PROTOCOL

Pilot Study of Loop Diuretics Among Individuals Receiving Hemodialysis

NCT number NCT04622709
Document Date 10/15/2020

Complete Title: Pilot study of loop diuretics among individuals receiving hemodialysis

Short Title: Loop diuretic pilot study

Drug or Device Name(s): Furosemide

FDA IND/IDE (if applicable): Exempt

Sponsor: NIH/NIDDK

Protocol Date: 10/15/2020

Amendment 1 Date: 1/07/2020 (Version 2.0)

Amendment 2 Date: 2/4/2020 (Version 2.1)

Amendment 3 Date: 03/09/2020 (Version 2.2)

Amendment 4 Date: 10/15/2020 (Version 2.3)

Sponsor: NIH/NIDDK

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Protocol Version: 2.3

Version Date: 10/15/2020

I confirm that I have read this protocol and understand it.

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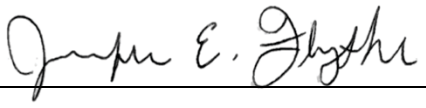
Principal Investigator Signature: 

Date: 10/15/2020

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
BP	Blood pressure
EHR	Electronic health record
ESKD	End-Stage Kidney Disease
FDA	Food and Drug Administration
HD	Hemodialysis
IDS	Investigational Drug Service
IDWG	Interdialytic weight gain
IND	Investigational new drug
Inner EAR	Inner Effectiveness of Auditory Rehabilitation
IRB	Institutional Review Board
PI	Principal investigator
SAE	Serious adverse event
UF	Ultrafiltration
UNC	University of North Carolina
U.S.	United States

PROTOCOL SYNOPSIS

Study Title	Pilot study of loop diuretics among individuals receiving hemodialysis (HD)
Funder	NIH/NIDDK
Clinical Phase	Phase II
Study Rationale	Individuals with end-stage kidney disease (ESKD) receiving HD have high mortality rates, driven largely by cardiovascular causes. Volume-related factors including volume overload, large interdialytic weight gains (IDWG), and higher ultrafiltration (UF) rates are critical, modifiable contributors to cardiovascular complications such as hypertension, arterial stiffness, left ventricular hypertrophy, and heart failure. Reversing volume overload has been shown to improve blood pressure (BP) and cardiac remodeling, and lower IDWGs and UF rates are associated with lower rates of intradialytic hypotension and less myocardial ischemia on cardiac imaging. While dietary restrictions and longer, more frequent HD can improve these volume-related factors, they add substantial burden for patients. Oral loop diuretics are one potential strategy to increase urine output in HD patients. Loop diuretics are often prescribed for volume control before HD initiation and to patients on peritoneal dialysis. Many, if not most, patients discontinue diuretics once they begin treatment with HD. Small studies suggest that high-dose oral furosemide may increase urine volume in HD patients. However, dose-related side effects raise concern about such doses. Observational data suggest that lower doses of diuretics are associated with both less IDWG and less intradialytic hypotension. Loop diuretic use after HD initiation is associated with lower hospitalization rates. Diuretics may represent a pragmatic, low-cost, and low-burden strategy to improve outcomes in HD patients. Lack of data on optimal furosemide dosing, safety, and acceptability are barriers to expanded use.
Study Objective(s)	*Primary* • To generate pilot data on the short-term and longer-term efficacy, safety, tolerability, and acceptability of furosemide in patients with HD-dependent ESKD. *Exploratory* • To generate data on the short-term and longer-term clinical outcomes in patients with HD-dependent ESKD receiving furosemide.
Test Article(s)	Oral furosemide (maximum possible dose 320 mg/day)
Study Design	This is a single center (multi-clinic), open-label, non-randomized pilot study to test whether oral furosemide is safe and effective at increasing urine volume in HD patients. The study will consist of 2 periods: a 6-week dose escalation period (period 1) and a subsequent 12-week follow-up period (period 2). During period 1, all participants will receive escalating doses of furosemide as tolerated, and we will examine the short-term safety, tolerability, and efficacy of furosemide. During period 2, all participants will continue the maximally tolerated period 1 furosemide dose, and we will examine the acceptability of and adherence to furosemide and the longer-term safety and efficacy of furosemide.
Population **Key criteria for Inclusion and Exclusion:**	*Inclusion Criteria* • Patient self-report of at least 1 cup urine/24-hours • Age ≥18 years • Receipt of thrice weekly in-center HD at a participating clinic (UNC-associated Carolina Dialysis- Carrboro, Siler City, Pittsboro, Sanford, and Lee County) • ≥60 days receiving in-center HD • Willingness to take study medication and undergo study testing

	• Ability to provide informed consent *Exclusion Criteria* • Known allergy to loop diuretic • History of poor adherence to HD or medical regimen per nephrologist • >1 hospitalization in prior 30-days • Frequent hypotension (systolic blood pressure (BP) <80 mmHg at >30% of HD treatments in prior 30-days) • Cirrhosis per nephrologist • Hearing disorder per nephrologist • Serum potassium <3.5 mEq/L, magnesium <1 mg/dL, or corrected calcium <8 mg/dL in prior 30-days • Taking a non-loop diuretic (e.g. spironolactone, eplerenone, ethacrynic acid, thiazides) • Taking an aminoglycoside, cisplatin, methotrexate, cyclosporine, ACTH, lithium, phenytoin, or oral/intravenous steroid • Natural licorice consumption • Prisoners, patients with significant mental illness • Pregnant patients and nursing mothers
Number Of Participants	30 (with enrollment up to 40 to ensure sample size)
Study Duration	Each participant's participation will last 22 weeks (4-week baseline and 18-week study periods) The entire study is expected to last 16 months.
Study Phases **Screening** **Study Treatment** **Follow-Up**	Screening: screening for eligibility and obtaining consent Baseline: medical history and other baseline assessments Study treatment and follow-up: dose escalation (period 1, 6-weeks) and follow-up (period 2, 12-weeks)
Efficacy Evaluations	*Short-term and longer-term efficacy (urine volume)* • Among participants with baseline 24-hour urine volume ≥200 mL: incidence of ≥25% increase in urine volume • Among participants with baseline 24-hour urine volume <200 mL: incidence of a ≥50 mL increase in urine volume to a urine volume of at least 100 mL/24-hours
Pharmacokinetic Evaluations	*N/A*
Safety Evaluations	*Short-term and longer-term safety (electrolytes, furosemide levels, BP, symptoms)* • Incidence of serum potassium <3.2 mEq/L • Incidence of serum magnesium <0.8 mg/dL • Incidence of serum corrected calcium <7.0 mg/dL • Incidence of serum furosemide level >12 microgram/L • Incidence of dialysis-associated 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) • Incidence of severe or very severe patient-reported rash, tinnitus, or hearing change) not attributable to causes other than furosemide • Incidence of 10-point decrease in Inner Effectiveness of Auditory Rehabilitation (Inner EAR) instrument score

Tolerability Evaluations	*Short-term and longer-term tolerability (symptoms)* • Incidence of severe or very severe cramping, dizziness/pre-syncope, unusual tiredness/weakness, chest pain, nausea, vomiting, or diarrhea not attributable to causes other than furosemide
Statistical And Analytic Plan	At designated study time-points, we will determine the change from baseline for efficacy measures (24-hour urine volume, fractional excretion of urea/ sodium) and safety measures (e.g. potassium, magnesium, hypotension). These measures and absolute change in electrolyte values will be analyzed via one-way repeated measures of analysis of variance to determine the presence of absence of dose-related differences. In addition, we will use mixed effects modeling to test the effect of increased dose and duration with the fixed effect of time (dose), random subject effect, and an unstructured repeated covariance type. We will use generalized estimating equations to assess categorical outcomes (sufficient increase in 24-h urine volume, hypokalemia, hypomagnesemia, hypotension, symptoms, Inner EAR score, patient acceptance). We will also consider binary outcomes as rates (events/time).
DATA AND SAFETY MONITORING PLAN	We will ensure participant safety through regular review by the research team. This will include review of study progress, listings of adverse events (AEs), and AE logs. We will also use an independent safety monitor to provide an additional layer of protection. The principal investigator (PI) will be responsible for quality assurance and quality control and will provide oversight of all research team members to assure adherence to the protocol.

