Pre-screening Visit*

Dialysis unit records and study center medical records will be reviewed to assess eligibility. The treating nephrologist for potentially eligible patients will be contacted to further assess eligibility and obtain permission to contact the patient. Patients remaining eligible after this assessment will be approached in person at the dialysis unit to confirm eligibility for and interest in participation. Participants signing IRB-approved informational flyers which expressly provide consent to be called, may be screened by telephone. Participants will be provided with a copy of study brochures and the consent form to review at their leisure, and they will be provided with study staff contact information so that they can reach the investigator or a designee with additional questions about the study.

6.2 *Screening Visit*

Eligible participants expressing interest in participation will be scheduled for a screening visit. This visit may take place at the local dialysis unit or at the study center. Informed consent will be obtained and documented, eligibility criteria will be confirmed, and the baseline visit will be scheduled during this

visit. Demographic data (age, sex, race), vital signs, edema assessment, medication use, dialysis labs, and medical history will be obtained following informed consent.

6.3 *Baseline Visit/Randomization Visit*

The baseline visit will be scheduled within 4 weeks of the screening visit. A physical examination will be conducted. Data on medication use, baseline medical conditions, vital signs and edema assessment will be collected. Dialysis prescription, dialysis labs, medications, and vital signs will be updated within 3 days prior to the baseline visit. Serum and plasma will be collected, aliquoted and frozen at -80°C. Additional blood will be drawn for testing of baseline chemistries and blood counts as well as local lab serum pregnancy testing (in pre-menopausal women). Baseline TDI echo and PET scans will be performed. The echo and PET scans are not required to occur on the same day, however, they both should be completed prior to randomization and drug distribution. If LVEF stratification needs to be determined from the baseline echo, then there may be up to a 72-hour delay between the baseline visit and randomization in order to allow for a limited reading of the study TDI echo. A clinical echocardiogram will be used for LVEF determination if available within 6 months of the baseline visit date. Once all the baseline activities have been completed, the patient will be randomized and a 1-week + 3 day supply of study medication will be dispensed. The baseline/randomization visit can be scheduled over two dates within a 10-day window.

6.4 *Week 1, 2, 3, 4, 8, 12, 16, 20, 24 Visits*

Participants will be contacted by telephone or in-person at the dialysis unit 1 week after the start of study medications for the occurrence of adverse events (hypotension, headache, gastrointestinal symptoms, and hospitalizations). Those participants tolerating study medication will have the dose increased to ISD/HY 20/35 mg or placebo for the next week. Dialysis treatment records will be reviewed for the occurrence of hypotension and standard of care dialysis labs will be reviewed at each visit. The dose of study drug may be reduced if a participant experiences dose-limiting adverse events, as outlined below.

Participants will be interviewed in-person at the dialysis unit at Week 2, Week 4 and Week 16 for the occurrence of adverse events (intra-dialytic hypotension, headache, gastrointestinal symptoms, and hospitalizations). Dialysis treatment records will be reviewed for the occurrence of hypotension and standard of care dialysis labs will be reviewed. Vital signs and edema will be assessed. In the absence of dose-limiting symptoms, the dose of study medications will be increased following the Week 2 visit. The dose of study drug may be reduced if a participant experiences dose-limiting adverse events, as outlined below. Following each visit, the appropriate dose of study drug will be delivered to study participants. At that time, pill containers will be returned and unused pills counted and destroyed. Participants will be contacted at Week 3 to collect adverse event information.

Following the Week 4 visit, participants will be contacted in-person or by telephone every 4 weeks at their dialysis facilities. Adverse events and tolerability will be reviewed. Dialysis treatment records will be analyzed for the occurrence of hypotension and standard of care dialysis labs will be reviewed. Pill containers will be returned and unused pills counted and destroyed after the Week 16 visit.

The IDS will be notified to proceed with refill of study medication for patients continuing in the study following the Week 1, 2, 4, and 16 visits.

Visit windows will be ± 3 days for the week 1, 2 and 4 visits and ± 7 days for week 8-30 visits.

