Safety and Cardiovascular Efficacy of Spironolactone in Dialysis-Dependent ESRD (SPin-D) Trial

A phase II randomized, double-blind, placebo-controlled, multi-center study of the safety and effects on cardiac structure and function of spironolactone in patients with hemodialysis-dependent ESRD

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INVESTIGATOR AGREEMENT:

I agree to conduct this study entitled, Safety and Cardiovascular Efficacy of Spironolactone in Dialysis-Dependent ESRD (SPin-D) Trial, according to protocol version 5.0 dated May 9, 2016. I also agree to conduct this study in compliance with: applicable federal, state and local regulations, Good Clinical Practices, and the requirements of my Institutional Review Board (IRB).

I understand that I may not implement this protocol or amendment without reading this document and receiving written IRB approval. I understand that I am not to make any changes to this protocol or amendment without the prior approval of the Steering Committee of the Hemodialysis Novel Therapies Consortium and the IRB.

I will provide copies of the protocol and amendment, any subsequent protocol amendments, and access to all pertinent information furnished by the Steering Committee of the Hemodialysis Novel Therapies Consortium to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the study protocol.

Principal Investigator Signature	**Date**
Name (Please Print)	**Institution**

Once signed, this original shall be maintained in the Regulatory Binder at the clinical center, with a copy emailed or faxed to the DCC Project Manager (215-573-6262).

Table of Contents

List of Abbreviations

ACE	angiotensin converting enzyme inhibitor
AE	adverse event
ARB	angiotensin receptor blocker
ALDO	aldosterone
ANCOVA	analysis of covariance
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 and Safety Monitoring Board
ESRD	end stage renal disease
FTP	file transfer protocol
GFR	glomerular filtration rate
HD	hemodialysis
Hg	mercury
HIPAA	Health Insurance Portability and Accounting Act
IDS	Investigational Drug Service
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	intention to treat
K	potassium
L	liter
LVMI	left ventricular mass index
mEq	milliequivalent
MM	millimeter
MG	milligram
MOP	Manual of Procedures
MRI	magnetic resonance imaging
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
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
SPL	spironolactone
TDI	tissue Doppler index
UAE	unanticipated adverse event

Study Summary

Title	**Safety and Cardiovascular Efficacy of Spironolactone in Dialysis-Dependent ESRD Trial**
Short Title	SPin-D Trial
NCT Identifier	NCT02285920
Phase	Phase II
Methodology	Randomized, double-blind, placebo-controlled 4-arm
Study Duration	2 years
Study Center(s)	*Brigham and Women's Hospital* *George Washington University* *University of Washington* *Vanderbilt University / Nashville VA Medical Center* *University of Pennsylvania (Data Coordinating Center)*
Objectives	• To generate pilot data on the safety and tolerability of spironolactone (SPL) in patients with dialysis-dependent ESRD. • To generate pilot estimates of the effect of SPL compared with placebo on cardiovascular efficacy parameters in patients with dialysis-dependent ESRD.
Number of Participants	125
Diagnosis and Main Inclusion Criteria	Dialysis-dependent ESRD
Study Product, Dose, Route, Regimen	Spironolactone 12.5, 25, or 50 mg, orally daily
Duration of administration	36 weeks
Reference therapy	Placebo
Major Outcomes	• Safety: hyperkalemia, hypotension • Tolerability: treatment adherence • Efficacy: change in mitral annular E′ velocity • Feasibility: recruitment
Statistical Methodology	Mixed effects linear regression models will be used to assess the direction and time averaged magnitude of change in efficacy parameters, with and without controlling for baseline covariates, to compare the effects of therapy. These mixed effects models will also be utilized to investigate the potential shift in overall potassium levels. The incidence of safety events, especially serious hyperkalemia, 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 Institutional research policies and procedures.

1.1. Background

End stage renal disease (ESRD) is characterized by cardiovascular disease (CVD) with a unique pathogenesis, the absence of effective therapies, and an extremely high rate of cardiovascular (CV) death[1-8]. Given the predicted growth in the dialysis-dependent ESRD population[9], the lack of effective therapy represents a critical public health challenge. New strategies for treating CVD in this setting are desperately needed.

The majority of CV deaths in dialysis-dependent ESRD result from sudden cardiac death rather than overt myocardial infarction. Only 5.3% of deaths in ESRD are due to myocardial infarction compared with 26.1% from arrhythmia and cardiac arrest[10]. Although an increase in the risk of sudden cardiac death is detectable in early stages of CKD, the risk is particularly marked in individuals with ESRD. For example, in a study of individuals with documented coronary artery disease, the relative risk of sudden cardiac death was 1.9 for those with stage 3-4 CKD compared to those with preserved renal function and 4.7 for those with stage 5 CKD. In dialysis-dependent ESRD the sudden cardiac death incidence was increased to 24 cases per 1000-patient years versus only 3.8 per 1000-patient years among individuals with preserved GFR[11]. This association between renal function and sudden cardiac death was independent of angiographic findings as well as standard CVD risk factors, suggesting a direct effect of impaired renal function on the risk of sudden cardiac death.

The disproportionate risk of sudden cardiac death relative to myocardial infarction suggests that neither faster progression of epicardial coronary artery disease nor an increased likelihood of plaque rupture and subsequent myocardial infarction fully explains the high incidence of sudden cardiac death in ESRD. Instead, the risk appears to be related to changes in the myocardium itself – specifically, an increase in the extent of myocardial fibrosis and a decrease in the supply of myocardial capillaries in the uremic myocardium.

Experimental models of CKD are characterized by greater microvascular loss in the heart than other organs[12], decreased myocardial capillary density, reduced ischemia tolerance, and increased infarct size after coronary artery ligation[12-17]. These changes are accompanied by hypertension-independent increases in left ventricular hypertrophy interstitial collagen, and myocardial fibrosis [12-14, 18]. Similar changes have been observed in patients with ESRD. In an autopsy study, ESRD patients with left ventricular hypertrophy but without coronary artery disease had fewer myocardial capillaries and more interstitial fibrosis than patients with preserved renal function[19]. Myocardial coronary flow reserve – a measure of microvascular supply and function – also declines in parallel with glomerular filtration rate[20-23] while coronary collateral vessels – the presence of which reduces the risk of death in CAD[24, 25] – are 41% less abundant in individuals with mild-moderate CKD than in those without CKD[26]. Left ventricular

mass, diastolic function and late gadolinium enhancement (a marker of myocardial scar and fibrosis) also increase dramatically as GFR declines and predict an increased risk of death [27-34].

The combination of left ventricular hypertrophy, increased interstitial collagen content, and microvascular rarefaction increases the distance between capillaries and cardiomyocytes[14, 19] and impairs myocardial oxygen delivery even when overt coronary obstruction is absent, thereby increasing the likelihood of myocyte necrosis with even minor reductions in coronary flow. The resultant hypoxia in combination with disruption of myocardial electrical circuits by both interstitial fibrosis and frank scar in areas of prior necrosis lowers the threshold for the generation of fatal arrhythmias and creates a favorable substrate for their propagation. Alterations in myocardial structure are appear to be a key factor underlying the high incidence of sudden cardiac death in patients with ESRD,[35, 36] and thus, inhibiting these changes may provide a potent, disease-specific strategy for improving CV outcomes in the setting of ESRD.

Role of Aldosterone. Aldosterone plays a key role in the development of myocardial fibrosis and capillary rarefaction. In the L-NAME model of hypertensive cardiomyopathy, for example, aldosterone blockade with spironolactone (SPL) largely reverses left ventricular hypertrophy[37]. SPL as well as a second aldosterone blocker, eplerenone, prevent both angiotensin-2 and L-NAME-induced myocardial necrosis, inflammation and fibrosis via sodium- and potassium-independent mechanisms[38-40], and both adrenalectomy and eplerenone inhibit myocardial fibrosis via blood pressure-independent mechanisms[41]. In models of CKD, SPL attenuates diastolic dysfunction and eliminates left ventricular fibrosis while strongly inhibiting collagen production in cultured cardiac fibroblasts[42]. These experimental findings are consistent with data from a placebo-controlled trial in humans in which SPL decreased collagen production, reduced left ventricular mass and improved left ventricular relaxation (diastolic function) in individuals with stage 2-3 CKD [43, 44], and with studies showing that aldosterone concentrations are elevated and associated with left ventricular hypertrophy in individuals with ESRD[45, 46].

Aldosterone plays a similarly important role in microvascular homeostasis. Aldosterone blockade exerts beneficial effects on the renal microvasculature[41, 47], and eplerenone dramatically increases microvascular density and tissue perfusion following induction of hind-limb ischemia in a murine model[48]. Finally, an ability of aldosterone blockade to preserve myocardial vessels has been demonstrated in experimental models showing that both adrenalectomy and eplerenone reduce L-NAME-induced fibrinoid necrosis of intra-myocardial vessels[41].

Potential Benefits of Aldosterone Antagonism. In patients with congestive heart failure, aldosterone blockade with SPL or eplerenone dramatically reduces overall and CV mortality[49, 50]. This effect appears to be associated with reduced myocardial collagen synthesis as evidenced by reduced circulating concentrations of serum procollagen type I carboxy-terminal peptide (PICP) and other collagen fragments, suggesting that beneficial effects of SPL and eplerenone are at least partly mediated via the inhibition of myocardial fibrosis and adverse cardiac remodeling [51, 52]. Similar effects have been observed in a randomized trial of stage 2-3 CKD patients in which SPL (25 mg per day for 40 weeks) reduced left ventricular mass index by 11.4% and the amino terminal peptide of procollagen 3 by 5%[43], and by a recent, open-label

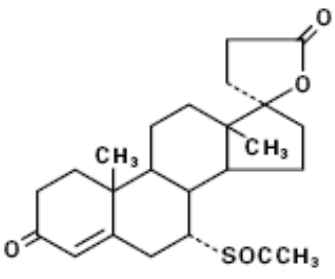
trial in which the administration of 25 mg of SPL daily to chronic dialysis patients was associated with a 60% reduction in death or cardiovascular hospitalizations[53]. Analogous changes in capillary supply are suggested by the 20% improvement in coronary flow reserve observed following just 4 weeks of SPL therapy in a study of diabetic patients[54].

Summary. Both experimental and clinical data strongly implicate aldosterone homeostasis in ESRD-associated CVD, providing a compelling rationale for assessing the safety, tolerability and therapeutic potential of aldosterone blockade using SPL to improve cardiovascular outcomes and overall mortality in the setting of dialysis-dependent ESRD. Although the cardiac effects of aldosterone blockade in ESRD have not been broadly assessed, preliminary studies in patients treated with maintenance dialysis suggest that SPL administered as a daily dose of 25 mg is effective for reducing blood pressure and has a good safety profile with a rate of serious hyperkalemia of less than 3% [53, 55-63]. Furthermore, as noted above, in a recently completed open-label trial comparing SPL and standard therapy in maintenance hemodialysis patients, SPL dramatically reduced overall mortality and the combined endpoint of mortality and cardiovascular hospitalization[53]. Lastly, two additional trials comparing SPL with placebo have been recently initiated in Europe[64, 65]. In light of the established body of evidence and emerging interest in the use of SPL in dialysis-dependent ESRD, the current protocol is a randomized, pilot trial designed to assess the safety and tolerability of SPL in maintenance hemodialysis patients and to generate pilot estimates of the effect of SPL compared with placebo on cardiovascular efficacy parameters in maintenance hemodialysis by assessing diastolic function, heart rate variability, and circulating markers of fibrosis. The trial will build on existing and emerging data about the use and therapeutic potential of SPL in dialysis-dependent ESRD. In particular, the study will generate important information about the feasibility of recruiting participants for a mortality-powered SPL trial in the United States and about the safety of SPL therapy US dialysis population under standard of care hemodialysis practices. In addition, to our knowledge, the proposed trial will be the first in the dialysis-dependent ESRD population to prospectively compare the safety and efficacy of multiple doses of SPL and placebo, and it will thereby provide critical preliminary data needed to choose an optimal dose of SPL in the dialysis population and to design large-scale trials testing whether SPL can effectively improve the poor cardiovascular outcomes in dialysis-dependent ESRD.

1.2. Investigational Agent

(Information obtained from the FDA Package Insert[66])

Aldactone (spironolactone) oral tablets contain 25 mg, 50 mg, or 100 mg of the aldosterone antagonist spironolactone,17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate, which has the following formula:



SPL is practically insoluble in water, soluble in alcohol, and freely soluble in benzene and in chloroform. Inactive ingredients include calcium sulfate, corn starch, flavor, hypromellose, iron oxide, magnesium stearate, polyethylene glycol, povidone, and titanium dioxide.