1 BACKGROUND AND RATIONALE

1.1 Introduction (Study rationale, brief study overview and setting)

Over 450,000 people in the United States (U.S.) require maintenance hemodialysis (HD) to sustain life. These individuals have exceedingly high mortality rates (166 deaths/1,000 person-years), driven largely by cardiovascular causes.[1] Volume-related factors including volume overload,[2-4] large interdialytic weight gains (IDWG),[5, 6] and higher ultrafiltration (UF) rates[7-9] are critical, modifiable contributors to cardiovascular complications such as hypertension, arterial stiffness, left ventricular hypertrophy, and heart failure. *Reversing* volume overload has been shown to improve blood pressure (BP) and cardiac remodeling, and lower IDWGs and UF rates are associated with lower rates of intradialytic hypotension and less myocardial ischemia on cardiac imaging.[10, 11] However, over 50% of HD patients are volume-overloaded,[2] and more than 35% have IDWGs exceeding 3.5% of body weight.[12] While dietary restrictions and longer, more frequent HD can improve these volume-related factors, patients are often averse and poorly adherent to dietary restrictions and are generally unwilling to increase HD treatment time.[13]

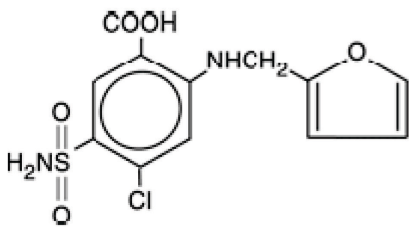
The use of oral loop diuretics is one potential strategy to increase urine output in HD patients. Loop diuretics are often prescribed for volume control before HD initiation and to patients on peritoneal dialysis.[14, 15] However diuretic use declines sharply after HD initiation. It is estimated that >50% of U.S. HD patients stop diuretics at HD initiation irrespective of urine output, and <25% of patients use diuretics 6 months into HD.[14, 16] A small study (N=13) suggests that high-dose oral furosemide (>1,000 mg/day) may increase urine volume in HD patients with urine output as low as 100 mL urine/day.[17] However, dose-related side effects raise concern about such doses. Observational data show associations between lower-dosed diuretics and lower IDWG and less intradialytic hypotension.[16] Loop diuretic use after HD initiation is associated with lower hospitalization rates.[18] Diuretics may represent a pragmatic, low-cost strategy to improve outcomes in HD patients. *[See grant proposal for additional detail and supporting studies.]*

Lack of data on optimal furosemide dosing, safety, and acceptability are barriers to expanded use. To fill this knowledge gap, we will conduct an 18-week single center (multi-clinic), open-label pilot study to test whether oral furosemide is safe and effective at increasing urine volume in HD patients. The study will consist of 2 periods: a 6-week dose escalation period (period 1) and a subsequent 12-week follow-up period (period 2). During period 1, all participants will receive escalating doses of furosemide as tolerated, and we will examine the short-term safety and efficacy of furosemide. During period 2, all participants will continue the maximally tolerated period 1 furosemide dose, and we will examine the acceptability of and adherence to furosemide and the longer-term safety and efficacy of furosemide. The study duration is 18 weeks.

We will recruit study participants from the University of North Carolina (UNC)-owned Siler City, Pittsboro, Sanford, Lee County, and Carrboro HD clinics. The study selection criteria are listed below. All recruitment activities and study visits will take place at the participant's dialysis clinic, scheduled to coincide with routine HD treatments. Laboratory (blood and urine electrolytes) samples will be transported to and analyzed at the UNC McClendon Laboratories in Chapel Hill, NC. Stored blood and urine samples will be housed in freezers at the UNC Kidney Center (5th floor Burnett-Womack Building, Chapel Hill, NC). Serum and urine furosemide levels will be analyzed in the UNC Kidney Center Hostetter Lab Center (5th floor Burnett-Womack Building, Chapel Hill, NC).

1.2 Name and Description of Investigational Product or Intervention

Study intervention: Oral furosemide



The information provided in this section was obtained from Food and Drug Administration (FDA) package insert.[19] Furosemide (Lasix®) is a diuretic which is an anthranilic acid derivative. Tablets contain furosemide (active ingredient) and lactose monohydrate NF, magnesium stearate NF, starch NF, talc USP, and colloidal silicon dioxide SF (inactive ingredients). Chemically, it is a 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid. It is available in 20, 40 and 80 mg tablets.

Furosemide is a white to off-white odorless crystalline power. It is practically insoluble in water, sparingly soluble in alcohol, freely soluble in dilute alkali solutions and insoluble in dilute acids.

Mechanism of action: Furosemide inhibits the absorption of sodium and chloride not only in the proximal and distal tubules but also in the loop of Henle. The action on the distal tubule is independent of any inhibitory effect on carbonic anhydrase and aldosterone.

Pharmacokinetics: The onset of diuresis following oral administration is within 1 hour. The peak effect occurs within the first or second hour. The duration of diuretic effect is 6 to 8 hours. In fasted normal men, the mean bioavailability of oral furosemide tablets is 64% of that from an intravenous injection of the drug. The terminal half-life of furosemide is approximately 2 hours. Peak plasma concentrations increase with increasing dose, but times-to-peak do not differ among doses. Furosemide is predominantly excreted unchanged in the urine.

Indications and usage: Furosemide is indicated in adults and pediatric patients for the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and kidney disease. Oral furosemide may also be used in adults for the treatment of hypertension alone or in combination with other antihypertensive agents.

Contraindications: Furosemide is contraindicated in patients with anuria and in patients with a history of hypersensitivity to furosemide.

Dosing and dose changes with rationale

During the 6-week dose escalation period (period 1), participants who are not taking oral furosemide at study entry will take 80 mg oral furosemide twice a day for 14 days and then, if the dose was tolerated, they will take 120 mg oral furosemide twice a day for 14 days and then, if the dose was tolerated, they will take 160 mg oral furosemide twice a day for 14 days. If a patient is taking oral furosemide at study entry, the participant will remain on his/her prescribed oral furosemide dose for the first 2 weeks of the dose escalation period. If a participant is taking another loop diuretic (e.g. bumetanide), we will calculate the corresponding oral furosemide dose (i.e. 1 mg bumetanide = 40 mg furosemide). The participant will discontinue the other loop diuretic and receive the equivalent dose of oral furosemide for the first 2 weeks of the dose escalation period. The first dose will then be increased by 50%, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day) at week 2 and, if tolerated, the second dose will be increased by 50% at week 4, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day). Establishing ceiling dose thresholds by increasing the prior dose by 30-50% is the recommended approach in heart failure patients per the American College of Cardiology Foundation/American Heart Association task force on practice guidelines.[20] The maximum proposed study dose is 160 mg twice daily (320 mg total per day), which is well below the maximum oral furosemide daily dose of 600 mg/day listed in the furosemide package insert.[19] In addition, the planned furosemide doses are consistent with available formulations (i.e. study doses can be provided using currently marketed furosemide tablet strengths) and standard clinically utilized/approved doses for the FDA-labeled indications. We are not testing higher doses due to the potential increased risk of AEs necessitating drug discontinuation.

Occurrence of any of the following events will end dose up-titration: 1) electrolyte abnormalities (serum potassium <3.2 mEq/L, serum magnesium <0.8 mEq/L, and/or serum corrected calcium <7 mg/dL); 2) side effects attributable to furosemide (severe or very severe rash, tinnitus, or hearing change); decrease in Inner Effectiveness of Auditory Rehabilitation (Inner EAR) instrument score by more than 10 points; and/or intradialytic systolic BP <80 mmHg that a study investigator attributes to study drug-induced hypovolemia); and/or 3) patient non-acceptance (defined as the

patient reporting unwillingness to take the study drug). If one of these events occur, the participant will return to the prior tolerated oral furosemide dose. If one of these events occurs at the lowest administered dose, oral furosemide will be stopped and not re-started.

During the 12-week follow-up period (period 2), participants who tolerated and accepted furosemide in period 1 will continue their maximally tolerated period 1 furosemide dose during the 12-weeks of follow-up. If a participant has electrolyte abnormalities or other side effects attributable to furosemide in period 2 (i.e. on their period 1 maximally tolerated dose), the dose will be lowered sequentially (i.e. dose de-escalation: 160 → 120 → 80 mg twice daily, or, if on alternative dosing due to prior diuretic use, decrease dose by 50%, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations, until tolerated). If side effects persist or if the participant chooses, oral furosemide will be stopped.