6.5 *Week 26 Visit*

At the Week 26 (end of treatment) visit, participants will be interviewed in person to review the occurrence of adverse events. Dialysis unit records will be reviewed as above. Pill containers will be returned and unused pills counted. Vital signs and edema will be assessed. Blood will be drawn for long-term storage, for central lab testing of chemistries and blood counts, and for local lab serum pregnancy tests (in pre-menopausal women). End of study TDI echo will be performed as will PET scans. If necessary, this visit can be split into 2 visits to promote scheduling of end of study testing.

6.6 *Week 30 Visit*

At Week 30, participants will be called or visited at the dialysis unit to determine whether participants are having any adverse effects from the study medications. Dialysis treatment records will be reviewed for the occurrence of hypotension and dialysis unit records will be reviewed.

6.7 *Hypotension Procedures*

In the event of recurrent intra-dialytic hypotension or serious hypotension not attributable to acute events (e.g. acute myocardial infarction, sepsis), the investigator will review the participant's non-study anti-hypertensive medications, dry weight, and volume status. The participant's clinician will be contacted to consider the following interventions listed in the preferred order of implementation:

1) Decrease non-study anti-hypertensive medication(s)
2) Increase dry weight if there is no evidence of peripheral or pulmonary edema
3) Reduce ultrafiltration rate by increasing dialysis session duration
4) Reduce dose of study medication if:
    a. Peripheral or pulmonary edema is present
        AND
    b. There are no other anti-hypertensive medications prescribed OR other anti-hypertensive medications should not be discontinued (e.g., beta blocker following myocardial infarction)
5) Discontinue study medication when reduction below minimal dose is required

Treating clinicians will be free to manage hypotension according to standard clinical practices and are not obligated to follow the study guidelines with respect to non-study medication, fluid removal rate, or dry weight. In contrast, dose reduction or discontinuation of study drug will be directly managed by study staff in accordance with the guideline. All enrolled participants will continue to be followed for the occurrence of adverse events and for end of study cardiac testing regardless of whether study medications are discontinued.

6.8 *Headache or Gastrointestinal Symptoms*

In the event of persistent headache, nausea, vomiting, diarrhea, or anorexia without alternative cause, the dose of study medication may be reduced.

6.9 *Imaging Studies*

6.9.1 Echocardiography

The primary echocardiographic endpoint, the early diastolic mitral annular velocity (E'), will be acquired using the apical 4-chamber view at both the septal and lateral positions at the junction of the LV wall with mitral annulus with the measurements averaged. TDI images will be acquired using steerable pulse wave TDI according to standard techniques (typically >100 frames/s with a 2 mm sampling volume). Other standard echocardiographic measurements such as early (E) and late (A) mitral inflow velocity, LV mass index and LV ejection fraction will be obtained during each study. Blinded analyses will be performed off-line using commercial software. In addition, 3 cardiac cycles of 2D TDI data will be obtained at high frequency in the 3 standard apical views for offline measurement of strain rate (SR) and strain using strain quantification software. Mean strain rate and peak systolic strain will be calculated by averaging across the basal segments of each wall.

6.9.2 PET Scan

Individuals will refrain from smoking, exercise, caffeine-containing beverages, or methyl-xanthines for 24 hours before PET studies. Vasoactive medications such as calcium channel blockers, beta-blockers or nitrates will also be withheld for 24 hours. All participants will have PET scans performed while in a semi-fasting state. PET scans will be performed according to the standard rest and stress scanning protocol at Brigham & Women's Hospital. In brief, a transmission scan will be acquired for correction of photon attenuation, and using ^{13}N ammonia (~0.286 mCi/kg), myocardial blood flow (MBF) will be measured at rest. This will be followed by a regadenoson infusion, adenosine infusion at a dose of 0.14/mg/kg/min (61) or a dipyridamole (0.56 mg/kg administered over 4 min) infusion, to induce hyperemia. At peak hyperemia, a second dose of ^{13}N ammonia will be injected and imaging repeated. Changes in systemic hemodynamics will be measured between baseline and hyperemia. Visual interpretation of the gated myocardial perfusion images will be performed semi-quantitatively using a standard 5-point scoring system and 17 myocardial segments. Summed rest (SRS) and stress scores (SSS) will be calculated as the sum of individual segmental scores, with the difference recorded as a summed difference score (SDS).