Mechanism of action: SPL is a specific pharmacologic antagonist of aldosterone, acting primarily through competitive binding of receptors at the aldosterone dependent sodium-potassium exchange site in the distal convoluted renal tubule. SPL causes increased amounts of sodium and water to be excreted, while potassium is retained. SPL acts both as a diuretic and as an antihypertensive drug by this mechanism. It may be given alone or with other diuretic agents which act more proximally in the renal tubule.

Aldosterone antagonist activity: Increased levels of the mineralocorticoid, aldosterone, are present in primary and secondary hyperaldosteronism. Edematous states in which secondary aldosteronism is usually involved include congestive heart failure, hepatic cirrhosis, and the nephrotic syndrome. By competing with aldosterone for receptor sites, SPL provides effective therapy for the edema and ascites in those conditions.

SPL is effective in lowering the systolic and diastolic blood pressure in patients with primary hyperaldosteronism. It is also effective in essential hypertension, even though aldosterone secretion may be within normal limits in essential hypertension.

Through its action in antagonizing the effect of aldosterone, SPL inhibits the exchange of sodium for potassium in the distal renal tubule and helps to prevent potassium loss.

Pharmacokinetics: SPL is rapidly and extensively metabolized. Sulfur-containing products are the predominant metabolites and are thought, together with SPL, to be primarily responsible for the therapeutic effects of the drug. The following pharmacokinetic data were obtained from 12 healthy volunteers following the administration of 100 mg of SPL (Spironolactone film-coated tablets) daily for 15 days. On the 15th day, spironolactone was given immediately after a low-fat breakfast and blood was drawn thereafter.

	Accumulation Factor: AUC (0-24 hr, day 15) /AUC (0-24 hr, day 1)	Mean Peak Serum Concentration	Mean (SD) Post-Steady State Half-life
7-α-(thiomethyl) spirolactone (TMS)	1.25	391 ng/mL at 3.2 hr	13.8 hr (6.4) (terminal)
6-β-hydroxy-7-α-(thiomethyl) spirolactone (HTMS)	1.50	125 ng/mL at 5.1 hr	15.0 hr (4.0) (terminal)
Canrenone (C)	1.41	181 ng/mL at 4.3 hr	16.5 hr (6.3) (terminal)
Spironolactone	1.30	80 ng/mL at 2.6 hr	Approximately 1.4 hr (0.5) (β half-life)

The pharmacological activity of SPL metabolites in man is not known. However, in a study of adrenalectomized rats, the anti-mineralocorticoid activities of the metabolites C, TMS, and HTMS, relative to SPL, were 1.10, 1.28, and 0.32, respectively. Relative to SPL, the binding affinities of these metabolites to the aldosterone receptors in rat kidney slices were 0.19, 0.86, and 0.06, respectively. In humans the potencies of TMS and 7-α-thiospirolactone in reversing the effects of the synthetic mineralocorticoid, fludrocortisone, on urinary electrolyte composition were 0.33 and 0.26, respectively, relative to SPL. However, since the serum concentrations of

these steroids were not determined, their incomplete absorption and/or first-pass metabolism could not be ruled out as a reason for their reduced *in vivo* activities. SPL and its metabolites are more than 90% bound to plasma proteins. The metabolites are excreted primarily in the urine and secondarily in bile. The effect of food on spironolactone absorption (two 100 mg SPL tablets) was assessed in a single-dose study of 9 healthy, drug-free volunteers. Food increased the bioavailability of un-metabolized spironolactone by almost 100%. The clinical importance of this finding is not known.

1.3. Clinical Data to Date

Heart Failure: The RALES trial was a multi-national trial that enrolled 1663 patients with NYHA class 4 heart failure and a median creatinine clearance of 57 mL/min to SPL 25 mg daily or placebo. The primary endpoint was time to all-cause mortality. RALES was terminated early because of a significant mortality benefit. Spironolactone reduced the risk of death by 30% compared to placebo ($p<0.001$; 95% confidence interval 18% to 40%). Spironolactone reduced the risk of cardiac death, primarily sudden death and death from progressive heart failure by 31% compared to placebo ($p <0.001$; 95% confidence interval 18% to 42%.[50]

Stage 2-3 CKD: Edwards *et al* randomized 112 patients with stage 2-3 CKD and controlled blood pressure on ACEI or ARB therapy to SPL 25 mg daily or placebo for 36 weeks. The co-primary endpoints of the study were change in LV mass and arterial stiffness measured by pulse wave velocity. No patients died during follow-up. LV mass increased by 3 gram in the placebo group but decreased by 13 gram in the SPL group ($P<0.01$). Similarly, pulse wave velocity decreased by 0.2 meter/second in the placebo group compared with a decrease of 0.8 meter/second in the SPL group ($P<0.01$).[44]

ESRD: Trials including 332 hemodialysis patients treated with SPL and 78 peritoneal dialysis patients suggest that it is associated with minimal changes in serum potassium and a serious hyperkalemia incidence of <3%[53, 55-63].

Gross *et al* conducted a randomized, crossover trial of 2 weeks of SPL 50 mg twice daily compared with placebo in 8 hemodialysis patients. Patients on ACEI or ARB were excluded. The mean change in systolic blood pressure was 11 mm Hg ($P<0.05$). Changes in potassium were not significantly different in placebo (4.7 ± 0.4 mEq/L to 4.7 ± 0.5 mEq/L) and SPL group 4.6 ± 0.7 to 5.0 ± 0.8, $P>0.05$)[55].

Hussain treated 15 hemodialysis patients with SPL 25 mg daily for 28 days[56]. Pre-enrollment ACE or ARB was continued throughout the treatment period in 6 of 15 patients. Mean serum potassium levels were 4.6 ± 0.6mEq/L at baseline and 4.7±0.6 mEq/L at study completion ($P=0.19$). Nine patients completed the trial with all serum potassium levels <5.6 mEq/L and 13 completed therapy with all potassium levels < 6.0 mEq/L. Of the remaining 2 patients, the peak potassium level was detected following a missed dialysis session.

Michea treated 9 hemodialysis patients with SPL 50 mg 3x/week for 2 weeks followed by 2 weeks of placebo therapy. During each study period participants were challenged with an oral potassium load. Individuals using ACEI or ARB were excluded from enrollment. In this study, there was no significant effect of SPL therapy on the rise in potassium concentration following

the potassium load. No patient developed hyperkalemia during the study period[61].

Saudan studied a low dose of SPL (12.5 mg 3x/weekly for 2 weeks, followed by 25 mg 3x/weekly for 2 weeks) in 14 hemodialysis patients[57]. ACEI or ARB was continued in 8 of 14 participants. SPL therapy resulted in a decrease of potassium from 5.0 ± 0.4 to 4.9 ± 0.4 mEq/L compared with a change from 4.8 ± 0.8 to 4.6 ± 0.7 mEq/L in a non-randomized concurrent control group. In a multi-variable, analysis adjusting for baseline potassium concentration, SPL therapy was associated with a decrease in serum potassium compared with placebo.

Taheri conducted a small, randomized trial of 16 hemodialysis patients with an ejection fraction <45%[58]. The treatment group received SPL 25 mg daily for 6 months and the control group was treated with placebo therapy. All patients were treated with ACEI and/or ARB.

SPL therapy was associated with a significant increase in ejection fraction compared with placebo therapy (6.2 ± 1.64% vs. 0.83 ± 0.49%, P=0.046) as well as a decrease in left ventricular mass compared with a small increase in the placebo group (P=0.02). Potassium concentrations increased by a mean of 1.02 ± 0.34 mEq/L in the SPL group compared with 0.08 ± 0.45, (P=0.004). However, only 1 patient developed frank hyperkalemia.

A larger randomized trial of 66 patients was conducted by Vukusich[59]. This study enrolled prevalent hemodialysis patients not taking ACEI or ARB and randomized patients to 50 mg of SPL 3x/week or placebo for 24 months. Carotid ultrasound was performed before and at the end of therapy. No patients died during therapy and there were no hyperkalemic events. In this study, SPL did not have a significant effect on blood pressure at 24 months (systolic blood pressure change of -2 mm Hg in the SPL group). Calculated carotid intimal-medial thickness progression rate for left and right segments in the placebo group were 0.06 ± 0.07 mm/year in the common carotid artery *versus* 0.01 ± 0.04 mm/year in the SPL group (P=0.003). These effects were consistent across other carotid artery segments. Plasma potassium concentration increased by 0.012 mEq/L per month during treatment (P = 0.001) in the SPL group, but did not change significantly in the placebo group.

Matsumoto administered SPL 25 mg daily for 6 months to 61 oligo-anuric prevalent, hemodialysis patients[60]. ACEI or ARB was used by 66% of patients during the study period. No patient discontinued therapy due to hyperkalemia or required ion-exhange therapy, although 3 patients discontinued therapy due to gynecomastia, and 3 additional patients discontinued therapy due to possible side-effects. Serum potassium rose from 4.96 ± 0.72 to 5.18 ± 0.72 during therapy. A serum potassium >6.8 mEq/L was not observed in any of the patients.

Flevari[62] conducted a sequential, non-randomized crossover trial of 14 stable dialysis patients without heart failure. Placebo was administered for 4 months followed by 4 months of SPL 25 mg post-dialysis 3 times per week. ACEI and ARB were continued if previously used. Pre-dialysis potassium levels increased from 4.4 ± 0.2 to 5.5 ± 0.3 meq/L during SPL therapy (P<0.05) and 2 patients required administration of potassium binding resins. Treatment with SPL was not associated with changes in left ventricular mass, systolic function, or the E/A ratio. However, there were significant decrements in systolic blood pressure from 145 ± 4.2 to 121 ± 2.3, and significant improvements in flow-mediated reactive forearm vasodilatation, and heart

rate variability.

McGill[63] studied 30 prevalent dialysis patients (mean time on dialysis 6 years). Thirteen individuals completed 9 months of SPL 25 mg daily. ACE inhibitor therapy was continued in 7/13 participants. The remaining 17 patients failed to complete the trial protocol. Reasons for non-completion were varied. Although only limited data on reasons for non-completion were presented, drug side-effects were not implicated in the published report. No potassium measurements of >6.0 mEq/L were observed. Hemodialysis prescriptions were adjusted on 2 occasions – for measurements of 5.7 and 5.9 mEq/L, respectively. There was no detectable pattern of change in serum potassium levels during the study. MRI measurements of LV mass did not decrease between baseline and follow-up in the small subset of individuals completing baseline and follow-up scans. However, there were non-significant decreases in relative wall thickness (from 0.60 ± 0.20% to 0.56 ± 0.20%) and end diastolic volume index (from 99 ± 27 mL/m^2 to 92 ± 33 mL/m^2) and a non-significant increase in ejection fraction (from 59 ± 10% to 60 ± 10%).

Masumoto[53] randomized 309 Japanese maintenance hemodialysis patients to 25 mg SPL daily or placebo. During a 3-year, open-label trial, 5.7% of patients in the SPL group reached the primary endpoint of combined death or CV hospitalization compared with 12.5% of patients in the control group (HR 0.40; 95% CI: 0.20 to 0.81). There was also a significant effect on the secondary outcome of all-cause mortality with 10 deaths (6.4%) in the treatment group and 30 (19.7%) in the control group (HR 0.36; 95% CI: 0.19 to 0.66). Serious hyperkalemia requiring drug discontinuation was rare and occurred in only 3 (1.9%) participants during the trial.

Finally, Ito randomized 158 peritoneal dialysis patients on concurrent ACEI or ARB therapy to open-label SPL or standard therapy[67]. LVMI assessed by echocardiography improved significantly at 6 months (P=0.03) and 2 years (P=0.01) in individuals taking SPL compared with control therapy. There was no detectable impact of SPL therapy on rate of change in residual renal function. Hyperkalemia (K>6.0 mEq/L) occurred in only 2 of 78 patients (2.6%) in the SPL group and 1 of 80 patients (1.3%) in the control group, P=0.62. None of the SPL participants experienced a K>6.0 mEq/L. Gynecomastia affected 11 of 78 (14.1%) SPL participants compared with 2 of 80 (2.5%) control patients (P=0.01), but drug discontinuation was required in only 2 participants.