Investigational New Drug (IND) exemption status

Oral furosemide is marketed as a prescription drug product and regulated by the U.S. FDA. Furosemide is indicated for edema and hypertension (signs and symptoms of volume overload). Its use in this study will be considered an on-label use. Our study is exempt from IND requirements for the following reasons:

- Oral furosemide is lawfully marketed in the U.S.
- The study is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use and is not intended to be used to support any other significant change in the labeling for the drug.
- The study is not intended to support a significant change in the advertising for the product.
- The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product. Specifically, the initial starting dose of oral furosemide in the proposed trial is 80mg twice daily for loop diuretic naïve patients and the maximum proposed study dose is 160 mg twice daily (320 mg total/day). The study doses of furosemide are well below the maximum daily dose specified in the furosemide package insert, 600 mg/day.[42] Moreover, the proposed furosemide dose escalation scheme is consistent with heart failure guidelines from the American College of Cardiology Foundation/American Heart Association task force on practice guidelines.[51]
- The study will be conducted in compliance with Institutional Review Board (IRB) review requirements set forth in 21 CFR Part 56 and informed consent requirements set forth in 21 CFR Part 50.
- The study will be conducted in compliance with 21 CFR 312.7 (i.e. the investigation is not intended to promote or commercialize the drug product).

1.3 Clinical Data to Date

Heart Failure: Most patients with heart failure and volume overload are initially treated with the combination of an oral loop diuretic (e.g. furosemide) and a low sodium diet. The diuretic response to furosemide is described by a threshold type dose-response curve linking the rate of diuretic excretion in the urine and the degree of natriuresis. There is virtually no increase in natriuresis until the threshold concentration of luminal diuretic is reached and, once the threshold is exceeded, the degree of natriuresis increases until a maximal ceiling is reached.[22, 23] As the threshold concentration differs across patients, it is necessary to determine an effective dose in individual patients. The usual starting dose in furosemide-naïve patients without kidney dysfunction is furosemide 20 to 40 mg once or twice daily, and subsequent dosing is determined by the diuretic response. It is customary to double the prior dose when titrating furosemide for a diuretic response.[20] For patients with normal glomerular filtration rate, maximal single oral doses of furosemide are 40 to 80 mg. For patients with kidney insufficiency (impaired glomerular filtration rate), a higher maximum dose of 160 to 200 mg of furosemide can be given (maximum total daily dose of 600 mg).[19] Dosing in patients who have received prior loop diuretic therapy is based upon the response to prior dosing amounts.[20]

No single furosemide dosing regimen (bolus versus continuous intravenous infusion; high dose verse lower dose oral or intravenous infusion) has been shown to be superior to others. A meta-analysis from the National Institute for

Health and Clinical Excellence Guidance (NICE) included 10 randomized controlled trials comparing various intravenous furosemide regimens.[24] Comparisons between furosemide intravenous bolus and continuous infusion showed no clear differences in outcomes such as weight loss, urine output, or change in kidney function (ototoxicity was not assessed). The Diuretic Optimization Strategies Evaluation (DOSE) study evaluated the optimal approach to diuretic dosing and route of administrations for patients with decompensated heart failure.[25] The trial randomly assigned 308 patients to receive furosemide administered intravenously via either a bolus every 12 hours or continuous infusion and at either a low dose (equivalent to the patient's previous oral dose) or a high dose (2.5 times the previous oral dose). The efficacy end point was the patients' global assessment of symptoms over the course of 72 hours and the safety end point was change in serum creatinine from baseline to 72 hours. There was no significant difference in efficacy or safety end points for bolus versus continuous infusion. Patients assigned to intravenous bolus therapy were more likely to require a dose increase at 48 hours; however, the total dose of furosemide over 72 hours in the bolus group was not significantly different from that in the continuous infusion group. High-dose furosemide, compared with low-dose furosemide, produced greater net fluid loss, weight loss, and relief from dyspnea but also more frequent transient worsening of renal function. There was no significant difference in patients' global assessment of symptoms in the high-dose group and the mean change in the serum creatinine was less than 0.1 mg/dL in both groups.

Dialysis-Dependent End-Stage Kidney Disease (ESKD)

Peritoneal dialysis: Over 40% of peritoneal dialysis patients are prescribed diuretics for volume control.[14, 15, 26] An increase in urine volume from diuretics helps patients receiving peritoneal dialysis improve or maintain their urine output. A randomized trial showed that the long-term use of furosemide at a daily dose of 250 mg led to greater urine volume compared to control. Both groups had similar mean urine volume at randomization (1020 vs. 1040 mL), but at 12-months, the mean daily urine volume in the furosemide group was 1070 mL compared to 733 mL in the control group.[17]

Hemodialysis: Diuretics are used less frequently in HD patients as compared to peritoneal dialysis patients in the U.S. Over 50% of U.S. HD patients stop diuretic therapy at HD initiation irrespective of residual urine output, and <25% of patients remain on diuretics 6 months after HD initiation.[14, 16] In contrast, the majority of patients in Europe and Japan, stay on diuretics after HD initiation.[16] There have been no large randomized trials of furosemide for the indications of volume overload or hypertension among individuals receiving maintenance HD. However, since diuretics promote urine output, they may offer more consistent volume control in HD patients with residual urine output. Mechanistic studies suggest that adaptive renal tubule changes preserve diuretic response even in advanced kidney disease.[22, 23] While there may be concerns about diuretic efficacy in patients with little urine output, a study of 13 HD patients receiving large doses of oral furosemide (>1,000 mg/day), showed that furosemide can increase urine volume in patients producing as little as 100 mL urine/day.[17] However, some participants had bullous dermatosis, underscoring the need to study furosemide safety and efficacy at lower doses as we will study in this pilot study. Using international Dialysis Outcomes and Practice Patterns Study (DOPPS) data, Bragg-Gresham showed an association between diuretic use and lower IDWG, fewer hypotensive and hyperkalemic events, and lower cardiovascular mortality.[16] Another retrospective cohort study showed that continuation (vs. discontinuation) of loop diuretics after HD initiation was associated with lower hospitalization rates, intradialytic hypotension frequency and IDWG.

Clinical trial data on optimal dosing, safety, tolerability, and effectiveness of loop diuretics are lacking. It is known that in comparison to non-dialysis patients, individuals with dialysis-dependent ESKD require higher doses of loop diuretics to achieve increases in urine output.[22] However, a "ceiling effect" has been observed. At doses where the fractional excretion of sodium plateaus, further dose increase does not provide additional diuretic effect.[22, 23, 27, 28] The optimal loop diuretic dose in HD patients is unknown and may differ based on baseline urine output. Second, diuretics can cause hypokalemia and hypomagnesemia, conditions associated with adverse cardiovascular outcomes.[19, 28-31] Moreover, high dose intravenous (constant rate of 25 mg/min) and very high dose oral (>800 mg/day) loop diuretics are associated with ototoxicity.[32-35] These effects have not been observed with oral furosemide administered at recommend

doses without interacting medications (e.g. cisplatin, aminoglycosides).[35] No studies have evaluated the safety of lower dose oral loop diuretics in HD patients. **Table 1** summarizes studies of diuretics and outcomes in dialysis patients (the majority of these studies evaluated loop diuretics, specifically furosemide).

Table 1. Studies of diuretics in dialysis patients.

Study (year)	Population (N; location)	Study design/ diuretic	Outcome with diuretic
Sibbel/Flythe (2019)[18]	Incident HD (7,072; U.S.)	Observational cohort: loop continued after HD initiation vs. not	• ↓ IDH • ↓ hospitalization • ↓ mortality (non-sig trend)
Bragg-Gresham (2007)[16]	Incident & prevalent HD (16,420; multinational)	Observational cohort: diuretic vs. not	• ↓ IDWG and ↓ IDH • ↓ hyperkalemia • ↓ CV mortality
van Olden (1992)[36]	Prevalent HD & 100 mL UO/day (13; Netherlands)	Interventional study: furosemide	• ↑ 24-h urine vol. & sodium
Flinn (2006)[37]	Prevalent PD (61; Canada)	Interventional study: furosemide vs. control	• ↓ anuria (non-sig trend)
Medcalf (2001)[17]	Incident PD (61; U.K.)	RCT: furosemide vs. control	• ↑ 24-h urine vol. & sodium • ↓ IDWG

Abbreviations: HD, hemodialysis; U.S., United States; IDH, intradialytic hypotension; non-sig., non-significant; IDWG, interdialytic weight gain; CV, cardiovascular; UO, urine output; vol., volume; PD, peritoneal dialysis; U.K., United Kingdom; RCT, randomized controlled trial

1.3.1. Risks

Risks of furosemide include electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypocalcemia), hypotension, allergic or toxicity-related symptoms (rash, tinnitus, and hearing change), and other symptoms (cramps, dizziness, unusual tiredness/weakness, chest pain, nausea, vomiting, diarrhea, and numbness/tingling). Furosemide-induced ototoxicity can lead to transient or permanent deafness. Ototoxicity primarily occurs with high-dose intravenous therapy (e.g. >240 mg/hr infusions) or at lower doses in patients with kidney function impairment or concurrent use of other ototoxins such as aminoglycosides.