Absolute MBF (in ml/g/min) will be computed blindly from the rest and stress images using commercial software and previously validated methods. Serially acquired transaxial images will be re-oriented into short axis slices of the LV and assembled into serial polar maps, and blood pool (arterial input function) and tissue time-activity curves will be generated. Regional and global rest and stress MBF will be calculated by fitting the ^{13}N time-activity curves to a two-compartment tracer kinetic model. CFR will be calculated as the ratio of absolute MBF at stress over rest for the entire left ventricle. In the case of flow defects the primary measure of CFR used will be the CFR over flow defect-free portion of the LV.

7 Statistical Plan

7.1 *Sample Size Determination*

Sample size considerations were framed using standard study design parameters to ensure 80% power to detect pre-specified effect sizes utilizing intermediate outcomes. However, for this early phase pilot study, the primary focus is directed at providing pilot efficacy, safety and feasibility information, with no attempt to create critical test result regions for standard hypothesis testing. Results will nevertheless provide preliminary estimates needed to guide design of subsequent, more robust studies and will provide information on safety and tolerability events occurring at high event rates which can be incorporated into the design of further studies.

With 8 participants per group, there will be 80% power to detect a change in CFR of 0.75 (unitless measure), assuming a common standard deviation for change in CFR of 0.5[138-143]. Assuming the common SD of E' is 2.0 cm/s and baseline is 5.8 ± 1.8 cm/s[39, 144-146], we will be able to detect a difference of 3.0 cm/s with 80% power. Changes of this magnitude have been observed in other settings[144, 147, 148]. Baseline variables will be presented using standard descriptive statistics according to distribution. $P<0.05$ will be considered significant without correction for multiple comparisons given the pilot nature of this proposal.

Although the detectable change in each effect measure is large, detectable differences are, as noted above, clinically relevant. Further, given the pilot nature of this study, additional trials will be required regardless of whether a significant difference in the efficacy measures is detected. Point estimates and standard deviations around these measures will provide the needed estimates of expected change so that more robust studies can be designed.

It is similarly noted that under the proposed sample size, estimates of safety and tolerability event rates will provide relatively crude estimates of the true event rates. Confidence intervals around estimated event rates based on various observed event rates within the treatment (or control arm) will be large and are provided below for a range of detected event rates (Table 1). As shown in Table 2, power to detect event rates ≥ 20% will be good while power to detect lower event rates within each study arm will be limited for events with a true rate <15%.

Table 1. Confidence interval for estimated event rates within each study arm

Observed Events	Observed Event Rate	Low Bound 95% CI	Upper Bound 95% CI
0	0.00	0	0.37
1	0.125	0.00	0.53
2	0.25	0.32	0.65
5	0.625	0.24	0.91
8	1.00	0.63	--

Table 2. Detectable event rates

True Event Rate	Power (Detection Probability)
0.05	0.34
0.10	0.57
0.15	0.73
0.20	0.83
0.25	0.90
0.30	0.94

7.2 *Randomization and Stratification*

To ensure balance in treatment assignments within potential confounders, a stratified randomization procedure, based on LVEF <=45% will be implemented. Within each of these strata, participants will be randomly allocated to the ISD/HY or placebo in a ratio of 1:1.

In order to maintain blinding, ISD, HY, and placebo preparations for each arm will have an identical appearance, and the same number of tablets will be prescribed for each participant. The treatment assignment code, corresponding to each treatment identifier number, will be known only to the IDS, until the completion of treatment and data collection on all participants. This information may also be known to the dispensing pharmacists. The study participants, and all other members of the investigative team, will remain blinded to the treatment assignment, including the investigator, the biostatisticians, the study nurses, and referring physicians.