Based on available experience in the ESRD population the incidence of hyperkalemia with SPL therapy appears to be low. Available data are summarized in **Table 1**. In addition to the studies described above, the ALCHEMIST trial, which randomizes participants to SPL or placebo, was recently initiated in France.[64] Despite some overlap, there are important differences between ALCHEMIST and the current trial. In particular, ALCHEMIST will enroll a more selective, less generalizable population as participants are required to have diabetes, left ventricular hypertrophy, history of cardiovascular disease, or low ejection fraction. The ALCHEMIST study will enroll patients exclusively in France and will therefore not provide data on the safety and efficacy of SPL within the context of standard of care dialysis practices in the United States or within the U.S. dialysis population. Thirdly, the ALCHEMIST study focuses on clinical endpoints comparing a single dose of SPL with placebo with only limited physiologic studies planned.

Thus, in contrast to the current study, ALCHEMIST is not expected to improve understanding of the biological and physiological effects of SPL, nor will it provide the comparative safety and efficacy data needed to define the optimal dose of SPL.

Because ALCHEMIST is not powered for the detection of differences in mortality, larger trials will be necessary to change the standard of care following its completion. Results of the current trial will contribute important information for the planning of definitive trials and for determining the optimal dose of SPL for patients treated with chronic hemodialysis. Nevertheless, all newly available data from ALCHEMIST or other SPL studies will be reviewed on an ongoing basis during conduct of the current trial. New data with the potential to alter the risks and benefits of participation in the current trial will be shared with the Data and Safety Monitoring Board which will be asked to make recommendations for continuing the study unchanged, modifying the protocol, or discontinuing the study.

Table 1. Hyperkalemia in Dialysis Studies of Spironolactone

Author	Design	Number of Participants	Hyperkalemia Definition	Hyperkalemia Incidence in SPL Group	Notes
Gross[55]	RCT, crossover design, placebo vs SPL 50 mg BID x 2 weeks	8	NA	NA	Non-significant change in potassium (P>0.05) a) placebo 4.7 ± 0.4 to 4.7± 0.5 mEq/L b) SPL 4.6 ± 0.7 to 5.0 ± 0.8.
Hussain[56]	Single arm study, SPL 25 mg QD x 28 days	15	≥6.0 mEq/L	2/15 (13.3%)	Hyperkalemia detected post missed dialysis session in both cases. Concurrent ACEI/ARB in 6/15 participants.
Michea[61]	Crossover, non-randomized, placebo or SPL 50 mg 3x/wk x 2 wks. Challenge with high potassium diet.	9	Undefined	0/9 (0.0%)	Mean potassium values during SPL therapy 4.56 ± 1.13 mEq/L.
Saudan[57]	Single arm study, SPL 12.5 mg 3x/week x 2 weeks to SPL 25 mg 3x/week x 2 weeks	14	NA	NA	SPL therapy resulted in a decrease in potassium from 5.0 ± 0.4 to 4.9 ± 0.4 mEq/L. ACEI/ARB continued in 8/14 participants.
Taheri[58]	RCT. Placebo vs. SPL 25 mg QD x 6 months	16	Undefined	0/8	ACEI/ARB in all participants.
Vukusich[59]	RCT. SPL 50 mg 3x/week vs. placebo x 24 months	66	Undefined	0/33	No concurrent ACEI/ARB.
Matsumoto[60]	Single arm study, SPL 25 mg QD x 6 months	61	>6.5 mEq/L	8/61(13.1%)	Concurrent ACEI/ARB use in 66%. All serum potassium measurements ≤6.8 mEq/L. Resin use not required in any participants.
Flevari[62]	Non-randomized crossover. Placebo vs. SPL 25 mg 3x/week x 4 months.	14	>6.0 mEq/L	2/14 (14.2%)	Concurrent ACEI/ARB allowed. 2 participants required potassium binding resins for K>6.0 mEq/L.
McGill[63]	Single arm study, SPL 25 mg QD x 9 months.	13	>6.0m mEq/L	0/13 (0.0%)	Concurrent ACEI in 7/13 participants. Additional 17 participants enrolled but did not complete study for reasons other than hyperkalemia.
Masumoto[53]	RCT. SPL 25 mg QD vs. placebo x 3 years.	309	Discontinuation due to hyperkalemia (criteria not specified)	3/157 (1.9%)	Non-blinded. Inclusion criteria required mean K<6.5 mEq/L in 2 months prior to randomization. Concurrent ACEI/ARB allowed.
Ito[67]	RCT. SPL 25 mg QD vs. standard therapy x 2 years.	158	≥6.0 mEq/L	2/78 (2.6%)	Non-blinded. Concurrent ARB in 100%.

1.4. Dose Rationale and Risk/Benefits

An SPL oral dose of 25 mg daily has been associated with improvement in mortality and ventricular function in a large, heart failure trial[50], and a 25 mg daily dose has also been associated with improvement in ventricular structure and function in mild to moderate CKD. Doses tested in ESRD have ranged from 25 mg 3x/week to 50 mg 2x/day. Although, a 25 mg daily dose has been the most frequently tested, and appears to be well-tolerated and associated with a minimal incidence of serious hyperkalemia as detailed in Section 1.3 and Table 1, the optimal dose in terms of safety and efficacy in the ESRD population remains uncertain. To our knowledge, no study to date has compared the relative safety, efficacy, or tolerability of multiple doses with each other or with placebo within the structure of a single trial. Thus, in order to generate comparative information on safety, efficacy and tolerability, the current study will compare 3 doses of SPL, representing a low dose (12.5 mg/day), a medium dose (25 mg/day) and a high dose (50 mg/day), with placebo therapy. Spironolactone will be administered once per day. On days in which dialysis is performed the drug will be taken post-dialysis to minimize effects of dialysis on drug metabolism. In addition to spanning a range from low to medium to high dose, the targeted doses of SPL are consistent with available tablet sizes and standard clinically utilized/approved doses for the FDA-labeled indications. Although additional doses could be tested, it is uncertain whether significantly greater knowledge would be generated through the addition of medium-high dose (e.g. 37.5 mg/day) compared with the current plan to test low, medium and high dosing strategies. Conversely, addition of an ultra-high dose group (e.g. >50 mg/day) is more likely to be associated with a high rate of adverse events necessitating drug discontinuation. Additional dosing arms also have the disadvantage of increased logistical complexity. Furthermore, in the absence of an increase in overall sample size, a smaller cohort size in each dose group could have the undesired effect of limiting power to detect differences in safety or efficacy across doses. The increase in the overall sample size required to increase power across 4 or more active dose groups would increase the costs, recruitment challenges, and probably the duration of the trial.

1.4.1. Risks

Risks of SPL include hyperkalemia, hypotension and intra-dialytic hypotensive symptoms. Other potential side effects include gynecomastia and gastrointestinal symptoms. Of particular concern in trials of SPL in patients with CKD is the risk of hyperkalemia and the potential for hyperkalemia-associated arrhythmias. Although the effects of SPL on potassium excretion are important, it is noteworthy that extracorporeal (dialytic) clearance, rather than renal or colonic clearance, provides the majority of potassium excretion in dialysis-dependent ESRD. This extracorporeal clearance should not be impaired by SPL therapy.

In the current trial, risks of hyperkalemia will be reduced through close monitoring of serum potassium concentrations through scheduled study-specific safety labs, and through standard clinical practice at the treating dialysis unit (monthly to bi-monthly in individuals with normal serum potassium, more frequently in individuals with hyperkalemia or hypokalemia). Manipulation of dietary intake of potassium, eliminating, when medically acceptable, concomitant use of medications with hyperkalemic effects, and adjustment of dialysate

potassium will also be used as additional safety measures (see **Section 6.8**). The use of low potassium dialysate is currently considered by many clinicians to be an appropriate approach for treating hyperkalemia, and dialysate potassium concentration may be altered according to standard practice of the treating clinicians throughout the course of the study. However, the study protocol will not mandate or recommend prophylactic changes to the dialysate prescription and calls for lowering dialysate potassium only as a second-line response to significant hyperkalemia after other measures fail to resolve hyperkalemia. The relative risks of arrhythmic death from hypokalemia, hyperkalemia and low dialysate potassium levels in hemodialysis patients is an active area of inquiry and uncertainty and further research into the best approach is warranted[68-70]. In the absence of definitive data on the optimal approach to dialytic management of serum potassium concentration, evaluation of the relative risks and benefits of SPL therapy within the context of standard clinical approaches to dialysate potassium prescription will increase the understanding of the true risks and benefits of SPL therapy within the context of standard clinical approaches to hemodialysis in the United States.

Given these uncertainties and equipoise regarding the ideal serum potassium concentration and ideal dialysate potassium in chronic hemodialysis patients, changes in dialysate potassium concentration will be recommended only as a secondary means of controlling serum potassium to be utilized only in the context of significant hyperkalemia. Use of 1 mEq/L potassium dialysate will be recommended only as a temporizing measure of last resort. The proportion of treatments requiring use of 1 mEq/L dialysate will be monitored as a secondary safety endpoint and compared across treatment groups.

1.4.2. Benefits

Benefits of SPL therapy in the dialysis population remain unproven at this time. Hypothesized benefits of SPL 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 and tolerability of multiple doses of chronic SPL therapy compared with placebo in maintenance hemodialysis patients and to assess the feasibility of conducting a full-scale, mortality-powered trial of SPL. The effects of SPL compared with placebo on multiple cardiovascular efficacy parameters will also be analyzed. The primary efficacy parameter will be the change in the E′ measurement on tissue Doppler echocardiography (TDI) as an index of diastolic function and a surrogate for myocardial fibrosis. Secondary cardiac parameters of interest that will be studied in the overall population or in sub-studies include heart rate variability, circulating markers of fibrosis, and coronary flow reserve (CFR) as an index of microvascular function. These parameters are designed to broaden insight into the potential effects of SPL on cardiac structure and function in individuals with dialysis-dependent ESRD and to assess the feasibility of conducting a full-scale, mortality-powered trial.

2.1. Primary Objectives

Safety

- To evaluate the safety of chronic spironolactone therapy in individuals with ESRD on HD.
    - Safety events will include the following:
        - Potassium level >6.5 mEq/L
        - 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)
        - Serious hyperkalemia defined as hyperkalemia requiring hospitalization, emergency/unscheduled dialysis, or emergency/unscheduled resin therapy
        - The combined incidence of potassium level >6.5 mEq/L or serious hyperkalemia defined as hyperkalemia requiring hospitalization, emergency/unscheduled dialysis, or emergency/unscheduled resin therapy
        - Treatment-emergent events defined as the combined incidence of death, myocardial infarction, stroke, hospitalizations, potassium level >6.5 mEq/L, serious hyperkalemia defined as hyperkalemia requiring hospitalization, emergency/unscheduled dialysis, or emergency/unscheduled resin therapy, and 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
        - The individual components of the treatment-emergent endpoint
        - Cardiovascular death
        - Hyperkalemia requiring adjustment in dialysate potassium concentration, or discontinuation of study medication
        - Proportion of dialysis sessions utilizing 1 mEq/L potassium dialysis solution bath
        - Within-patient variability in serum potassium concentration
        - Symptomatic inter- or intra-dialytic hypotension

Tolerability

- To evaluate the tolerability of chronic spironolactone therapy in individuals with ESRD treated with 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 chronic blockade of aldosterone with SPL improves diastolic function in individuals with ESRD on HD. 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.

Feasibility

- To assess feasibility of conducting a full-scale mortality-powered 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 SPL therapy in ESRD by analysis of the following parameters:

- Change between baseline and 36 weeks in left ventricular mass index (LVMI)
- Change between baseline and 36 weeks in ejection fraction
- Change between baseline and 36 weeks in myocardial strain and strain rate
- Change between baseline and 36 weeks in circulating indices of tissue fibrosis such as procollagen type I carboxy-terminal peptide (PICP), galectin-3, soluble ST2, GDF-15
- Change between baseline and 36 weeks in circulating indices of systemic inflammation such as CRP, IL-1, IL-2, IL-6, IL-10, IL-18, TNF-alpha, albumin, sVCAM-1, leptin, adiponectin
- Change between baseline and 36 weeks in circulating indices of oxidative stress such as F2 isoprostanes and isofurans
- Change between baseline and 36 weeks in coronary flow reserve
- Association between change in coronary flow reserve (CFR) and change in E′
- Heart rate variability
- Arrhythmia

3. Study Design

3.1. General Design

This is a randomized, placebo-controlled 4-arm trial that will compare placebo with 3 doses of SPL. Participants will be randomized in a 2:1:1:1 fashion to placebo or SPL, with equal proportions randomized to each of 3 SPL doses: 12.5, 25 or 50 mg once daily. As shown in **Figure 1**, the study will be conducted in 2 phases – a dose escalation phase and a treatment phase.