We have chosen our study selection criteria to exclude patients who are at increased risk for furosemide side effects including those with frequent hypotension, cirrhosis, a hearing disorder, or electrolyte abnormalities in the prior 30 days (hypokalemia defined as serum potassium <3.5 mEq/L, hypomagnesemia, defined as serum magnesium <1 mg/dL, and hypocalcemia, defined as serum corrected calcium < 8 mg/dL), and those taking an interacting drug or substance including an aminoglycoside, cisplatin, methotrexate, cyclosporine, adrenocorticotropic hormone (ACTH), lithium, phenytoin, oral/intravenous steroid, or natural licorice.

In the current trial, risks related to furosemide will be reduced through close monitoring of serum electrolytes, patient symptoms (including hearing), BP, and serum and urine furosemide levels. In addition, we will withdraw participants from the study if any of the following occurs during the course of the study: 1) pregnancy; 2) initiation of a non-loop diuretic (spironolactone, eplerenone, ethacrynic acid, thiazides); 3) initiation of an interacting drug (aminoglycoside, methotrexate, cyclosporine, ACTH, lithium, phenytoin, or oral/intravenous steroid); and/or 4) new diagnosis of cirrhosis.

Occurrence of any of the following events will end dose up-titration (defined below): 1) electrolyte abnormalities, 2) side effects attributable to furosemide (e.g. hypotension, allergic or toxicity-related symptoms), and/or 3) patient non-acceptance. If one of these events occur, the participant will return to the prior tolerated furosemide dose. If one of these events occurs at the lowest administered dose, furosemide will be stopped and not re-started.

- Serum potassium <3.2 mEq/L
- Serum magnesium <0.8 mEq/L
- Serum corrected calcium <7.0 mg/dL
- Systolic BP <80 mmHg that a study investigator attributes to study drug-induced hypovolemia

- Patient desires not to continue furosemide
- Patient-reported symptom of severe or very severe
    - Rash
    - Tinnitus
    - Other self-reported symptom plausibly attributed to furosemide
    - Hearing change (also evaluated with the Inner Effectiveness of Auditory Rehabilitation (Inner EAR) instrument*)

*In addition to self-reported hearing change, we will monitor hearing with the Inner EAR instrument. The Inner EAR instrument measures hearing-related function and has good intra-rater reliability, internal consistency, discriminant validity, and responsiveness to change. Instrument scores correlate with pure tone audiometry.[38-40] Scores range from 0 to 100 points. We will refer participants with ≥10-point decline in Inner EAR scores or self-reported hearing loss (any severity) after furosemide initiation and/or dose titration for audiometry testing. Audiometry for all participants is impractical (i.e. expensive, additional clinic visits) and unnecessary given low risk of hearing complications at the proposed doses (maximum 320 mg/ day).[32-34]

*See **Section 7** for additional safety information (e.g. monitoring, adverse events and serious adverse events).*

1.3.2 Benefits

Benefits of furosemide in the HD population remain unproven at this time. Hypothesized benefits of furosemide therapy include improved volume status, BP control, and a decrease in volume-related symptoms during dialysis.

2 STUDY OBJECTIVE

The overall objective of this study is to test whether oral furosemide is safe and effective at increasing urine volume in HD patients.

2.1 Primary Objectives

Period 1 (dose escalation = short-term outcomes; 6-weeks) and Period 2 (follow-up = longer-term outcomes; 12-weeks)

Short-term and longer-term efficacy: To test the hypothesis that furosemide will increase 24-hour urine volume at its maximally tolerated dose in individuals with HD-dependent ESKD. 24-hour urine volumes will be used as the primary endpoint for both short-term and longer-term efficacy assessments.

Short-term and longer-term safety: To evaluate the short-term (6-weeks) and longer-term (12-weeks) safety of escalating doses of furosemide in individuals with HD-dependent ESKD. Safety outcomes will include the following:

- Serum potassium level <3.2 mEq/L
- Serum magnesium level <0.8 mg/dL
- Serum corrected calcium level <7 mg/dL
- Serum furosemide level >12 microgram/L
- Serious dialysis-associated 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)
- Severe or very severe rash, tinnitus, or hearing change not attributable to causes other than furosemide
- Inner EAR score decrease by ≥10 points

Short-term and longer-term tolerability: To evaluate the short-term and longer-term tolerability of furosemide in individuals with HD-dependent ESKD. Tolerability will be assessed by:

- Severe or very severe cramping, dizziness/pre-syncope, unusual tiredness/weakness, chest pain, nausea, vomiting, or diarrhea not attributable to causes other than furosemide

Short-term and longer-term acceptability: To evaluate the short-term and longer-term acceptability of furosemide in individuals with HD-dependent ESKD. Acceptability will be assessed by:

- Participant affirmative response to question: "If recommended, would you be willing to stay on the dose of furosemide you have received during the last week?"
- Recruitment rates
- Drop-out rates

Short-term adherence: To evaluate short-term and longer-term adherence to furosemide among individuals with HD-dependent ESKD. Adherence will be assessed by:

- Pill counts
- Serum and urine furosemide levels

2.2 Exploratory Objectives

Period 1 (dose escalation = short-term outcomes) and Period 2 (follow-up = longer-term outcomes)

Short-term and longer-term clinical outcomes: Exploratory objectives are designed to expand understanding of the effect of furosemide on clinical outcomes in HD-dependent ESKD. We will analyze the following:

- 24-hour creatinine clearance
- 24-hour fractional excretion of urea
- 24-hour fractional excretion of sodium
- Pre-HD systolic BP
- Intradialytic hypotension (systolic BP <80 mmHg)[21]
- Intradialytic hypotension (systolic BP <90 mmHg)[41]
- IDWG
- UF volume
- UF rate
- Target weight achievement
- Post-HD weight-target weight difference
- Extra HD treatments
- Hospitalizations

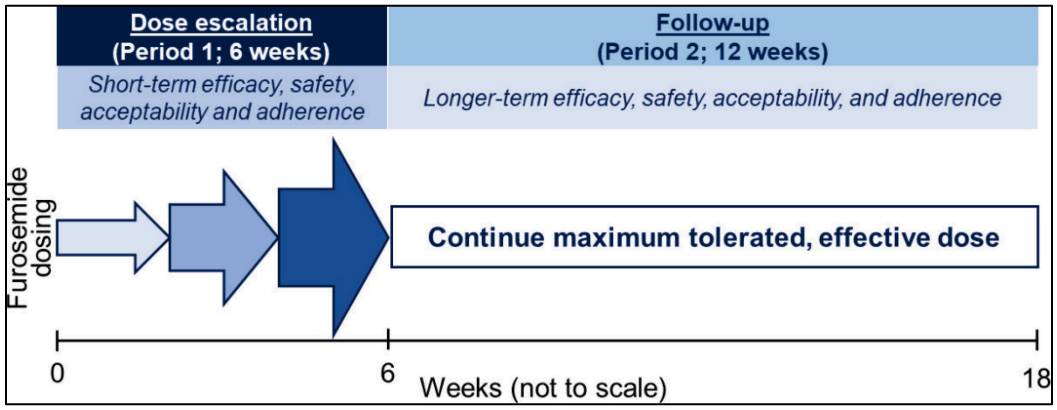
3 INVESTIGATIONAL PLAN

3.1 Study Design (Figure 1)

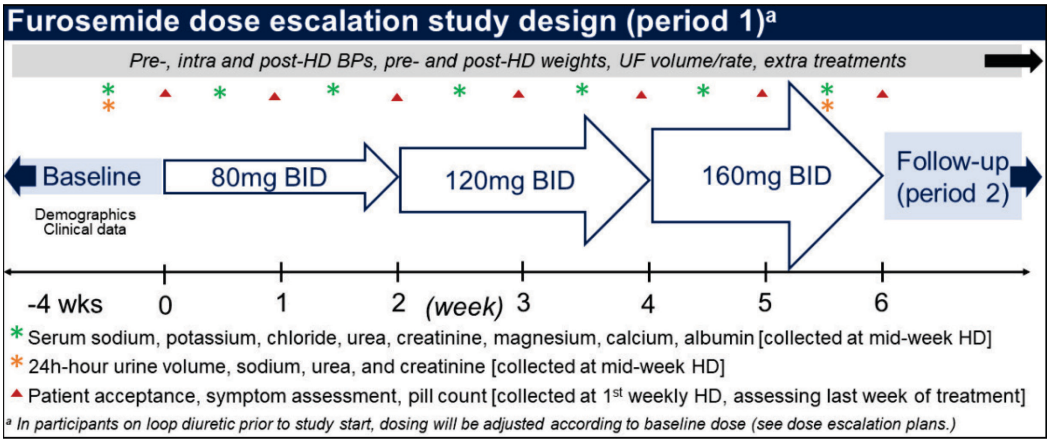
This is a single center (multi-clinic), open-label, non-randomized pilot study to test whether oral furosemide is safe and effective at increasing urine volume in HD patients. The study will consist of 2 periods: a 6-week dose escalation period (period 1) and a subsequent 12-week follow-up period (period 2). During period 1, all participants will receive escalating doses of furosemide as tolerated, and we will examine the short-term safety and efficacy of furosemide. During period 2, all participants will continue the maximally tolerated period 1 furosemide dose, and we will examine the acceptability of and adherence to furosemide and the longer-term safety and efficacy of furosemide. The study duration is 18 weeks.