7.3 *Intent-to-treat Analysis and Missing Data*

An intent-to-treat analysis, in which all available data on all randomized participants are included, will be used for the primary comparison of treatments. All attempts will be made to keep missing data to a minimum, and participants who withdraw from treatment will be encouraged to continue on study in order to provide complete follow-up information. Thus, irrespective of withdrawal from treatment, all participants should continue to be followed with all scheduled outcome evaluations until the end of the study. However, it is expected that up to 10% of the randomized participants may withdraw prior to the final assessment of response at 26 weeks. These participants will be included in the denominator for evaluation of the response rates defined for the primary endpoint.

The characteristics at the time of randomization for those participants without complete follow-up will be examined; however, there will be limited statistical power to detect any but major differences between these participants and those with complete follow-up. In addition, in order to assess the potential biases introduced by differential withdrawal among treatment arms, a comparison of withdrawal rates and/or time to withdrawal will be included as an ancillary analysis to the primary endpoint comparison.

Secondary analyses will examine the all-treated, as-treated, and per-protocol populations as defined below. Given the pilot nature of this study and small sample size, no corrections will be made for

multiplicity in these additional analyses. The possibility of false-positive results is acknowledged and results of these analyses will be considered strictly exploratory.

Nevertheless, these secondary analyses may provide important, hypothesis-forming insights into mechanism of action and optimal design for further studies in the event that significant deviations from the protocol or dropout from the study are felt to have had an important impact on the results.

The analysis populations are defined as follows:

- All-randomized / intention-to-treat (ITT) population: Any participant randomized into the study, regardless of whether study drug was received.
- As-treated population: The as-treated population is the same as the ITT population (i.e., any participant randomized into the study regardless of whether study drug was received). However, for the as-treated analysis, patients in the active drug group who did not receive at least one dose of study drug will be classified into the placebo arm.
- All-treated population: Any participant randomized into the study who received at least one dose of study drug.
- Per-protocol population: Any participant who was appropriately randomized, and received the protocol-dictated study drug exposure (≥75% of prescribed doses) and endpoint assessments through 24 ± 2 weeks.

Because dose-related efficacy and safety are important questions of interest in this study, the ITT analysis will be supplemented with an analysis of the as-treated population. Although ITT approaches provide the least-biased analysis of treatment efficacy and safety, as-treated analyses provide important complementary information on biological effectiveness of therapy (e.g. theoretical efficacy if drug were tolerated by all participants) and on the effects of actual doses used that is not captured by (ITT) analyses in which the unit of analysis is a randomized therapy that may not have been used by individual participants[149]. For this reason, as-treated approaches provide important complementary information to ITT analyses and are typically mandated as an important secondary analyses of clinical trials by the FDA[149]. Given a treatment protocol that allows for dose reduction of study medications, the as-treated analysis will provide critical information in this trial for assessing dose-related efficacy and the incidence of adverse events such as hypotension. As-treated analyses will assign treatment dose according to the dose used at the time of an adverse event (safety analyses) or according to the mean dose received during the trial (efficacy analysis).

In general, missing data will not be imputed. Every effort will be made to use statistical methods that are robust to missingness, and the number of participants included with each analysis will be given with the results.

7.4 *Statistical Methods*

In addition to the analyses described subsequently, descriptive statistics will be used during the course of the project as part of data management procedures for monitoring data quality. A brief overview of some of the statistical methods that may be used at the time of analysis, both for descriptive purposes and in more comprehensive analysis of the primary research questions, is summarized in the following

sections. It is recognized that these methods may be revised and additional ones considered as the details of the specific analyses are developed.

Given the pilot nature of this study, the primary objectives of this study are to derive pilot estimates of efficacy and safety. As noted above, given the small sample size, the power to detect small to medium effect sizes or adverse event rates will be limited. However, the point estimates and confidence intervals around effect sizes and adverse event rates are expected to provide information needed for the planning of future studies.