All participants randomized to SPL will initiate treatment at 12.5 mg daily. Participants will be evaluated weekly for tolerability, hypotension and hyperkalemia. At 2 weeks, if the initial dose has been tolerated, participants randomized to the 25 or 50 mg SPL arm will increase the dose to 25 mg for 2 weeks with weekly evaluation for tolerability, hypotension and hyperkalemia. Participants randomized to 12.5 mg SPL or placebo arm will continue on the same dose with weekly evaluation for tolerability, hypotension and hyperkalemia.

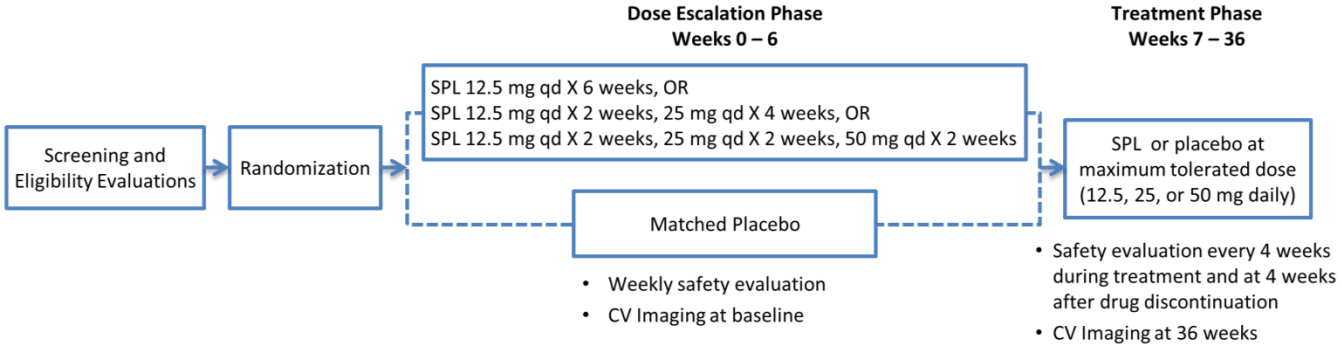
At 4 weeks, participants randomized to the 50 mg SPL arm will increase the dose to 50 mg if the 25 mg dose was tolerated with weekly evaluations for tolerability, hypotension and hyperkalemia

until 6 weeks. Participants randomized to 12.5 mg, 25 mg, or placebo will continue on the same dose with weekly evaluation for tolerability, hypotension and hyperkalemia until 6 weeks.

In order to maintain the blind, during the dose escalation phase all study participants, regardless of randomized assignment, will receive new study drug kits every 2 weeks and will be evaluated weekly.

At the end of the dose escalation phase, participants will continue treatment based on the randomized dose assignment for an additional 30 weeks (treatment phase) such that the total duration of study medication is 36 weeks. As described in **Sections 6.8 and 6.9**, the dose of SPL or placebo will be decreased during the dose-escalation and follow-up phases for hypotension, hyperkalemia or other dose limiting side-effects.

Figure 1. Study Design



3.2. Study Endpoints

3.2.1. Primary Endpoints

Safety

The primary safety endpoints are:

1) The incidence of serum potassium >6.5 mEq/L
2) The incidence of 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).

Tolerability

Tolerability will be assessed on the basis of whether participants can continue the assigned dose throughout the entire treatment period. Any reduction in dose of study medication will be considered a failure of primary tolerability.

Efficacy

The primary efficacy parameters will be measured between baseline and 36 weeks: 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.

E′ has been chosen to be the primary echocardiographic endpoint because the intra-observer coefficient of variation is low[71] and multiple studies demonstrate a high correlation with myocardial fibrosis[72-74], a primary biologic endpoint of interest. E′, for example, parallels the accumulation of myocardial collagen and fibrosis in the senescence prone mouse[75], and correlates with endomyocardial fibrosis after human cardiac transplant[76] as well as the presence of late gadolinium enhancement[77] – a highly-validated measure of myocardial scar – across multiple disease states. Furthermore, abnormalities in TDI have been strongly linked to mortality in multiple disease states[78-80] 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[34, 81].

In contrast to other echocardiographic measures, E′ (or the ratio of E/E′) is minimally affected by intravascular volume status[82-84] – 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 strain rate and strain may allow detection of subtle changes in myocardial function due to fibrotic processes[85]. Finally, these measures are known to respond to therapeutic interventions that exert anti-fibrotic effects[86]. 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.

Feasibility

An objective of this study is to assess the feasibility of conducting a full-scale mortality-powered trial. Feasibility will be assessed based on recruitment, dropout and loss to follow-up rates.

3.2.2. Secondary Endpoints

Safety

- Serious hyperkalemia defined as hyperkalemia requiring hospitalization, emergency/unscheduled dialysis, or emergency/unscheduled resin therapy
- The combined incidence of potassium level >6.5 mEq/L or serious hyperkalemia defined as hyperkalemia requiring hospitalization, emergency/unscheduled dialysis, or emergency/unscheduled resin therapy
- Treatment-emergent events defined as the combined incidence of death, myocardial infarction, stroke hospitalization, potassium level >6.5 mEq/L, serious hyperkalemia, and serious hypotension
- The individual components of the treatment-emergent endpoint
- Cardiovascular death
- Hyperkalemia requiring adjustment in dialysate potassium or discontinuation of study medication
- Potassium ≥6.0 mEq/L
- Mean serum potassium during follow-up
- Proportion of dialysis sessions utilizing 1 mEq/L potassium dialysis solution bath
- Within-patient variability in serum potassium concentration

- Mean pre-dialysis blood pressure during follow-up
- Symptomatic inter- or recurrent intra-dialytic hypotension
    - Symptomatic inter-dialytic hypotension is defined as systolic blood pressure <90 mm Hg or hypotension requiring adjustment in blood pressure medications or treatment in an emergency or hospitalized setting
    - Recurrent intra-dialytic hypotension is defined as systolic blood pressure <80 mm Hg during ≥3 dialysis sessions per 30-day period or treatment for either hypotension or symptoms of hypotension during ≥3 dialysis sessions per 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.

Efficacy

Secondary outcome measures include other echocardiographic markers of systolic and diastolic function, circulating markers of fibrosis,heart rate variability and arrhythmias (sub-study), and coronary flow reserve (CFR) as assessed by myocardial positron emission tomography (PET)(sub-study).

- Change in left ventricular mass index (LVMI) between 0 and 36 weeks
- Change in ejection fraction between 0 and 36 weeks
- Change in myocardial strain and strain rate between 0 and 36 weeks
- Change in cardiac fibrosis markers between baseline and 36 weeks
- Change in inflammatory markers between baseline and 36 weeks
- Change in oxidative stress markers between baseline and 36 weeks
- Change in CFR between baseline and 36 weeks (sub-study)
- Association between change in CFR and change in E′ (sub-study)
- Heart rate variability assessed by Medtronic SEEQ monitor (sub-study)
- Arrhythmias assessed by Medtronic SEEQ monitor (sub-study)

4. Participant Selection and Withdrawal

4.1. Inclusion Criteria

a) Maintenance hemodialysis therapy for end-stage renal disease
b) Age 18-85 years
c) ≥3 calendar months since dialysis initiation
   If a patient has been on dialysis for ≥3 but less than 6 calendar months, there must be:
   i) no hospitalizations during the 6 weeks prior to screening and
   ii) no change in EDW within 2 weeks of the screening date.
d) For women of childbearing potential, willingness to use a highly effective method of birth control for up to 4 weeks after the last dose to study drug. See **Section 4.3.1** for definition of childbearing potential and acceptable methods of birth control.
e) Ability to provide informed consent

4.2. Exclusion Criteria

a) Serum potassium ≥6.5 mEq/L within the 3 months prior to screening
b) Serum potassium level ≥6.0 mEq/L within 2 weeks prior to the baseline visit. If a potassium value is not available through routine clinical care during this 2-week period a potassium measurement will be performed as a research test.
c) Unscheduled dialysis for hyperkalemia within the 3 months prior to screening
d) Pre-dialysis systolic blood pressure <100 mm Hg within 2 weeks prior to screening or at the baseline visit
e) 2 or more dialysis sessions within the month prior to screening with either 2 intra-dialytic measurements of systolic blood pressure <80 mm Hg
f) Current dual use of ACEI and ARB
g) Current use of digoxin
h) Current use of spironolactone or epleronone
i) Allergy to spironolactone
j) Inability to maintain dialysis machine blood flow ≥300 mL/min during any of the most recent 3 dialysis sessions prior to the screening visit as an indicator of vascular access dysfunction
k) Mitral valve repair or replacement
l) Severe mitral valve disease by echocardiography, coronary angiography or cardiac magnetic resonance imaging
m) Anticipated kidney transplant, change to peritoneal dialysis, or transfer to another dialysis unit within 9 months
n) Expected survival <9 months
o) Pregnancy, anticipated pregnancy, or breastfeeding
p) Incarceration
q) Participation in another intervention study

4.2.1. Exclusion Criteria for SEEQ Heart Rhythm Monitoring Sub-study Only

a) symptomatic arrhythmias for which heart rhythm monitoring is clinically indicated

4.3. Participant Recruitment and Screening

Participants at dialysis units affiliated with investigator and co-investigator practices will be screened for eligibility. In addition to active screening of dialysis unit patient rosters by study personnel, informational handouts and brochures may be disseminated at affiliated dialysis units in order to allow potential participants to learn about the study and to contact investigators 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. Prior to enrollment, each participant's treating nephrologist will be contacted to assess suitability for enrollment.

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 (referred to hereafter as the Clinical Center), according to investigator and participant preferences. No study-specific testing is required to confirm eligibility, except a serum pregnancy test for women of childbearing potential (see **Section 4.3.1**), and serum potassium if a measurement is not available through clinical measurements within the specified time window. A Baseline/Randomization visit will be scheduled within 30 days of the screening visit.

4.3.1. Women of Childbearing Potential

Women of childbearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Postmenopausal is defined as:

- Amenorrhea for ≥12 consecutive months without another cause or
- Women with irregular menstrual periods and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL or
- Women on hormone replacement therapy (HRT)

Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or who are practicing abstinence, or have a partner who is sterile (e.g., vasectomy) should be considered to be of childbearing potential.

Acceptable methods of highly effective birth control include:

- Condom with spermicide
- Diaphragm and spermicide
- Cervical cap and spermicide
- Hormonal contraception

4.4. Early Withdrawal of Participants and Early Termination of Study Medication

4.4.1. Early Withdrawal of Participants

Early withdrawals will be discouraged and participants who are not willing or able to continue study medication will be encouraged to remain in the study and continue study evaluations. In the case of withdrawal of consent, every attempt will be made to obtain consent to continue monitoring 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. If a patient becomes pregnant, she will be withdrawn from the study but will be followed for specific pregnancy outcomes.

4.4.2. Early Termination of Study Medication

Study medication will be discontinued by the research team in the following circumstances because of the potential for compromising patient safety:

- Allergy to or documented intolerance of study medications
- Poor adherence with dialysis schedule compromising ability to follow serum potassium on a monthly basis
- Organ transplantation
- Change to a different dialysis modality
- Initiation of digoxin therapy
- Transfer to a non-participating dialysis unit

If study medication is discontinued study visits and procedures will continue unless the participant withdraws consent for follow-up.

5. Study Drug

5.1. Description

Study drug will be administered as matched, gel-encapsulated capsules containing 12.5 mg, 25 mg or 50 mg of spironolactone (Spironolactone,17-hydroxy-7α-mercapto-3-oxo-17α-pregn-4-ene-21-carboxylic acid γ-lactone acetate) or placebo (methylcellulose powder).