Figure 1. Study design.

Panel A. Study overview including both study periods (dose escalation and follow-up).



Panel B. Period 1 dose escalation schematic.



3.2 Allocation to Treatment Groups and Blinding

All participants will receive oral furosemide. Participants and investigators are unblinded.

3.3 Study Duration, Enrollment and Number of Participants

The study duration is 18 weeks and consists of a 6-week dose escalation period (period 1) and a subsequent 12-week follow-up period (period 2). Our goal sample size is 36 participants in period 1 with the goal of >30 participants continuing on to period 2. We will recruit up to 40 participants to account for dropout and will recruit additional participants if fewer than 30 participants are eligible for period 2.

All participants will be recruited from the University of North Carolina (UNC)-owned Siler City, Pittsboro, Sanford, Lee County, and Carrboro "Carolina Dialysis" HD clinics. We will screen individuals for eligibility if they self-report, on average, at least 1-cup urine/day. We selected this threshold based on a recent DOPPS analysis showing an association between self-reported urine volume of ≥1 cup/day and lower interdialytic weight gain (IDWG) and mortality.[42] Our selection criteria exclude patients unlikely to respond to diuretics (i.e. minimal urine output), patients at increased risk for furosemide side effects (e.g. cirrhosis, electrolyte abnormalities, and concurrent use of interacting medications or substances), and patients likely to have urine output decline during the study (i.e. within the first 60-days of HD therapy). We will recruit patients 1:3, incident (60-180 days on HD): prevalent (>180 days on HD) to ensure study of both populations while accounting for the challenge of recruiting incident patients (e.g. fewer eligible patients, research reticence early in therapy). See ***Section 3.4*** for participant inclusion and exclusion criteria and ***Section 9*** for enrollment practices.

3.4 Study Population

Participant Inclusion Criteria

- Patient self-report of at least 1 cup urine/24-hours[42]
- Age ≥18 years
- Receipt of thrice weekly in-center HD at a participating clinic (UNC-associated Carolina Dialysis- Carrboro, Siler City, Pittsboro, Sanford, and Lee County)
- ≥60 days receiving in-center HD
- Willingness to take study medication and undergo study testing
- Ability to provide informed consent

Participant Exclusion Criteria

- Known allergy to loop diuretic
- History of poor adherence to HD or medical regimen per nephrologist
- >1 hospitalization in prior 30-days
- Frequent hypotension (systolic BP <80 mmHg at >30% of HD treatments in prior 30-days)[21]
- Cirrhosis per nephrologist
- Hearing disorder per nephrologist
- Serum potassium <3.5 mEq/L, magnesium <1 mg/dL, or corrected calcium <8 mg/dL in prior 30-days
- Taking a non-loop diuretic (e.g. spironolactone, eplerenone, ethacrynic acid, thiazides)
- Taking an aminoglycoside, cisplatin, methotrexate, cyclosporine, ACTH, lithium, phenytoin, or oral/intravenous steroid
- Natural licorice consumption
- Prisoners, patients with significant mental illness
- Pregnant patients and nursing mothers

3.5 Study Drug/ Intervention

3.5.1 Description

Furosemide, the study drug, will be administered as tablets containing 20 mg, 40 mg or 80 mg furosemide.

3.5.2 Dosing and treatment regimen

During the 6-week dose escalation period (period 1), participants who are not taking oral furosemide at study entry will take 80 mg oral furosemide twice a day for 14 days and then, if the dose was tolerated, they will take 120 mg oral furosemide twice a day for 14 days and then, if the dose was tolerated, they will take 160 mg oral furosemide twice a day for 14 days (**Figure 1 Panel B**). If a patient is taking oral furosemide at study entry, the participant will remain on his/her prescribed oral furosemide dose for the first 2 weeks of the dose escalation period. If a participant is taking another loop diuretic (e.g. bumetanide), we will calculate the corresponding oral furosemide dose (i.e. 1 mg bumetanide = 40 mg furosemide). The participant will discontinue the other loop diuretic and receive the equivalent dose of oral furosemide for the first 2 weeks of the dose escalation period. The first dose will then be increased by 50%, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day) at week 2 and, if tolerated, the second dose will be increased by 50% at week 4, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day). Establishing ceiling dose thresholds by increasing the prior dose by 30-50% is the recommended approach in heart failure patients.[20]

Occurrence of any of the following events will end dose up-titration: 1) electrolyte abnormalities (serum potassium <3.2 mEq/L, serum magnesium <0.8 mEq/L, and/or serum corrected calcium <7 mg/dL); 2) side effects attributable to furosemide (severe or very severe rash, tinnitus, or hearing change); decrease in Inner Effectiveness of Auditory Rehabilitation (Inner EAR) instrument score by more than 10 points; and/or intradialytic systolic BP <80 mmHg that a

study investigator attributes to study drug-induced hypovolemia); and/or 3) patient non-acceptance (defined as the patient reporting unwillingness to take the study drug). If one of these events occur, the participant will return to the prior tolerated oral furosemide dose. If one of these events occurs at the lowest administered dose, oral furosemide will be stopped and not re-started.

During the 12-week follow-up period (period 2), participants who tolerated and accepted furosemide in period 1 will continue their maximally tolerated period 1 furosemide dose during the 12-weeks of follow-up. If a participant has electrolyte abnormalities or other side effects attributable to furosemide in period 2 (i.e. on their period 1 maximally tolerated dose), the dose will be lowered sequentially (i.e. dose de-escalation: 160 → 120 → 80 mg twice daily, or, if on alternative dosing due to prior diuretic use, decrease dose by 50%, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations, until tolerated). If side effects persist or if the participant chooses, oral furosemide will be stopped.

3.5.3 Preparation and administration of study drug

The study drug, furosemide, will be provided by the UNC Investigational Drug Service (IDS) which will be the Central Pharmacy for the study. Study drug kits will contain 2-week supplies during the dose escalation period (period 1) and 4-week supplies during the follow-up period (period 2). The PI will request the next supply of furosemide from IDS at each 2-week interval. Unless there is a contraindication (e.g. hypokalemia, serious hypotension), the investigator will escalate the furosemide dosing as described in ***Sections 1.2 and 3.5.2***. If up-titration is contraindicated, the PI will notify IDS. If down-titration or discontinuation is indicated, the PI will notify IDS. At the end of period 1, participants will be provided with a 4-week supply of furosemide at the indicated dose (week 6). At the week 10 and 14 visits, additional 4-week supplies of furosemide at the indicated dose will be provided.

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 8 visit, the participant will remain at the current dose of study drug for the remainder of the study and the follow-up period (i.e., the 12-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 8, the treatment phase will be 10 weeks rather than 12 weeks.

3.5.4 Participant adherence monitoring

Study drug containers will be returned to study coordinators at the conclusion of each 2-week period during dose escalation (weeks 2, 4 and 6), and at the conclusion of each 4-week period during follow-up (weeks 10, 14 and 18). Pill counts will be performed to assess participant adherence with prescribed study drug.

3.5.5 Concomitant therapy

Medication use (both oral and intravenous) will be collected at baseline and throughout the course of the study. Non-loop diuretic (e.g. spironolactone, eplerenone, ethacrynic acid, thiazides), aminoglycoside, cisplatin, methotrexate, cyclosporine, ACTH, lithium, phenytoin, and oral/intravenous steroid use are exclusion criteria. If these medications are initiated during follow-up the treating clinician will be contacted by the research team. If the prohibited drug cannot be discontinued, study medication will be withdrawn, and participants will continue to be followed.