7.4.1 Descriptive Analysis

Standard descriptive statistics will be used to summarize baseline characteristics and study outcome measures at each follow-up visit, both overall and within each treatment group. Examination of baseline characteristics will include estimates of the distribution of age, race, and other demographic characteristics, lab measures and study center. Summary statistics such as means, medians, and ranges will be produced for all measured variables. Frequencies will be computed for all categorical and ordinal variables. Graphical methods including stem-and-leaf diagrams and boxplots will be used to examine distributions, identify potential influential points, and guide in the choice of transformations, if warranted. The balance of baseline measures across the treatment groups will be compared using appropriate k-sample tests, including X-Squared tests and Fisher's exact tests.

Analysis of Primary Efficacy and Safety Outcomes Mean ± standard deviation or median (interquartile values) of baseline and end of study measurement in the primary endpoint E' will be presented. Change in E' velocity or CFR at 26 weeks will be assessed and reported quantitatively, and descriptive statistics for absolute and % change will be provided. Mixed effects linear regression models with baseline covariate adjustments will be used for assessment of treatment effects. Model assumptions regarding homoscedasticity and normality will be examined using standard techniques.

For the safety endpoints, tables with percent incidence and incidence rate (for events that can occur more than once during follow-up) will be prepared. Differences in incidence and incidence-rate will be assessed using logistic regression or Poisson regression with factors for assigned treatment and the stratification variable. In particular, for safety endpoints, we will investigate the potential shift in blood pressure in control and treatment groups with monthly measure of blood pressure. We will use mixed effects linear regression models to assess the direction and time averaged magnitude of change in potassium, with and without controlling for baseline covariates. The proportion of serious hypotension in treatment and control groups will be compared with Chi-square test or Fisher's exact test.

The primary analysis will examine the intention to treat population. Exploratory analyses will be repeated in the as-treated, all-treated and per-protocol populations (see **Section 7.3**) using analogous techniques. $P<0.05$ will be considered significant in all analyses.

7.4.2 Secondary Analyses

A number of secondary analyses will be conducted to evaluate the secondary efficacy outcomes. Secondary efficacy parameters include angiogenesis inhibitor concentration, circulating markers of

fibrosis, additional echocardiographic parameters, and additional PET parameters including change in resting and hyperemic myocardial blood flows between baseline and 26 weeks and change in LVMI between 0 and 26 weeks. Analysis for these secondary outcomes will be similar to that for the primary outcome. Distribution of the secondary parameters will be examined and appropriate transformation will be applied. Because beta blockers may have effects on cardiac function, analyses will be performed to explore differences in efficacy outcomes between participants with and without beta blocker use at baseline.

7.4.3 Interim Analysis

No interim analysis is planned.

8 Safety and Adverse Events

8.1 *Definitions*

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Participants or Others and Adverse Events, Office on Human Research Protection (OHRP) Guidance. http://www.hhs.gov/ohrp/policy/advevntguid.html

8.1.1 Adverse Event

An *adverse event (AE)* is any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (for example, abnormal physical examination or laboratory finding), symptom, or disease, temporally associated with the participant's involvement in the research, whether or not considered related to the participant's participation in the research.

8.1.2 Serious Adverse Event

A *serious adverse event* (SAE*)* is any AE that is:

- fatal or results in death
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- results in congenital anomalies or birth defects
- an important medical event*

*Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance.

8.1.3 Unanticipated Problem Involving Risk to Participants or Others

An Unanticipated Problem is any incident, experience, or outcome that meets **all** of the following criteria:

- it is unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the IRB-approved research protocol and informed consent document and the characteristics of the participant population being studied;

- it is related or possibly related to participation in the research; possibly related means that there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research, and
- it suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

8.1.4 Pre-Existing Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

8.2 *Adverse Event Reporting Period*

The study period during which adverse events must be tracked and reported is defined as the period from the initiation of study procedures to study completion.

8.2.1 Post-study Adverse Events

All unresolved adverse events will be followed by the investigator until the events are resolved, the participant is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each participant to report any subsequent event(s) that the participant, or the participant's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the Data Coordinating Center (DCC) of any death or adverse event occurring at any time after a participant has discontinued or terminated study participation that may reasonably be related to the study.