5.2. Treatment Regimen

As depicted in **Figure 1**, 125 participants will be randomized in a 2:1:1:1 fashion to either placebo or SPL (12.5, 25, or 50 mg), with an equal proportion in each SPL group. SPL will be matched with placebo and administered orally, once daily, post-dialysis. Participants will be instructed that, on days of dialysis treatment, the dose should be taken after the dialysis session. All patients will be started on SPL 12.5 mg once daily or matched placebo. Potassium levels will be checked 3-5 days and 2 weeks after initiation of study drug. Dialysis run-sheets will be evaluated weekly for the occurrence of adverse events such as hypotension and gastrointestinal symptoms. Adjustment to dosage of concomitant medications, the dialysis prescription, or study drug for hypotension or hyperkalemia will be made as described in **Section 6.8 and Section 6.9.**

At 2 weeks, in the absence of dose-limiting hyperkalemia or hypotension, subjects randomized to the 25 or 50 mg SPL arm will increase the dose to 25 mg and those randomized to 12.5 mg will continue that dose. At 4 weeks, in the absence of dose-limiting hyperkalemia or hypotension, participants randomized to 50 mg SPL will increase the dose to 50 mg SPL, and participants randomized to 12.5 mg or 25 mg will continue the same dose.

In order to maintain the blind, all study participants, regardless of randomized assignment, will receive a new study drug supply every 2 weeks during the dose escalation phase. For all participants, serum potassium levels will be checked at 3-5 days and at 2 weeks after the new study drug supply is dispensed, and weekly evaluations will be performed for tolerability, hypotension and hyperkalemia.

At the end of the dose escalation phase, participants will continue treatment based on the

randomized dose assignment for an additional 30 weeks (treatment phase) such that the total duration of study medication is 36 weeks. As described in **Sections 6.8 and 6.9**, the dose of SPL or placebo will be decreased or discontinued during the dose-escalation and treatment phases for hypotension, hyperkalemia or other dose limiting side-effects.

5.3. Method for Assigning Participants to Treatment Groups

The DCC will generate randomization schedules stratified by 1) participating recruitment site; 2) dialysis vintage (≥ 1 vs. < 1 year on dialysis) and 3) angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) use. 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 sites will be unable to predict assignments. Both longer dialysis vintage and use of ACEI or ARB increase the likelihood of hyperkalemia through loss of residual renal function and drug effects, respectively, and thus, are potential confounders that should be balanced across treatment groups.
All randomization schedules will remain confidential, and known only to authorized members of the DCC staff and the central pharmacy.

All relevant screening and eligibility confirmation data will be entered into the centralized data management system in advance of randomization in order for an eligible participant to be randomized. When the study team agrees that the patient should be randomized, and the "Ready to Randomize Report" confirms that all eligibility criteria have been met, the Study Coordinator will implement the web-based randomization assignment managed centrally at the DCC. This centralized randomization process will provide a randomized treatment assignment for that participant within the relevant stratum, linked with a specific study drug kit prepared at the central pharmacy. This kit will be provided to the local investigative team for distribution at the randomization/baseline visit.

5.4. Preparation and Administration of Study Drug and Maintenance of Blind

Study drug and matching placebo will be prepared by the University of Pennsylvania Investigational Drug Service (IDS) which will be the Central Pharmacy for this study. Study drug kits will contain 2-week supplies during the dose escalation phase, a 6-week supply for weeks 6-12, and 12-week supplies for weeks 13-24 and weeks 25-36.

Study drug will be prepared using gelatin encapsulation or equivalent methods such that all study drug, regardless of type (SPL or placebo) and dose are identical in appearance. During encapsulation, SPL tablets will remain intact and will not be altered. All dose levels will be administered as a single capsule. IDS personnel will have access to the randomization scheme and code in order to prepare study drug kits containing the correct dose of SPL or placebo. 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. Randomized treatment assignments and the dosages provided in study drug kits will not be known by the Clinical Center research teams or the Clinical Center Research Pharmacy staff. Only the Investigational Drug Service personnel and the database programmer at the DCC will be unblinded.

During the 6-week dose escalation phase, a study drug request form signed by a Clinical Center investigator will be entered into the data management system at each 2-week interval to request the next supply of study drug. Unless there is a contraindication to a dose increase (e.g. hypotension or hyperkalemia), the investigator will indicate on the request form approval for dose escalation. 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 SPL (or placebo) will be generated. At the end of the dose titration period, participants will be provided with a 6 week supply of study medication at the indicated dose. At the 12-week and 24-week visits, study drug request forms signed by the Clinical Center investigator will be submitted through the Data Management System to request 12 week supplies of study medication.

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 SPL (or placebo) will be provided. In the event that a participant is already receiving the lowest dose of SPL, placebo will be substituted for the first two down-titrations. As only 3 doses of SPL (12.5, 25 and 50 mg/day) are specified, study medication will be discontinued in the event that a 3rd down titration of dose is required (see **Table 1**). For participants already on placebo, a new supply of placebo will be provided in order to maintain blinding. 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 a total of two weeks. If, due to considerable delays in dose escalation, a participant has not reached the final dose assignment by their Week 12 visit, the participant will remain at the current dose of study drug for the remainder of the study and the treatment phase (i.e., the 30-week post-escalation period) 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 26 weeks rather than 30 weeks.

Table 1. Study Drug Down-Titration Scheme

	Masked Study Drug	**Masked Study Drug**	**Masked Study Drug**	**Masked Study Drug**
Randomized Assignment	Placebo	12.5 mg	25 mg	50 mg
1st Down Titration	Placebo	Placebo	12.5 mg	25 mg
2nd Down Titration	Placebo	Placebo	Placebo	12.5 mg
3rd Down Titration	Discontinue	Discontinue	Discontinue	Discontinue

5.5. Participant Adherence Monitoring

Study drug containers will be returned to site investigators at the conclusion of each 2-week period during dose escalation (weeks 2, 4 and 6), and at the Week 12, 24 and 36 visits. Pill counts will be performed to assess participant adherence with prescribed study medication.

5.6. Concomitant Therapy

Medication use (both oral and intravenous) will be collected at baseline and throughout the course of the study. Digoxin use, dual ACE inhibitor/ARB therapy and non-selective beta blocker use are exclusion criteria. If these medications are initiated during follow-up the treating clinician will be contacted by the research team. For digoxin or dual ACEI/ARB use, if the prohibited drug cannot be discontinued, study medication will be withdrawn and participants will continue to be followed. For non-selective beta blocker use, it is anticipated that the treating clinician will be comfortable substituting a selective beta blocker but if not, study drug will be discontinued and the participant will continue to be followed.

5.7. Packaging

Study drug will be distributed in patient-specific containers with 17 capsules for every 2-week period during the dose escalation phase, 49 capsules for the weeks 6-12, and 91 capsules for weeks 12-24 and weeks 24-36. The extra capsules allow for continued dosing in the event of a short delay in dispensing the subsequent study drug supply. Labeling will include patient name, patient ID, protocol number, study-center, expiration date, and prescribing physician (study investigator).

5.8. Blinding of Study Drug

Generic SPL will be purchased, and matching placebos will be prepared by the University of Pennsylvania IDS. The IDS will send study drug to the Clinical Center research pharmacies and the identity SPL and matched placebo will be concealed from both investigators and participants. Only the IDS and the database programmer will be unblinded. See **Section 8.5** for information on unblinding procedures, if needed.

5.9. Receiving, Storing, Dispensing and Returning Study Drug

5.9.1. Receiving Study Drug Supplies

The University of Pennsylvania IDS will ship study drug to the Clinical Center research pharmacies and will maintain inventory at the pharmacies based on study drug utilization. Each Clinical Center research pharmacy will be responsible for maintaining detailed records regarding the receipt of study drug. General Study Product Accountability, Patient Specific Study Product Accountability, and if necessary, Shipment Tracking Accountability Logs will be maintained by the site pharmacist to document study drug use. Documentation includes study product receipt, storage, dispensing, and final disposition. Study product will be inventoried at least once per month within 30 days of the count for the previous month.

5.9.2. Study Drug Storage

SPL and matched placebo will be stored at <25ºC. Study supplies will be stored in the central IDS pharmacy at the University of Pennsylvania until shipment to the Clinical Center research pharmacy. Study drug will be stored at the Clinical Center's research pharmacy until distribution to the participant.

5.9.3. Dispensing Study Drug

The Clinical Center pharmacist or trained site designee will dispense study drug to the participant and complete a dispensing/accountability log for each participant. Designated site personnel with responsibility for dispensing and reconciling study drug will have appropriate training and documentation of this training will be maintained in the site′s regulatory binder.

5.9.4. Return or Destruction of Study Drug

Study drug containers will be returned by participants at the end of each 2-week, 6-week, or 12-week period and remaining pills will be counted to assess adherence. Unused supplies of study drug will be destroyed by the Clinical Center research pharmacy once the pill count is completed and documented. In the event that a participant does not return study drug containers he/she will be instructed to stop taking any study drug from the non-returned container.

6. Study Procedures

A schedule of study visits and procedures is provided in the Study Procedures Table in **Section 15.1** (Attachments).

6.1. Pre-screening Activities

Dialysis unit records and study center medical records will be reviewed by Clinical Center study personnel to assess eligibility. The treating nephrologist for a potentially eligible patient will be contacted to further assess eligibility and obtain permission to contact the patient. Patients remaining eligible after these procedures will be approached in person at the dialysis unit to confirm eligibility for and interest in participation. Participants signing IRB-approved informational flyers that indicate willingness to be called by study personnel may be pre-screened by telephone.

6.2. Screening Visit (Day −30 to Day 0)

Potential participants expressing interest in enrolling in the study will be scheduled for a Screening visit. This visit may take place at the local dialysis unit or at the Clinical Center and may take place over more than 1 visit. Screening activities must be completed within 30 days prior to the baseline visit.

Informed consent will be obtained and documented before any study procedures are performed. Demographic data (age, sex, race), 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), dialysis unit labs (chemistries, intact parathyroid hormone, complete blood count), and dialysis prescription will be collected during Screening. Blood will be drawn for serum pregnancy testing in women of childbearing potential. Participants will perform an inter-dialytic urine collection prior to or within seven days following the baseline visit, for assessment of residual renal function. The urine collection will begin immediately after dialysis and continue until the start of the next dialysis session, and will be performed during one of the 44-hour (approximately) inter-dialytic periods rather than during the 68-hour (approximately) inter-dialytic period.

Potential participants will be excluded if there is any serum potassium value of ≥6.5 mEq/L within 3 months prior to the Baseline/Randomization visit or if there is any serum potassium value ≥6 mEq/L within 2 weeks prior to the Baseline/Randomization visit. It is required that there be at least one serum potassium value obtained within 2 weeks prior to Baseline. The potassium measurement can be obtained either through routine clinical testing or as a research test. Participants who do not meet eligibility can be rescreened one additional time.

If the participant has agreed to participate in the heart rhythm monitoring sub-study, the first monitoring will be performed between the screening and baseline/randomization visits. The participant will wear the Medtronic SEEQ heart monitor for 7 consecutive days. Participants will receive the monitor kit from the research team and will return the kit after the monitoring period.

The heart rhythm data will be transmitted in batches to the DCC and will not be analyzed until a participant has completed participation in the study. Reports will not be generated for clinical use by the investigators, treating physicians, or participants. Participants will be informed of this at the time of enrollment and will be instructed to report to the research team or their treating physician any concerning symptoms they have while wearing the heart rhythm monitor. The same approach will be used for heart rhythm monitoring that takes place at 6 weeks and 32-36 weeks.

6.3. Baseline/Randomization Visit (Day 0)

The Baseline/Randomization visit will take place on a non-dialysis day during the short (approximately 44-hour) inter-dialytic interval. At this visit, medication use, vital signs, a brief physical examination and adverse events will be recorded, and eligibility will be confirmed. Once eligibility has been confirmed, randomization to treatment group will be performed through the centralized Data Management System.

Serum and plasma will be collected, aliquoted, and shipped for batched measurement of cardiac and inflammatory markers and for long-term storage. Whole blood will be collected for DNA extraction. Blood will also be drawn for local measurement of chemistries and complete blood count. If blood cannot be collected on the day of the Baseline/Randomization visit (Day 0), it should be collected the following day prior to the dialysis session and before the initiation of study drug. If blood for batched analyses, long-term storage, and DNA extraction cannot be obtained on the day of randomization or the following day, it is acceptable to obtain the blood within the 10 days prior to the Baseline/Randomization visit, and, if on a dialysis day, prior to initiation of the dialysis session. However, the blood for local measurement of chemistries and

complete blood count will be obtained on the day of or the day after the Baseline/Randomization visit rather than within 10 days prior.

Tissue Doppler Index echocardiography (TDI echo) will be performed on the day of the Baseline/Randomization visit. If the TDI echo cannot be performed at that visit (Day 0), it should be completed as soon as possible thereafter and within 7 days after the Baseline/Randomization visit. The echo must be performed on a non-dialysis day during the short (approximately 44 hour) inter-dialytic interval. A subset of participants will have positron emission tomography (PET scan) (after exclusion of pregnancy).