3.5.6 Packaging

Furosemide will be distributed in patient-specific containers with the specified number of tablets (based on dosing listed in ***Sections 1.2 and 3.5.2***) for every 2-week period during the dose escalation period (period 1) and the specified number

of tablets (based on dosing listed in ***Sections 1.2 and 3.5.2***) for every 4-week period during the follow-up period (period 2). Labeling will include patient name, patient ID, protocol number, expiration date, and prescribing physician (PI).

3.5.7 Blinding of study drug

None

3.5.8 Receiving, storing, dispensing and returning study drug

The UNC IDS will maintain detailed records regarding furosemide dispensing, general study product accountability and patient specific study product accountability. Documentation includes study product storage, dispensing and final disposition.

3.5.9 Study drug storage

Furosemide will be stored at 25°C at the central UNC IDS pharmacy.

3.5.10 Dispensing study drug

The UNC IDS pharmacist or trained site designee will dispense the study drug to the study coordinator and complete a dispending/accountability log. Furosemide will be dispensed in well-closed, light-resistant containers. A study coordinator will dispense the study drug to the participant at the dialysis clinic at the below-described study visits and complete a dispensing/accountability log. The study coordinators will have appropriate training for dispensing and reconciling the study drug, and documentation of this training will be maintained in the study's regulatory binder.

3.5.11 Return or destruction of study drug

Study drug containers will be returned by participants at the end of each 2-week (dose escalation, period 1) and 4-week (follow-up, period 2) and remaining pills will be counted to assess adherence. Unused supplies of study drug will be returned to IDS 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.

3.5.12 Participant access to study drug at study closure

Participants will have access to furosemide at study closure if deemed appropriate by their treating nephrologists.

4 STUDY PROCEDURES

4.1 Overview (Tables 2 and 3)

All study visits will occur at the participant's dialysis clinic at routinely scheduled HD treatments.

- There will be one study visit during the baseline period.

- <u>Dose escalation period (period 1):</u> Blood samples will be collected weekly at mid-week treatment visits (i.e. Wednesday or Thursday during weeks -1, 1, 2, 3, 4, 5 and 6) prior to scheduled dose initiation or dose titration. Urine samples will be collected twice during the dose escalation period at mid-week treatment visits (i.e. Wednesday or Thursday during weeks -1 and 5). Study drug visits will be conducted every two weeks at the first weekly treatment (i.e. Monday or Tuesday during weeks 0, 2, 4, and 6). This structure was selected so that blood results will be collected and analyzed the week prior to study drug dose titration. As such, test results will be available prior to potential dose titration. Adherence and symptom assessment visits will be conducted weekly at the first treatment of each week (i.e. Monday or Tuesday during weeks 1, 2, 3, 4, 5 and 6). Hearing

assessments will be conducted every two weeks at the first weekly treatment (i.e. Monday or Tuesday during weeks 2, 4, and 6).

- Follow-up period (period 2): Blood samples will be collected at mid-week treatment visits every 4 weeks (i.e. Wednesday or Thursday during weeks 9, 13 and 17). Study drug visits will be conducted at the first treatment of each of the subsequent weeks (i.e. Monday or Tuesday during weeks 10 and 14). As in the dose escalation period, this structure was selected so that blood results will be available prior to additional study drug administration. Study adherence and symptom assessment visits will be conducted every 2 weeks at the first weekly HD treatment in the scheduled week (i.e. Monday or Tuesday during weeks 8, 10, 12, 14, 16, and 18). Hearing assessments will be conducted every four weeks at the first weekly treatment (i.e. Monday or Tuesday during weeks 10, 14, and 18). Urine sample visits will be conducted at the mid-week treatments of the 12th week and final week of the follow-up period (i.e. Wednesday or Thursday during weeks 12 and 18). All remaining study drug will be returned at the first weekly treatment (i.e. Monday or Tuesday) of week 19. A final pill count (adherence assessment) will be performed upon study drug return (week 19).

- All HD treatments occurring during the 18-week study period will be considered study treatments, and all associated clinical data (BP, pre-HD and post-HD weights and dialysis treatment data such as UF volume, UF rate and dialysate composition) will be collected from the electronic health record (EHR).

Table 2. Study visits by type.

Procedures	Baseline clinical assessment (Prior to study start, weeks -4 to -2)	Baseline electrolyte, urine, symptom, and hearing assessment (week -1)	Study drug visit (weeks 0, 2, 4, 6, 10, 14,)	Follow-up blood sample (weeks 1, 2, 3, 4, 5, 6, 9, 13, 17)	Follow-up urine sample (weeks -1, 5, 12, and 18)	Adherence and symptom assessment (weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 18)	Hearing assessment (weeks 2, 4, 6, 10, 14, and 18 weeks)	Clinical data collected at every HD treatment during study period (weeks -4 to 18)	Study drug return (week 19)
Complete medical history	X								
Pregnancy status inquiry	X								
Serum electrolytes and furosemide level		X		X					
24-hour urine		X			X				
Symptom assessment		X				X			
Hearing assessment		X					X		
Study drug administration			X						
Patient acceptance assessment									
Drop-out rates	X							X	
Pill counts						X			X
End of trial study drug return									X
BP monitoring	X							X	
Weight monitoring	X							X	
UF volume/rate	X							X	
Extra HD treatments	X							X	
Hospitalizations	X							X	

Table 2. Study visits by week.

Week(s)	-4 to -2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Procedures																						
Complete	X																					

medical history																						
Pregnancy status inquiry	X																					
Serum electrolytes and furosemide level		X		X	X	X	X	X	X			X				X				X		
24-hour urine		X						X							X						X	
Symptom assessment		X		X	X	X	X	X	X		X		X		X		X		X		X	
Hearing assessment		X			X		X		X				X				X				X	
Study drug administration			X		X		X		X				X				X					
Patient acceptance assessment				X	X	X	X	X	X		X		X		X		X		X		X	
Drop-out rates	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pill counts				X	X	X	X	X	X		X		X		X		X		X		X	X
Study drug return																						X
BP monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Weight monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
UF volume/ rate	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Extra HD treatments	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Hospitalizations	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

4.2 Screening/Baseline Visit procedures

Screening

The PI will communicate with potential participants to determine whether they fit the inclusion/exclusion criteria and would like to participate in the study. If patients are interested in participating, a signed HIPAA waiver will be obtained to allow the PI to review the individual's medical record and confirm eligibility status. The study baseline period will begin within 2 weeks of enrollment.

Baseline

Written informed consent will be obtained from study participants prior to the implementation of any study procedures. The participant will receive a signed and dated informed consent form. See ***Section 10.3.2*** for details of consent process. The PI will verify inclusion/ exclusion criteria. A pregnancy inquiry will occur and a complete medical history will be obtained in weeks -4 to -2 of the baseline period. In the one week prior to start (week -1), the following data will be obtained: serum electrolytes, 24-hour urine collection, symptom assessment, and hearing assessment.

4.3 Intervention/Treatment procedures (by visits)

During the 6-week dose titration period (period 1), study drug administration visits will occur every 2 weeks on the first day of the participant's weekly, routinely scheduled HD treatments (i.e. Monday or Tuesday during weeks 0, 2, 4, and 6). During the 12-week follow-up period (period 2), study drug administration visits will occur every 4 weeks on the first day of the participant's weekly, routinely scheduled HD treatments (i.e. Monday or Tuesday during weeks 10, 14 and 18).

Dose-titration (period 1)

<u>Week 0:</u>

A. Participants who are not taking oral furosemide at study entry will receive 2-weeks supply of 80 mg oral furosemide twice daily dosing.

B. Participants who are taking oral furosemide at study entry will receive 2-weeks supply of his/her prescribed oral furosemide dose.
C. Participants who are taking another loop diuretic (e.g. bumetanide) at study entry will receive 2-weeks supply of an equivalent dose of furosemide (1mg bumetanide = 40 mg furosemide).

Week 2:

A. Participants who were not taking oral furosemide at study entry and who tolerated the 80 mg twice daily dose (i.e. no hypokalemia, serious hypotension, symptoms, etc.) will receive 2-weeks supply of 120 mg oral furosemide twice daily dosing.
B. Participants who were taking oral furosemide at study entry and tolerated the first 2 weeks of dosing will receive 2-weeks supply of furosemide dose 50% higher than initial dose, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day).
C. Participants who were taking another loop diuretic at study entry and tolerated the first 2 weeks of furosemide dosing will receive 2-weeks supply of furosemide dose 50% higher than their initial dose, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day).
D. Participants who did not tolerate the weeks 0 and 1 dose of furosemide will stop furosemide, and it will not be restarted. See below for parameters for determining intolerance.