8.3 *Recording of Adverse Events*

At each contact with the participant, the investigator or site designee will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on adverse events will be recorded in the source document, and also on the adverse event log case report form (CRF). All signs, symptoms, and abnormal diagnostic procedure results relating to the same event will be recorded under one diagnosis name.

8.3.1 Anticipated Adverse Events

The following adverse events are anticipated in the hemodialysis population and are not considered Unanticipated Problems. Note that the designation as "Anticipated" does not imply that the event is not an SAE but relates to the regulatory definition of Unanticipated Problems as provided in **Section 8.1.3**.

- Death
- Coronary Ischemia including:
    - Unstable angina
    - Acute MI
    - Coronary revascularization
- Heart failure hospitalization or exacerbation

- Cardiac arrest
- Cardiac arrhythmia (ventricular or atrial)
- Peripheral vascular revascularization
- Amputation
- Hypotension
- Vomiting
- Vascular Access Events Including:
    - Catheter exchange, removal or declotting
    - Arteriovenous graft or fistula complications
        - Clotting
        - Stenosis
        - Revascularization
        - Infection
- Infections Including:
    - Pneumonia
    - Bacteremia
    - Hemodialysis vascular access infection

The following adverse events are anticipated in patients treated with ISD, HY:

- Hypotension
- Headache
- Gastrointestinal symptoms including diarrhea, constipation and nausea
- Rash
- Edema
- Flushing
- Nasal congestion
- Light-headedness

8.3.2 Non-Reportable Events

The hemodialysis population is characterized by frequent laboratory testing and a high rate of peri-dialytic hypotensive events requiring change in the dialysis prescription, adjustment of dry weight or change in dialysis-related medications. Due to the unique nature of this population, the following events are considered routine aspects of chronic dialysis therapy and they will not be considered to meet the criteria of SAE in this study except as noted:

- Anemia—will be reported only when hemoglobin <8.0 mg/dL
- Hyperphosphatemia—will be reported only when phosphate >9.5 mg/dL
- Hypocalcemia—will be reported only when serum calcium <7.0 mg/dL
- Hypercalcemia—will be reported only when serum calcium >11.0 mg/dL
- Hyperparathyroidism—will be reported only when PTH>1000 pg/mL
- Hypotension—will be reported only when requiring emergency room visit or hospitalization

- Hyperkalemia—will be reported only when >7.0 mEq/L
- Hypokalemia—will be reported only when <3.0 mEq/L

8.4 *Reporting of Serious Adverse Events and Unanticipated Problems*

Study sites are required to report SAEs to the DCC within 24 hours of first knowledge of the event. To report such events, an SAE form will be completed by the investigator and faxed or emailed to the DCC. The DCC will facilitate the timely medical review and reporting of the event, and provide reports to the NIDDK and the Data and Safety Monitoring Board (DSMB) in accordance with DSMB-approved study policies and regulatory requirements (see **Section 8.5.1** for details of the DSMB).

The investigator will keep a copy of the SAE form on file at the study site. At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Participant number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study participation

Within the following 7 days, the investigator will provide further information on the SAE or the unanticipated problem in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic information that will assist in the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the DCC.

If a participant becomes pregnant while participating in the study it will be reported as an adverse event and will trigger the collection of additional documentation about the pregnancy. Pregnancy outcomes will be collected, including the outcome of the infant and if the pregnancy was terminated. This information will be submitted to the University of Pennsylvania IRB, and to the local site IRB as required.

SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.4.1 Investigator Reporting to the IRB

The site investigator will report SAEs and Unanticipated problems to the clinical center IRB in accordance with the reporting requirements of the local IRB or with the Office of Human Research Protections (OHRP) guidelines, whichever is sooner. OHRP recommends that:

1) Unanticipated problems that are serious adverse events should be reported to the IRB within 1 week of the investigator becoming aware of the event; and

2) Any other unanticipated problem should be reported to the IRB within 2 weeks of the investigator becoming aware of the problem.

Reporting Process

Unanticipated problems posing risks to participants or others as noted above will be reported using the appropriate IRB-designated form or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be maintained in the Clinical Center Investigator's study file.