A 2-week supply of study drug will be dispensed and the participant will be instructed to start the study drug the following day, after dialysis.

6.4. Week 1, Week 2, Week 3, Week 4, Week 5, and Week 6 Visits (window for each: -4 to +3 days)

Blood will be obtained for measurement of serum potassium at 3-5 days and 2 weeks after the initiation of each new study drug kit during the dose escalation phase. Participants will be interviewed weekly in person or by telephone for the occurrence of serious adverse events and adverse events of interest. Adverse events of interest include hypotension, gastrointestinal symptoms, gynecomastia/breast tenderness, hyperkalemia and rash (**see Section 8.3.1**). Dialysis unit records will be reviewed for inter-current laboratory values, the occurrence of hypotension, and changes to the dialysis prescription. The dose of study drug may be reduced if a participant experiences dose-limiting adverse events, as outlined in **Section 6.8 and 6.9**.

A new supply of study drug will be dispensed and used pill containers returned at the week 2, week 4, and week 6 visits as described in sections **5.9.3 and 5.9.4**. Pill counts will be performed to assess participant adherence with study medication, and unused study drug will be destroyed by the Clinical Center research pharmacies once the pill count has been documented.

At the end of the dose escalation phase, participants in the heart rhythm monitoring sub-study will undergo heart rhythm monitoring using the Medtronic SEEQ device. The participant will wear the Medtronic SEEQ heart monitor for 7 consecutive days. Participants will receive the monitor kit from the research team and will return the kit after the monitoring period.

6.5. Week 8 (-4 to +3 days), Week 12, Week 16, Week 20, Week 24, Week 28, and Week 32 Visits (-7 to +7 days for all except Week 8)

Participants will be contacted at weeks 8, 12, 16, 20, 24, 28 and 32 either in person or by telephone to review the occurrence of serious adverse events and adverse events of interest as described in **Section 6.4**. Dialysis unit records will be reviewed for inter-current laboratory values, the occurrence of hypotension, and changes to the dialysis prescription. If a serum potassium value is not available in the participant's dialysis unit record, corresponding to the date of the monthly study visit (plus window), blood will be obtained from the participant for measurement of serum potassium. The dose of study drug will be reduced for participants experiencing dose-limiting adverse events as outlined in **Sections 6.8 and 6.9**.

New study drug will be dispensed and used pill containers returned at the week 12 and week 24 visits as described in **Sections 5.9.3 and 5.9.4**. Pill counts will be performed to assess participant adherence with study medication, and unused study drug will be destroyed once the pill count has been documented.

Participants in the heart rhythm monitoring sub-study will undergo heart rhythm monitoring using the Medtronic SEEQ device for a continuous 7 day period between Weeks 32 and 36. Participants will receive the monitor kit from the research team and will return the kit after the monitoring period.

6.6. Week 36 Visit (End of Treatment) (-7 to +7 days)

At the Week 36 visit, participants will be interviewed in person to review the occurrence of adverse events as described in **Section 6.4**. Dialysis unit records will be reviewed for inter-current laboratory values, the occurrence of hypotension, and changes to the dialysis prescription. Pill containers will be returned and unused pills counted and destroyed. Serum and plasma will be collected, aliquoted, and shipped for batched measurement of cardiac and inflammatory markers and for long-term storage. Blood will also be drawn for local measurement of chemistries, complete blood count and, for women of childbearing potential, serum pregnancy testing. If the blood collection is performed on a dialysis day, the collection should take place prior to the dialysis session. End-of- study TDI echo will be performed and a subset of participants will have a PET scan performed (following exclusion of pregnancy).

6.6.1. Early Withdrawal Visit

If a patient withdraws from the trial before the Week 36 milestone or if it becomes known that a participant will not be available for an in-person visit at Week 36 (e.g., due to relocation), an Early Withdrawal visit will be scheduled to conduct the final comprehensive assessments that otherwise would be performed at Week 36. For participants in the heart rhythm monitoring sub-study, the assessments will include heart rhythm monitoring using the Medtronic SEEQ device for 7 continuous days at the time of early withdrawal, if possible. Patients who stop taking study medication are not considered early withdrawals and will be asked to continue to provide follow-up data on the study schedule and to participate in the final comprehensive assessment at Week 36.

6.7. Week 40 Visit (-7 to +7 days)

At the Week 40 visit (4 weeks after end of treatment), patients will be interviewed in person or by telephone to review the occurrence of adverse events as described in **Section 6.4**. Dialysis unit records will be reviewed for inter-current laboratory values, the occurrence of hypotension, and changes to the dialysis prescription.

6.8. Hypotension Management

In the event of recurrent intra-dialytic hypotension or serious hypotension not attributable to acute events (see **Section 3.2.2** for definitions of intra-dialytic hypotension and serious hypotension), study investigators 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 drug 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 at the 3rd dose reduction

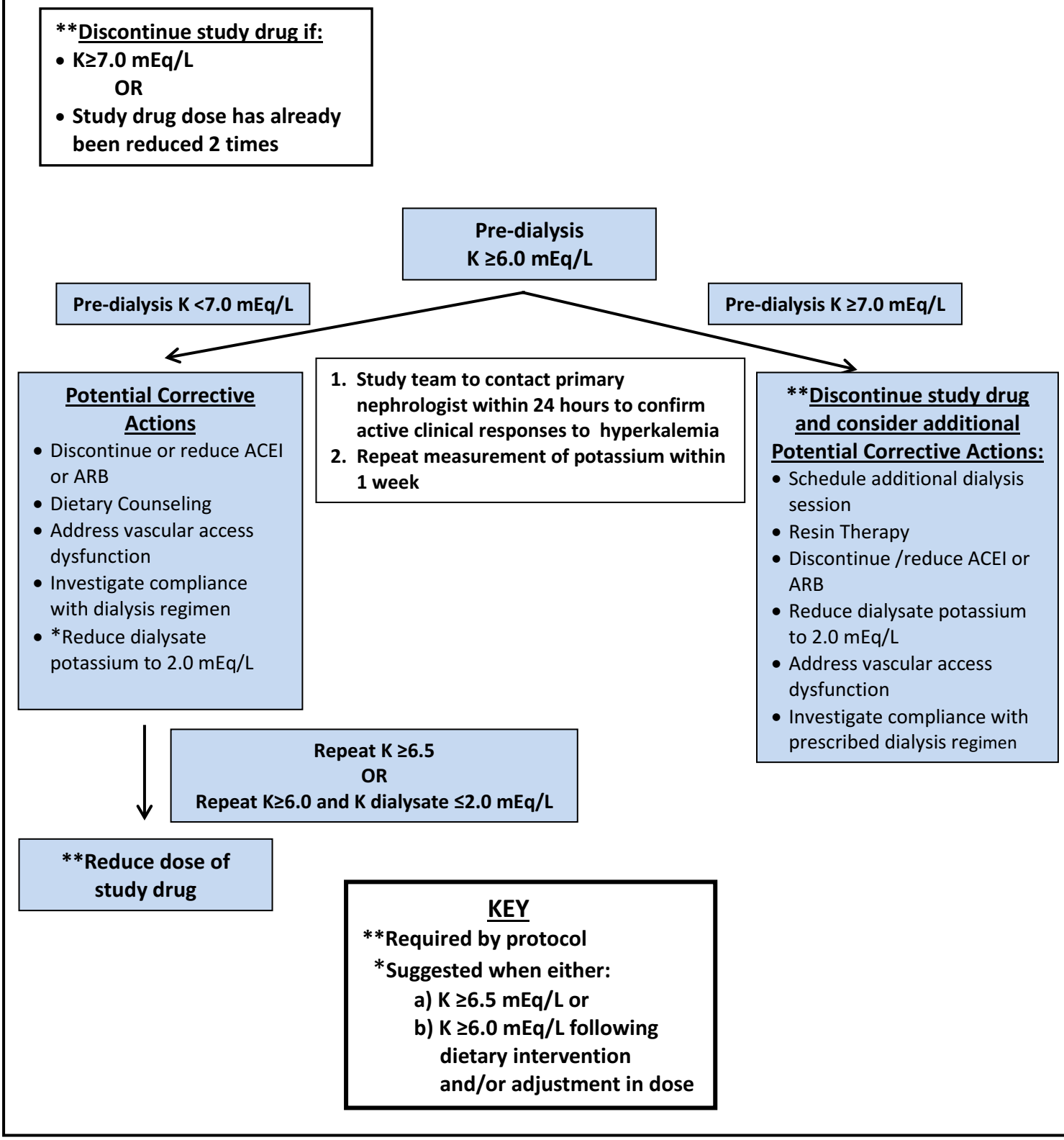
Treating clinicians will be free to manage hypotension according to standard clinical practices and are not obligated to follow the study guidelines with respect to non-study medication, fluid removal rate, or dry weight. In contrast, dose reduction or discontinuation of study drug will be directly managed by study staff in accordance with the guideline.

6.9. Hyperkalemia Management

Serum potassium will be measured at 3-5 days and 2 weeks after study drug initiation or dose increase as well as monthly throughout the duration of treatment. In order to minimize the risks of hyperkalemia, all participants will be provided with standardized, routine printed dietary information about dietary sources of potassium at the Baseline/Randomization visit. Treating clinicians will continue to manage the dialysate potassium concentration according to local standards. A hyperkalemia management guideline will be provided to treating clinicians. Study staff will monitor potassium values and contact treating clinicians in the event of hyperkalemia to review the management guidelines and ensure that hyperkalemia is being actively managed. Treating clinicians will be free to manage hyperkalemia according to local standards. However, study drug dose reductions or discontinuation will be directly managed by study investigators in accordance with the Hyperkalemia Management algorithm (see **Figure 2**).

As shown in **Figure 2**, study drug will be discontinued if serum potassium is ≥7.0 mEq/L. Study drug will also be discontinued if a 3rd study drug dose reduction is required. Study drug dose will be reduced if serum potassium is 6.0 – 6.9 mEq/L and either a) remains ≥6.5 mEq/L on repeat measurement or b) remains ≥6 mEq/L on repeat measurement despite a dialysate potassium bath concentration of ≤2.0 mEq/L. Reversible causes of hyperkalemia should be considered and addressed when serum potassium is ≥6.0 mEq/L even if there are changes to study drug dosing. Reversible causes of hyperkalemia include vascular access dysfunction, missed dialysis sessions, shortened or technically compromised dialysis sessions, dietary indiscretion and ACEI or ARB use. In general, these reversible causes should be addressed prior to reducing the dialysate potassium concentration. Use of 1.0 mEq/L dialysate potassium is not recommended. However, treating clinicians are not prohibited from using low potassium dialysate solutions in accordance with local practices. Serum potassium will be rechecked within one week when potassium is ≥6.0 mEq/L. Results of serum potassium and recommendations for change in dialysis orders will be conveyed to treating clinicians and the dialysis unit nursing staff.

Figure 2. Management of Hyperkalemia



6.10. Gynecomastia, Gastrointestinal Symptoms or Rash

In the event of persistent gynecomastia/breast tenderness, nausea, vomiting, diarrhea, or anorexia without alternative cause, the dose of study medication may be reduced. Study drug should be discontinued if there is a rash that is not attributable to another cause.

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 assessing safety and feasibility, with no attempt to create critical test result regions for standard hypothesis testing.

From preliminary studies, we assumed estimated baseline parameters of E′ to be 5.8 ± 1.8 cm/s.[34, 43, 87, 88] Assuming a correlation ≥ 0.2 between pre and post-therapy E′, and a 10% dropout rate of study participants by the end of the study, a sample size of 125 (2:3; 50 patients for the placebo arm and 25 per SPL dose x 3 active treatment arms) will provide 80% power to detect effect size of 0.7SD (1.3 cm/sec) difference in change from baseline to 9 month endpoint in E′ , equivalent to a 22% average reduction across the 3 active SPL treatment arms. Short-term studies with SPL in diabetes were consistent with an effect of this magnitude[54].

For the safety endpoint of hyperkalemia incidence, we assumed a 5% incidence in the placebo arm. With a sample size of 125, derived under the effect size considerations noted previously, this design provides 80% power to detect an average incidence of 22% across the 3 SPL dose arms. Moreover, this same sample size also provides 80% power to detect a small difference of 0.1 mEq/L in time-averaged serum potassium concentration, treated as a continuous variable, between placebo and treatment arms.