Week 4:

A. Participants who were not taking oral furosemide at study entry and who tolerated the 120 mg twice daily dose (i.e. no hypokalemia, serious hypotension, symptoms, etc.) will receive 2-weeks supply of 160 mg oral furosemide twice daily dosing.
B. Participants who were taking oral furosemide at study entry and tolerated the last 2 weeks of dosing will receive 2-weeks supply of furosemide dose 50% higher than initial dose, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day).
C. Participants who were taking another loop diuretic at study entry and tolerated the last 2 weeks of furosemide dosing will receive 2-weeks supply of furosemide dose 50% higher than initial dose, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations (not to exceed 320 mg/day).
D. Participants who did not tolerate the weeks 2 and 3 dose of furosemide will return to the prior tolerated oral furosemide dose. See below for parameters for determining intolerance.

Week 6:

A. Participants who were not taking oral furosemide at study entry and who tolerated the 160 mg twice daily dose (i.e. no hypokalemia, serious hypotension, symptoms, etc.) will receive 4-weeks supply of 160 mg oral furosemide twice daily dosing.
B. Participants who were taking oral furosemide at study entry and tolerated the last 2-weeks of dosing will receive 4-weeks supply of that same furosemide dose (not to exceed 320 mg/day).
C. Participants who were taking another loop diuretic at study entry and tolerated the last 2-weeks of furosemide dosing will receive 4-weeks supply of that same furosemide dose (not to exceed 320 mg/day).
D. Participants who did not tolerate the weeks 4 and 5 dose of furosemide will return to the prior tolerated oral furosemide dose. See below for parameters for determining intolerance.

As specified in ***Section 1.3.1,*** occurrence of any of the following events will end dose up-titration: 1) electrolyte abnormalities (serum potassium <3.2 mEq/L, serum magnesium <0.8 mEq/L, and/or serum corrected calcium <7 mg/dL); 2) side effects attributable to furosemide (severe or very severe rash, tinnitus, or hearing change); decrease in Inner Effectiveness of Auditory Rehabilitation (Inner EAR) instrument score by more than 10 points; and/or intradialytic systolic BP <80 mmHg that a study investigator attributes to study drug-induced hypovolemia); and/or 3) patient non-

acceptance (defined as the patient reporting unwillingness to take the study drug). If one of these events occur, the participant will return to the prior tolerated oral furosemide dose. If one of these events occurs at the lowest administered dose, oral furosemide will be stopped and not re-started.

The window for each dose-titration period intervention/treatment visit is -4 to +4 days.

Follow-up (period 2)

Weeks 10 and 14: Participants who tolerated (i.e. no hypokalemia, serious hypotension, symptoms, etc.) and accepted furosemide in period will continue their maximally tolerated period 1 dose during the 12-weeks of follow-up. At weeks 10 and 14, they will receive additional 4-weeks supplies of this furosemide dose.

As specified in ***Section 1.3.1***, if a participant has electrolyte abnormalities or other side effects attributable to furosemide in period 2 (i.e. on their period 1 maximally tolerated dose), the dose will be lowered sequentially (i.e. dose de-escalation: 160 → 120 → 80 mg twice daily, or, if on alternative dosing due to prior diuretic use, decrease dose by 50%, rounded to the nearest whole tablet strength of existing (i.e. available) furosemide tablet formulations, until tolerated). If side effects persist or if the participant chooses, oral furosemide will be stopped.

Week 19: At the first weekly treatment (i.e. Monday or Tuesday) of study week 19, participants will return all unused study drug to a study coordinator.

The window for each follow-up period intervention/treatment visit is -4 to +4 days.

4.4 Follow- up procedures (by visit types)

All study visits will occur at the time of routinely scheduled HD treatments at the patient's usual outpatient dialysis clinic. ***Section 5*** describes the measurements and assessments in detail.

Blood samples (weeks 1, 2, 3, 4, 5, 6, 10, 14, and 18): Blood will be obtained for the measurement of serum sodium, potassium, chloride, urea, creatinine, magnesium, calcium, albumin, and furosemide level. An additional 2.5 mL blood sample will be drawn, frozen, and stored in UNC Kidney Center -80° freezers for future testing as needed.

Urine samples (weeks -1, 5, 12, and 18): 24-hour urine collections will be obtained for the measurement of urine volume, sodium, urea, creatinine, and furosemide level. An aliquot of urine will be taken from the sample after the before-listed measurements are performed and be frozen and stored in UNC Kidney Center -80° freezers for future testing as needed.

Adherence assessments (weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 18): Study staff will complete pill counts.

Acceptance assessments (weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 18): Study staff will ask the participant the acceptance question.

Symptom assessments (weeks 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 18): Study staff will administer surveys evaluating patient-reported cramping, dizziness/pre-syncope, unusual tiredness/weakness, chest pain, rash, nausea, vomiting, diarrhea, tinnitus, hearing change, and numbness/tingling.

Hearing assessments (weeks 2, 4, 6, 10, 14, and 18): Study staff will administer the Inner EAR instrument.

Clinical data (weeks -4 to 18): HD treatment-level data (BPs, weights, dialysis prescription) will be obtained from the EHR.

4.5 Participant Completion/ Withdrawal procedures

If a participant withdraws from the study before Week 18, an early withdrawal visit will be scheduled to conduct the week 18 assessments (24-hour urine, symptom assessment, hearing assessment, acceptance assessment, pill count, and return of study drug). 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 study visit at week 18.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Measurements

All study evaluation and measurements will occur at study visits that occur at the time of routinely scheduled HD treatments at the patient's usual outpatient dialysis clinic. ***Section 4.4*** describes the timing and frequency of the measurements.

Baseline measurements

Complete medical history and clinical data: The participant's medical history will be taken from the EHR and confirmed verbally with the patient. The medical history will include a review of co-morbid conditions, medications, recent laboratory results (electrolytes, albumin, complete blood count, Kt/V, iron stores, bone-mineral tests), and dialysis treatment data (BPs (systolic and diastolic), weights (pre- and post-HD), UF volumes/rates, dialysate composition (sodium, potassium, bicarbonate, calcium), dialysate and blood flow rates, and vascular access type.

Serum blood tests: The following tests will be assessed via blood samples: sodium, potassium, chloride, urea, creatinine, magnesium, calcium, albumin and serum furosemide level. An additional 2.5 mL blood sample will be drawn, frozen, and stored in UNC Kidney Center -80° freezers for future testing as needed. Blood samples will be collected via the vascular access by trained dialysis clinic personnel.

24-hour urine collection: The following tests will be assessed via 24-hour urine collections: urine volume, sodium, urea, creatinine, and urine furosemide level. An aliquot of urine will be taken from the sample after the before-listed measurements are performed and be frozen and stored in UNC Kidney Center -80° freezers for future testing as needed. Urine samples will be collected by the patient in 24-hour urine collection containers. Containers will be provided by and returned to study staff at the dialysis clinic at specified study visits.

Symptom assessment: Study staff will administer a symptom questionnaire (***Appendix, Section 13.1***) to participants at specified study visits.

Hearing assessment: Study staff will administer the Inner Ear instrument (***Appendix, Section 13.2***) to participants at specified study visits.

Follow-up measurements

Serum blood tests: The following tests will be assessed via blood samples: sodium, potassium, chloride, urea, creatinine, magnesium, calcium, albumin and serum furosemide level. An additional 2.5 mL blood sample will be drawn, frozen, and stored in UNC Kidney Center -80° freezers for future testing as needed. Blood samples will be collected via the vascular access by trained dialysis clinic personnel.

24-hour urine collection: The following tests will be assessed via 24-hour urine collections: urine volume, sodium, urea, creatinine, and urine furosemide level. An aliquot of urine will be taken from the sample after the before-listed measurements are performed and be frozen and stored in UNC Kidney Center -80° freezers for future testing as needed. Urine samples will be collected by the patient in 24-hour urine collection containers. Containers will be provided by and returned to study staff at the dialysis clinic at specified study visits.

Symptom assessment: Study staff will administer a symptom questionnaire (***Appendix, Section 13.1***) to participants at specified study visits.

Hearing assessment: Study staff will administer the Inner Ear instrument (***Appendix, Section 13.2***) to participants at specified study visits.

Pill counts: Study staff will perform pill counts at specified study visits.