Other Reportable Events:

- Any adverse event that would cause the study's Steering Committee to modify the protocol or informed consent form, or would prompt other action by the IRB to assure protection of human participants.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency.
- Breach of confidentiality
- Change to the protocol made without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under 45 CFR part 46 subpart C and the investigator believes it is in the best interest of the participant to remain in the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of participants.

8.4.2 DCC Notification to Study Investigator

The DCC will notify the study principal investigator, in a written safety report, of any adverse event that meets the criteria of an unanticipated and related event as described in **Section 8.1.3**.

8.5 *Medical Monitoring*

The Clinical Center Principal Investigator will be responsible for overseeing the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Independent Data and Safety Monitoring Board (DSMB)

The information provided in this section of the protocol is a general description of the DSMB responsibilities and processes. A DSMB charter for the Hemodialysis Novel Therapies Consortium includes additional detail.

A DSMB has been established by the NIDDK and provides input to the Institute. The DSMB is comprised of individuals with expertise in clinical trials design and methodology, biostatistics, clinical nephrology and other relevant medical specialties. The DSMB members are not affiliated with the study and are appointed by the NIDDK. DSMB members will be free of conflicts of interest that could be affected by the outcomes of the study. During the study, DSMB members who develop real or perceived conflicts of interest that impact objectivity will disclose them to NIDDK project officers, who will arrange for replacement of the member, if indicated.

The DSMB will review the protocol before initiation of the study. After initial approval during the course of the study, the primary responsibilities of the DSMB will be to:

1) Review safety data and provide input to protect the safety of the study participants;
2) Provide input on major changes to the research protocol and plans for data and safety monitoring;
3) Provide input on the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the study site, and other factors that may affect study outcomes;
4) Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the need for continuation of the study, safety of the participants or the ethics of the study;
5) Provide input on modification of the study protocol or possible early termination of the study because of attainment of study objectives, safety concerns, or inadequate performance (such as enrollment and retention problems).

9 Data Management

An internet based data management system will be used to collect, store and manage study data. Site personnel will enter data directly into the database using electronic case report forms (CRFs) developed by the PI, statistician, and study investigator. Data entry screens will incorporate range and logical edit checks, within and across forms. Data entry will be followed with manual and programmed checks and edits for errors and omissions. A data monitoring plan will serve as a reference guide for the development of case report forms (paper and electronic versions), data management conventions, reporting, data dictionaries, supporting meta data, as well as project closeout activities, communication and coordination plans among the study teams and staff.

9.1.1 Quality Control Activities

Manual of Procedures (MOP) - The MOP will describe the sequence of study conduct and provide detailed instruction for the performance of screening, baseline, enrollment, treatment allocation and follow-up procedures. The MOP will provide instruction in case report form completion, use of the

electronic data management system, and collection, documentation and transfer of specimens and tests to laboratories and reading centers.

Internal quality control procedures - A data validation plan, rule set specifications, and programming logic to implement data validation rules will be implemented.

9.1.2 Routine reports

The study team will develop a set of standard enrollment, tracking, quality review, and safety monitoring reports. Adverse event reports, DSMB reports and reports for statistical analysis will be developed and produced on an appropriate schedule.

9.2 *Data Security*

The data management system will be designed to prevent unauthorized access to trial data and to prevent data loss due to equipment failure or catastrophic events. The procedures to do so encompass user account management, user privilege assignment, data loss prevention (database backup), and DMS change management. User access will be controlled by assignment of confidential usernames, passwords.

Study data collected at the clinical sites will be entered into a web-based electronic data capture system. This data management system uses a secure connection between the client browser at the clinical site and the web server at the DCC. Data transmitted over this connection is authenticated by the use of digital certificates and is encrypted as it travels the Internet.

The statistical project team will collaborate with the Investigational Drug Service (IDS) to protect the blinding of treatment assignments and electronic access to information that could indirectly or directly lead to unblinding treatment assignment or codes. Internal access to such information is stored in password-protected files.