7.2. Randomization and Stratification

To ensure balance in treatment assignments within potential confounders, a stratified randomization procedure, blocking on four (4) strata formed by the 2 x 2 cross-classification of 1) dialysis vintage (≥ 1 vs. < 1 year on dialysis) by 2) ACEI or ARB use (yes, no), within Clinical Centers will be implemented within a web-based randomization module deployed centrally within the DCC. Within each of these strata, participants will be randomly allocated to the placebo and the three SPL dose arms with the ratio of 2:1:1:1.

The treatment assignment code, corresponding to each treatment identifier number, will be known only to the person serving as the DCC Quality Assurance Director and the IDS, until the completion of treatment and data collection on all participants. At the end of a patient's study phase, the participants and the treating physicians will be asked to guess their treatment groups, and provide the basis for their judgments for analysis later, to determine whether the blinding has been broken. However, except in the case of emergency unmasking, the treatment codes will not be identified until the DSMB has approved unblinding in preparation for the public dissemination of results.

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 36 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:

- All-randomized population/intention-to-treat (ITT): Any participant randomized into the study, regardless of whether they received study drug.
- As-treated population: Any participant randomized into the study regardless of whether they received study drug. As-treated participants will be analyzed according to the dose of study drug actually received.
- All-treated population: Any participant randomized into the study that 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 36 ± 2 weeks.

Because dose-related efficacy and safety are primary 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[89]. 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[89]. 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 hyperkalemia. 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 study 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.

7.4.1. Descriptive Analyses and Primary Efficacy and Safety Analyses

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 theresuch 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 three treatment groups will be compared using appropriate k-sample tests, including Kruskal-Wallis 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 at 36 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 primary safety endpoint, 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 change in potassium level in placebo and treatment groups with monthly measure of potassium. Mixed effects linear regression models will be used to assess the direction and time averaged magnitude of change in potassium, with and without controlling for baseline covariates. The proportion of serious hyperkalemia in treatment and placebo groups will be compared with Chi-square test or Fisher's exact test.

The primary analysis will examine the intention to treat population. All analyses will be repeated in the as-treated, all-treated and per-protocol populations (see **Section 7.3**). Secondary endpoint analyses will be presented 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 cytokine concentration/markers of inflammation, circulating markers of fibrosis, aldosterone concentration, heart rate variability (sub-study), arrhythmias (sub-study), additional echocardiographic parameters including change in resting and hyperemic myocardial blood flows between baseline and 36 weeks and change in LVMI between 0 and 36 weeks, and coronary flow reserve (CFR) on myocardial PET (sub-study). 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.

7.4.3. Interim Analysis

No interim efficacy 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 exam 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 Problems Involving Risk to Participants or Others

(See also Section 8.3.4)

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

- 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;
- 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
- 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.

8.1.5. General Physical Examination Findings

At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

8.2. Adverse Event Tracking Period

The study period during which adverse events must be tracked and reported is normally defined as the period from the initiation of study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is 4 weeks following the last dose of study drug.

8.2.1. Post-study Adverse Event

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 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. The sponsor (NIDDK) will also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a participant that has participated in this 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. Adverse Events of Interest

The following adverse events are anticipated in patients treated with spironolactone and will be recorded on the appropriate Adverse Event of Interest CRF.

- Hyperkalemia
- Hypotension
- Gynecomastia
- Breast tenderness
- Gastrointestinal symptoms including: diarrhea, constipation, and nausea
- Rash

If the event meets the definition of serious as defined in **Section 8.1.2**, it will be recorded on an SAE form in addition to the appropriate adverse event of special interest CRF.

Participants will be contacted weekly during dose escalation and monthly while on treatment to assess for the occurrence of Adverse Events of Interest. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

8.3.2. Trial-Defined Events

The hemodialysis population undergoes frequent laboratory testing and has a high rate of peri-dialytic hypotensive events requiring changes 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 will not be recorded as an adverse event except as noted:

- Anemia – will be reported when hemoglobin is <8.0 mg/dL
- Hyperkalemia – will be reported when potassium level ≥6.0 mEq/L
- Hyperphosphatemia – will be reported when phosphorous >9.5 mg/dL
- Hypocalcemia – will be reported when serum calcium <7.0 mg/dL
- Hypercalcemia – will be reported when serum calcium >11.0 mg/dL
- Hyperparathyroidism – will be reported when PTH >1000 pg/mL
- Hypotension – will be reported when it meets the criteria for recurrent or symptomatic intra- or inter-dialytic hypotension as defined in **Section 3.2.2**.

8.3.3. Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if:

- The laboratory abnormality is not otherwise refuted by a repeat test that was performed specifically to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity

- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.
- The abnormality meets the criteria in **Section 8.3.2.**

8.3.4. Anticipated Adverse Events

The following adverse events are anticipated in the hemodialysis population and will not be considered to be 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
- Vascular Access Events Including:
    - Catheter exchange, removal or declotting
    - Arteriovenous graft or fistula complications
        - Clotting
        - Stenosis
        - Revascularization
        - Infection
- Infections Including:
    - Pneumonia
    - Vascular access infection
    - Bacteremia
    - Clostridium difficile infection

8.4. Reporting of Serious Adverse Events and Unanticipated Problems

Study sites are required to report SAEs and Unanticipated Problems to the DCC within 24 hours of first knowledge of the event. To report such events, an SAE form or an Unanticipated Problem form will be completed by the investigator and faxed to the DCC. The DCC will facilitate the timely medical review and reporting of the event to the NIDDK, the DSMB, and the study sites in accordance with DSMB-approved study policies and IRB requirements.

The investigator will keep a copy of the SAE form / Unanticipated Problem 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 treatment

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 or Unanticipated Problem Form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the DCC.

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.

If a patient becomes pregnant while participating in the trial it will be reported as an adverse event and will trigger the collection of additional information about the pregnancy on the Pregnancy Report Form. Pregnancy outcomes will be collected, including the outcome of the infant and if the pregnancy was terminated. Study Staff should complete the Pregnancy Report form when they learn about a pregnancy, and follow-up with the participant and update the form when additional information is available about the baby. The Pregnancy Report form will be submitted to the University of Pennsylvania IRB, and to the local site IRB as required.

8.4.1. Investigator Reporting to the IRB

Site investigators will report SAEs and Unanticipated problems to their 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 experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, 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.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken 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 Subpart C and the investigator believes it is in the best interest of the participant to remain on 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 Participating Investigators

The DCC will notify all Clinical Center principal investigators, in a written safety report, of any adverse event that meets the criteria for an unanticipated problem as described in **Section 8.1.3**.

8.5. Unblinding Procedures

Unblinding of study drug may be considered when necessary for safety purposes. Examples of these circumstances may include, but are not limited to, serious allergic reactions such as Stevens-Johnson syndrome, or overdose of study drug. In the event that un-blinding of study drug is viewed by the Clinical Center Principal Investigator, or an appropriate designee, as necessary for safety purposes, the investigator will contact the DCC. The circumstances of the event and rationale for unblinding will be described to the DCC Principal Investigator, or an appropriate designee, who may authorize unblinding if deemed necessary to preserve participant safety. Following this, an SAE report will be submitted within 24 hours to the DCC and to the local IRB as described in **Sections 8.4 and 8.4.1.** The circumstances and need for unblinding should also be appropriately documented in the source documents.

8.6. Stopping Rules

Given that the primary focus of this pilot trial is on safety, tolerability and feasibility, there are no pre-specified stopping rules for efficacy outcomes. However, the Consortium investigators will monitor all safety endpoints for evidence of differential safety effects, and routinely update the NIDDK and the DSMB. The DSMB may recommend study termination on the basis of unacceptable increases in adverse events or external data.

8.7. Medical Monitoring

Each 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 (See **Section 9**). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.7.1. 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. The DSMB charter for the Hemodialysis Novel Therapies Consortium is provided as an attachment in **Section 15.2**.

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 prior to initiation of the study. After initial approval during the course of the study, the primary responsibilities of the DSMB will be to:

- Review safety data and provide input to protect the safety of the study participants;
- Provide input on major changes to the research protocol and plans for data and safety monitoring;
- 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 sites, and other factors that may affect study outcomes;
- 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;
- 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 registration system designed by the DCC will be used for all of the pilot and feasibility studies of the Hemodialysis Novel Therapies Consortium in order to promote uniformity across studies. The central registration system will include a randomization module for each study that will confirm eligibility. Central participant registration will also allow the DCC to generate recruitment reports across concurrent studies.

An Oracle Clinical data management system designed by the DCC will be used for the collection, storage and management of data. Site personnel will enter data directly using Oracle Clinical Remote Data Capture. Electronic case report forms (eCRFs) will incorporate range and logical edit checks, both within and across forms. Data entry will be followed daily with manual and programmed checks and edits for errors and omissions.

9.1. Data Quality

The DCC will collaborate with the Clinical Center investigators to establish parameters for primary and secondary outcomes, safety, and descriptive values. The data management team will use a data validation plan, rule set specifications, and programming logic to implement data validation rules. The DCC staff will interact with Clinical Center study staff to verify queried data and track all queries to resolution.

9.1.1. Quality Control Activities

The Quality Control Committee and the DCC will develop a quality assurance and control plan that ensures that study data are as precise and reliable as possible.

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.

Training and certification procedures – The DCC will conduct a training session before the study starts to train and certify personnel in the performance of study procedures.

Site visits – Site visits will be conducted as outlined in the Study Monitoring Plan. Findings from site visits will be used to resolve problems and develop corrective action plans.

External data sources – The DCC will monitor quality control of data received from study 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 DCC 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 and passwords.

Study data collected at the Clinical Centers will be entered into Oracle Clinical. This data management system uses a secure connection between the client browser at the Clinical Center 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 to the DCC.

Where applicable, electronic files containing data from hand held devices, central laboratories, or central reading centers will be transferred to the DCC using secure FTP technology. The DCC team will maintain a secure FTP server. The files transmitted using this method will be encrypted during the exchange.

The DCC project team will collaborate with the IDS and the biostatistics team 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. Documentation is stored in the locked files of the IDS at the University of Pennsylvania. Within the DCC this information is locked in files to which only department managers have access.

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:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- 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 that 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.

9.2.2. Data Linkage

Participants will be asked to consent to provide their Social Security Number (SSN) to facilitate access to long term clinical outcomes after study participation has ended, in national databases at the Social Security Administration, the Center for Medicare and Medicaid Services (CMS) and the United States Renal Data Systems (USRDS). Providing the SSN and access to national databases is optional, and is not required for participation in the study.

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: dialysis unit records, hospital records, clinical and office charts, laboratory reports, memoranda, participant 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. Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. "N/D" will be used to indicate on the CRF that a procedure was not done or a question was not asked rather than leaving a space blank. "N/A" will be used to indicate that an item is not applicable to the individual case. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. Erasing or white-out will not be used for errors. For clarification of illegible or uncertain entries, the clarification will be printed above the item, and the clarification will be initialed and dated.

9.3.2. Maintaining Anonymity of Submitted Medical Records

Clinical site personnel will de-identify all medical records before sending them to the DCC by obliterating any Protected Health Information (PHI). Upon receipt, DCC personnel will review the records to ensure that no PHI is visible.

If a SSN is provided by the participant during the informed consent process, it will be maintained in a restricted-access section of the data management system, and only DCC personnel who are directly involved in the collection of long term clinical outcomes data from national databases will be granted access. Once the necessary outcome data are obtained, the data will be linked to the participant's study ID number. The SSN will be excluded from any shared data.

9.3.3. Data and Biosample Sharing

Research results will be made available to the scientific community and public in a timely manner. The primary method by which data will be shared with the scientific community will be through peer-reviewed publications and presentation at scientific and professional society meetings. In addition, data and results will be submitted to the NIH in the annual progress reports required under the terms and conditions of the funding award. This study will also be registered with clinicaltrials.gov prior to initiation.

Data from the 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 investigators.

A portion of the serum and plasma collected at Baseline and Week 36, as well as extracted DNA, will be submitted to the NIDDK Biosample Repository for future investigations. The NIH Data and Biosample Repositories will meet all NIH standards, and will provide data and/or specimens to researchers in accordance with IRB, HIPAA, and NIH procedures that protect the confidentiality of participants.

9.3.4. Records Retention

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

10. Study Compliance

10.1. Protocol Deviations and Exceptions

A protocol deviation is any change or alteration to the IRB-approved protocol without prospective IRB approval. A protocol exception is a one time, intentional change or alteration to the IRB-approved protocol that is approved by the IRB prior to implementation.