9.2.1 Confidentiality

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

a. What protected health information (PHI) will be collected from participants in this study
b. Who will have access to that information and why
c. Who will use or disclose that information
d. The rights of a research participant to revoke their authorization for use of their PHI.

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

9.3 *Source Documents*

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

9.3.1 Maintaining Anonymity of Submitted Medical Records

Clinical site personnel will de-identify all medical records before sending them to the data coordinating center.

9.3.2 Data Sharing

It is required that research results generated under sponsorship by NIH are made available to the scientific community and public in a timely manner. The primary method by which data are shared with the scientific community is through peer-reviewed publications and presentation at meetings. In addition, data and results created from NIH supported research will be submitted to NIH in the annual progress reports required under the terms and conditions of this award. This study will also be registered with clinicaltrials.gov.

Data from this study will be submitted to the NIDDK Data Repository in accordance with the NIDDK Data Sharing policy. The policy requires that data sets be transferred no later than 2 years after study completion or 1 year after publication of the primary results, whichever comes first. Through the repository, the study data will be made available to external investigator.

9.3.3 Records Retention

The site investigator will retain study documents, including participant files and Investigational Binders, for at least 5 years after the close of the study, or longer depending on site institutional requirements.

10 Study Monitoring, Auditing, and Inspecting

10.1 *Study Monitoring Plan*

Given the small size and single center nature of the study, there will be no external monitoring. However, the principal investigator will allocate adequate time for monitoring activities, study binders, and case report forms with study personnel.

10.2 *Auditing and Inspecting*

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the NIH, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study

data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All participants for this study will be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a participant, using the EC/IRB-approved consent form, must be obtained before that participant undergoes any study procedure. The consent form must be signed by the participant or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 *Funding Source*

This study is financed through a grant from the National Institute of Diabetes and Digestive and Kidney Diseases of the U.S. National Institutes of Health.

12.2 *Conflict of Interest*

The study investigator will follow the conflict of interest policies of the National Institutes of Health as well as their home institution. Any investigator who has a potential conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study

12.3 *Participant Stipend*

Participants will be compensated $400 plus travel expenses for time and effort required to participate in the study. Stipends will be distributed in equal payments after the completion of the first and second PET scans.

13 Publication Plan

Study results will be published in peer reviewed manuscript and presented in preliminary form at annual meetings of national societies or research meetings. Every effort will be made to publish results within a year of final receipt of locked study data sets.

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15 Visit & Procedure Schedule

Visit #		1	2	3	4	5	6	7	8	9	10	11	12	13
Event/Procedure	**Pre-screen**	**Screen**	**Baseline/Rand**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 8**	**Wk 12**	**Wk 16**	**Wk 20**	**Wk 24**	**Wk 26**	**Wk 30**
		In-person visit	In-person visit (within 4 wks)	Phone/ in-person contact (day 6-8)	In-person visit	Phone/ in-person contact	In-person visit	Phone/ in-person contact	Phone/ in-person contact	In-person visit	Phone/ in-person contact	Phone/ in-person contact	In-person visit (+/- 2 wks)	Phone/ in-person contact
Eligibility Criteria	X	X												
Informed Consent		X												
Serum Pregnancy[1]		X	X rpt										X	
Randomization			X											
Demographics		X												
Medical History		X												
Concomitant Meds		X	X				X	X	X	X	X	X	X	
Vital signs & edema assessment		X	X		X		X			X			X	
Physical exam			X											
Central Labs [Chem panel 10, CBC]			X										X	
Serum/Plasma for Long-Term Storage			X										X	
Review of Dialysis Unit Labs and Records[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Drug Distribution			1 wk supply	1 wk supply	2 wk supply	prn	12 wk supply			10 wk supply				
Dose Escalation				+	+									
Drug Reconciliation				X	X		X			X			X	
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X
Dose Assessment [prn]				X	X	X	X			X				
PET Scan			X										X	
TDI-Echo Scan			X										X	

[1]Women of child-bearing age only

[2]Dialysis unit laboratories and records include intravenous medications, KT/V, PTH, CBC, chemistries, iron studies, and dialysis run sheet blood pressures, and dialysis prescriptions