Major Deviation: Any change/alteration that has or has the potential to: 1) adversely affect the rights, welfare or safety of the subjects, 2) adversely affect the integrity of the research data, or 3) affect the subject's willingness to participate.

Examples of Major Deviations

- Failure to obtain informed consent (i.e., no documentation of informed consent or informed consent was obtained after initiation of study procedures)
- Informed consent obtained by someone not approved to obtain consent for the protocol
- Use of invalid consent form (i.e., consent form without IRB approval stamp, or outdated/expired consent form)
- Enrollment of a participant who is ineligible for the study
- Performing a research procedure not in the approved protocol
- Failure to report serious adverse event to sponsor/DCC
- Study medication dispensing or dosing error
- Failure to follow the approved study protocol that affects participant safety or data integrity (e.g., failure to properly schedule study visits or failure to perform laboratory tests)
- Continuing research activities after IRB approval has expired
- Use of recruitment procedures that have not been approved by the IRB
- Participant giving study medication to a third-party
- Enrolling significantly more participants than approved in the IRB protocol

Minor Deviation: Any change/alteration that has not or does not have the potential to: 1) adversely affect the rights, welfare or safety of the subjects, 2) adversely affect the integrity of the research data, or 3) affect the subject's willingness to participate.

Examples of Minor Deviations

- Delay in a study visit beyond the protocol-defined window
- Failure to obtain a blood sample for laboratory measurements that are not related to safety monitoring

10.2. Study Monitoring Plan

A monitoring plan that includes formal visits to the Clinical Centers by members of the Consortium (DCC, Clinical Center investigators and study coordinators, and NIDDK representatives) will be developed by the Consortium Executive Committee. Clinical Center investigators will allocate adequate time for such monitoring activities. The Principal Investigator will also ensure that the monitor and other compliance or quality assurance reviewers are given access to study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and have adequate space to conduct the monitoring visit.

10.3. Auditing and Inspecting

The DCC and Clinical Center investigators 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 DCC and Clinical Center investigators 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 will 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. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the Clinical Center investigator and a copy of this decision will be provided to the sponsor before commencement of the study at the site.

All study participants will be provided a consent form describing the study and providing sufficient information to make an informed decision about participating in the study. The consent form will be submitted with the protocol for review and approval by the EC/IRB. 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 grants from the National Institute of Diabetes and Digestive and Kidney Diseases of the U.S. National Institutes of Health.

12.2. Conflict of Interest

All investigators 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 Stipends or Payments

Participants may be compensated for parking and time and effort required to participate in the study. Compensation approaches will be determined by the Clinical Centers and approved the local EC/IRB.

13. Publication Plan

Neither the complete, nor any part of, the results of the study carried out under this protocol, nor any of the information provided by the Hemodialysis Novel Therapies Consortium for the purposes of performing the study, will be published or passed on to any third party without the consent of the Consortium Executive Committee. Any investigator involved with this study is obligated to provide the Data Coordinating Center with complete test results and all data derived from the study.

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15. Attachments

15.1. Study Procedures

	SCREENING		BASELINE	DOSE ESCALATION PHASE WEEKS 1 – 6						TREATMENT PHASE WEEKS 7 – 36								FOLLOW-UP	PRN
Location	Facility or Study Center		Study Center	In person or phone						In person or phone							In person	Phone	as needed
Procedure	Prescreening Activities	Screening Visit	Baseline/Rand Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	prn
Visit Window (days)		Day -30 to 0	Day 0	Day -4 to +3	Day -4 to +3	Day -4 to +3	Day -4 to +3	Day -4 to +3	Day -4 to +3	Day -4 to +3	Day -7 to +7	Day -7 to +7	Day -7 to +7	Day -7 to +7	Day -7 to +7	Day-7 to +7	Day -7 to +7	Day -7 to +7	
Preliminary eligibility assessment	X																		
Informed consent		X																	
Confirm eligibility		X	X																
Demographic info & medical history		X	X																
Concomitant medications			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Serum pregnancy		X															X		X
Vital signs			X														X		
Focused physical exam			X																
Inter-dialytic urine			X[1]																
Randomization			X																
Chemistries, CBC, LFTs			X								X			X			X		
Serum Potassium[2]		X		X	X	X	X	X	X	X	X	X	X	X	X	X			X
Dispense study medication			X		X		X		X		X			X					X
Reconcile study medication					X		X		X		X			X			X		X
TDI Echocardiography			X														X		
Review adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review facility clinical info & lab results		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collect serum/plasma for storage[3]			X														X		
Dose adjustment (prn)				X						X									X
PET Scan (substudy)			X[4]														X[4]		
SEEQ Heart Monitor		X							X							X[5]	X[5]		

[1] Inter-dialytic urine collection is performed before or within 7 days after the Baseline/Randomization visit.
[2] Serum potassium value that is obtained for clinical purposes can be used if the collection date falls within the visit window.
[3] At Baseline, blood will also be collected for DNA extraction.
[4] Serum pregnancy test performed prior to PET scan at Baseline/Randomization and Week 36.
[5] SEEQ Heart Monitoring should occur between the Week 32 and Week 36 Visits.

15.2. NIDDK DSMB Charter

Data and Safety Monitoring Board (DSMB) Charter
HD Novel Therapies Consortium

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) to monitor patient safety and evaluate the efficacy of the interventions. The HD Novel Therapies Consortium – Spin-D Protocol/Trial is funded by the NIDDK.

DSMB RESPONSIBILITIES

The initial responsibility of the DSMB will be to review the study protocols, consent documents and plans for data safety monitoring, and approve the initiation of these clinical trials. After this approval, and at periodic intervals during the course of the trials, the DSMB responsibilities are to:

- review and approve major changes in the research protocol, informed consent documents and plans for data safety and monitoring, including all proposed revisions;
- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that may affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- protect the safety of the study participants;
- report on the safety and progress of the trial;
- make recommendations to the NIDDK, the Steering Committee and, if required, to the Food and Drug Administration (FDA) and the Institution Review Boards (IRBs) concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- if appropriate, conduct interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis and have the approval of the DSMB;
- ensure the confidentiality of the trial data and the results of monitoring;
- assist the NIDDK by commenting on any problems related to study conduct, enrollment, sample size, and/or data collection.

MEMBERSHIP

The DSMB will consist of at least eight members. Five participating members will constitute a quorum. The members have been appointed by the NIDDK. Members of the DSMB shall have no financial, scientific, or other conflict of interest with the studies. Collaborators or associates of the investigators in this trial are not eligible to serve on the DSMB. Written documentation attesting to absence of conflict of interest is required.

Dr. Paul Palevsky of University of Pittsburgh School of Medicine has been selected by the NIDDK to serve as the DSMB Chairperson for the remainder of the study. He is responsible for overseeing the meetings and developing the agenda in consultation with the NIDDK Program Directors, Dr. Paul Kimmel and Dr. John Kusek. Dr. Kimmel will serve as the DSMB Executive Secretary. The Chairperson is the contact person for the DSMB. Other NIDDK official (s) or

NIDDK appointee (s) may serve as an ex-officio member (s) of the DSMB. The DCC, University of Pennsylvania, shall provide the logistical management for the DSMB, in coordination with NIDDK (Dr. Yining Xie, as point of contact). Whenever possible, Dr. Robert Star, Director of the Division of Kidney, Urology and Hematology of NIDDK will also attend meetings.

BOARD PROCESS

The DSMB will meet a minimum of once a year at the call of the Chair, with advance approval of the NIDDK Program Director. An NIDDK representative will be present at every meeting.

Meetings shall be closed to the public because discussions may address confidential patient data. Meetings are attended, when appropriate, by the principal investigator and members of his/her staff. Meetings may be convened as conference calls/webinars as well as in person. An emergency meeting of the DSMB may be called at any time by the Chairperson or by the NIDDK Program Director should questions of patient safety arise. The DSMB Chairperson should contact the NIDDK Program Director prior to convening the meeting.

MEETING FORMAT

An appropriate format for DSMB meetings consists of open, closed and executive sessions. This format may be modified as needed. A brief closed and/or an executive session will usually be held before the open session.

Open Session:

The members of the DSMB, the NIDDK staff, the steering committee, including the study biostatistician will attend the open session. Issues discussed will include the conduct and progress of the study, including patient recruitment, data quality, general adherence and toxicity issues, compliance with protocol, and any other logistical matters that may affect either the conduct or outcome of the study. Protocol amendments may also be presented in this session.

Closed Session:

The closed session will be attended by voting DSMB members, representatives from the NIDDK, or its appointees, and the study biostatistician. **The discussion at the closed session is completely confidential.**

Analyses of blinded outcome data are reviewed by masked intervention groups, including baseline characteristics, primary and secondary outcomes, adverse events, adherence and dropouts, and examination of any relevant subgroups. However, the DSMB may request unmasking of the data for either safety or efficacy concerns.

Executive Session:

The executive session will be attended by voting DSMB members, and the NIDDK Staff, or its appointees.

The DSMB will discuss information presented to it during the closed and open sessions and decide whether to recommend continuation or termination, protocol modification or other changes to the conduct of the study in the Executive Session. The DSMB can become unblinded if trends develop either for benefit or harm to the participants.

Should the DSMB decide to issue a termination recommendation, a full vote of the DSMB will be required. In the event of a split vote, majority vote will rule and a minority report should be appended. Reasons for early termination may include:

- Serious adverse effects in the entire intervention group or in a dominating subgroup;
- Greater than expected beneficial effects;
- A statistically significant difference by the end of the study is improbable;
- Logistical or data quality problems so severe that correction is not feasible.

Final Open Session (optional):

The final session may be attended by voting DSMB members, steering committee members, the study biostatistician or other study members, and the NIDDK staff.

The Chairperson of the DSMB or the NIDDK Staff shall report on the recommendations of the DSMB regarding study continuation and concerns regarding the conduct of the study. Requests regarding data presentation for subsequent meetings will be made. Scheduling of the next DSMB meeting may be discussed.

REPORTS

Interim Reports: Interim reports will be prepared by the Data Coordinating Center, located at the University of Pennsylvania. The reports will be distributed to the DSMB and the NIDDK Program Director at least 7 days prior to a scheduled meeting. These interim reports are numbered and provided in sealed envelopes within an express mailing package or by secure email as the DSMB prefers. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be directed by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts:

Part 1 (**Open Session Report**) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status. This report is generally shared with all investigators involved with the clinical trial. The reports contained in this section may include:

- Comparison of Target Enrollment to Actual Enrollment by Month
- Comparison of Target Enrollment to Actual Enrollment by Site
- Overall Subject Status by Site, including: Subjects Screened, Enrolled, Active, Completed and Terminated
- Demographic and Key Baseline Characteristics by Group
- Treatment Duration for Subjects who Discontinue Therapy
- Adverse Events/Serious Adverse Events by Site and Subject

Part 2 (**Closed Session Report**) may contain data on study outcomes, including safety data, including serious adverse events or termination. Data will be presented by blinded treatment groups; however, the DSMB may request that the treatment groups be unblinded to ensure that there are no untoward treatment effects. This report should not be viewed by any members of the clinical trial except the designated study statistician.

Reports from the DSMB: A formal report containing the recommendations for continuation or modifications of the study, prepared by the Executive Secretary with concurrence of the DSMB, will be sent to the Chair of the Steering Committee and the DCC PI. This report will also contain any recommendations of the NIDDK in reference to the DSMB recommendations. It is the

responsibility of the DCC PI to distribute this report to all other PIs and to assure that copies are submitted to all the IRBs associated with the study.

Each report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by formal majority vote. A termination recommendation may be made by the DSMB at any time by majority vote. The NIDDK is responsible for notifying the Chair of the Steering Committee of a decision to terminate the study. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report. The report should not include unblinded data, discussion of the unblinded data, or any other confidential data.

Mailings to the DSMB: On a scheduled basis, (as agreed upon by the DSMB) blinded safety data should be communicated to all DSMB members and the NIDDK Program Director. Any concerns noted by the DSMB should be brought to the attention of the NIDDK Program Director.

Access to Interim Data: Access to the accumulating endpoint data should be limited to as small a group as possible. Limiting the access to interim data to the DSMB members relieves the investigator of the burden of deciding whether it is ethical to continue to randomize patients and helps protect the study from bias in patient entry and/or evaluation.

CONFIDENTIALITY

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.