Acceptance assessment: Study staff will ask participants the following yes/no/unsure question: "If recommended, would you be willing to stay on the dose of furosemide you have received during the last week?"

Clinical data: The following data will be obtained from the EHR: HD treatment BPs (systolic and diastolic), weights (pre- and post-HD), UF volumes/rates, dialysate composition (sodium, potassium, bicarbonate, calcium), dialysate and blood flow rates, and vascular access type.

5.2 Specimen preparation, handling and storage

All blood samples (2.5 mL tubes) will be collected via the vascular access by HD staff. Samples will be transported (on the day they are drawn by study staff) to UNC McClendon Laboratories and UNC Hostetter Laboratory for processing and storage (extra sample) at -80°C in UNC Kidney Center freezers (5th floor Burnett-Womack building). 24-hour urine samples will be collected in 24-hour urine jugs provided by and returned to study staff at the dialysis clinic. Urine samples will be transported to UNC McClendon Laboratories and UNC Hostetter Laboratory on ice for processing. Following processing, an aliquot of urine will be taken from the sample after the before-listed measurements are performed and be frozen and stored in UNC Kidney Center -80° freezers.

6 STATISTICAL CONSIDERATIONS

The short-term endpoints will be measured in the 6-week dose titration period (period 1).
The longer-term endpoints will be measured in the 12-week follow-up period (period 2).

6.1 Primary Endpoints

Short-term and longer-term efficacy- urine volume (assessed separately)

- Among participants with baseline 24-hour urine volume ≥200 mL: incidence of ≥25% increase in urine volume
- Among participants with baseline 24-hour urine volume <200 mL: incidence of a ≥50 mL increase in urine volume to a urine volume of at least 100 mL/24-hours

Short-term and longer-term safety- electrolytes (assessed separately)

- Incidence of serum potassium <3.2 mEq/L
- Incidence of serum magnesium <0.8 mg/dL
- Incidence of serum corrected calcium <7.0 mg/dL

Short-term and longer-term safety- furosemide levels

- Incidence of serum furosemide level >12 microgram/L

Short-term and longer-term safety- BP

- Incidence of dialysis-associated 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)[21]

Short-term and longer-term safety- symptoms (assessed separately)

- Incidence of severe or very severe patient-reported rash, tinnitus, or hearing change) not attributable to causes other than furosemide
- Incidence of 10-point decrease in Inner EAR instrument score

Short-term and longer-term tolerability- symptoms (assessed separately)

- Incidence of severe or very severe patient-reported cramping, dizziness/pre-syncope, unusual tiredness/weakness, chest pain, nausea, vomiting, or diarrhea not attributable to causes other than furosemide

Short-term and longer-term acceptability

- Incidence of an affirmative response to the furosemide acceptance question

Short term- and longer-term adherence

- Incidence of participants having <20% of furosemide pills remaining at pill counts

6.2 Exploratory Endpoints

Short-term and longer-term efficacy- alternative definitions (assessed separately)

- Absolute change in urine volume from baseline (continuous in mL urine)
- Percentage change in urine volume from baseline (continuous in %)

Short-term and longer-term safety- electrolytes- alternative definitions (assessed separately)

- Absolute change in serum potassium from baseline (continuous in mEq/L)
- Absolute change in serum magnesium from baseline (continuous in mg/dL)
- Absolute change in serum corrected calcium from baseline (continuous in mg/dL)
- Percentage change in serum potassium from baseline (continuous in %)
- Percentage change in serum magnesium from baseline (continuous in %)
- Percentage change in serum corrected calcium from baseline (continuous in %)

Short-term and longer-term safety- BP- alternative definitions (assessed separately)

- Incidence of intradialytic systolic BP <80 mmHg[21]
- Incidence of intradialytic systolic BP <90 mmHg[41]
- Lowest systolic BP during HD (continuous in mmHg)

Short-term and longer-term acceptability alternative definitions (assessed separately)

- Number (percentage) of participants withdrawing from the study
- Number (percentage) period 1 participants not continuing to period 2

Short-term and longer-term adherence alternative definitions (assessed separately)

- Number (percentage) of furosemide tablets not taken (# tablets remaining/# tablets provided)
- Serum furosemide level (continuous in microgram/L and binary, present vs. absent)
- Urine furosemide level (continuous in microgram/L and binary, present vs. absent)

Clinical endpoints (assessed separately)

- Change in 24-hour creatinine clearance from baseline (continuous in ml/min/1.73m^2)
- Percentage change in 24-hour creatinine clearance from baseline (continuous in %)
- Change in 24-hour fractional excretion of sodium from baseline (continuous in %)

- Change in 24-hour fractional excretion of urea from baseline (continuous in %)
- Percentage change in 24-hour fractional excretion of urea from baseline (continuous in %)
- Pre-HD systolic BP (mmHg on a per-HD treatment basis)
- IDWG (kg on a per-HD treatment basis)
- UF volume (mL on a per-HD treatment basis)
- UF rate (mL/h/kg on a per-HD treatment basis)
- Target weight achievement (post-HD weight (kg) within 1kg of prescribed target weight) (binary on a per-HD treatment basis)
- Post-HD weight- target weight difference (post-HD weight – prescribed dry/target weight, kg on a per-HD treatment basis)
- Extra HD treatments (number)
- Hospitalizations (number)

6.3 Statistical Methods

6.3.1 Overview

All data analyses will be preceded by extensive data checking and verification to resolve the reasons for missing values, inconsistencies, and out-of-expected-range values. All statistical estimates of population parameters will be tabulated along with corresponding confidence intervals (CIs) and will be based on the convenience sample of patients participating in the study. The primary focus of the analyses will be the magnitudes and directions of the point- and interval-estimates. All analyses will be in intention-to-treat analyses. All reported p-values will be 2-sided; <0.05 representing significance. All hypothesis test results (i.e. p-values) that are deemed not statistically significant (defined by a p-value ≥0.5) will be reported as inconclusive. Analyses will be performed with SAS 9.4 (Cary, NC) or a comparable statistical programming software package.

6.3.2 Missing data

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 relevant, scheduled outcome evaluations until the end of the study. The characteristics at the time of study start 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. Missing data variables will be identified in the data management system with a missing data indicator such that they can be easily identified. Reasons for missing data will be captured at each study visit and entered into the study database. No imputation of missing data will be performed.

6.3.3 Descriptive analyses

Examination of baseline characteristics will include estimates of the distribution of age, race, and other demographic characteristics, lab measures, and dialysis clinic. We will report baseline patient characteristics as counts (%) for categorical variables, and depending on the distribution, as means ± standard deviations (SDs) or medians [interquartile ranges] for continuous 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.

6.3.4 Primary analyses (each assessed separately)

At designated study time-points, we will determine the [data type; see **Section 6.1** for definitions]:

- change from baseline for the short and longer-term efficacy measure [binary] (24-hour urine volume);
- value of short and longer-term safety electrolyte measures [binary] (serum potassium, magnesium, corrected calcium);

- value of serum furosemide levels [binary] (short and longer-term safety furosemide level measure);
- incidence of dialysis-associated hypotension [binary] (short and longer-term safety blood pressure);
- incidence of severe or very severe patient-reported allergic or toxicity-related symptoms [binary] (short and longer-term safety symptom measures);
- change from baseline of Inner EAR instrument score [binary] (short and longer-term safety symptom measure);
- incidence of severe or very severe patient reported symptoms [binary] (short and longer-term tolerability symptom measures);
- incidence of patient-reported acceptance of furosemide [binary] (short and longer-term acceptability measure);
- incidence of adherence by pill counts [binary] (short and longer-term adherence measure).

Change measures and value measures (e.g. 24-hour urine volume, serum electrolytes, serum furosemide level) will be analyzed via one-way repeated measures of analysis of variance to determine the presence of absence of dose-related differences. In addition, we will use mixed effects modeling to test the effect of increased dose and duration with the fixed effect of time (dose), random subject effect, and an unstructured repeated covariance type. We will use generalized estimating equations to assess categorical outcomes (e.g. sufficient increase in 24-h urine volume, hypokalemia, hypomagnesemia, hypotension, symptoms, Inner EAR score, patient acceptance). We will also consider binary outcomes as rates (events/time).

6.3.5 Exploratory analyses (each assessed separately)

At designated study time-points, we will determine the [data type; see **Section 6.2** for definitions]:

- Absolute change from baseline for the short and longer-term efficacy measure [continuous] (24-hour urine volume);
- percentage change from baseline for the short and longer-term efficacy measure [continuous] (24-hour urine volume);
- absolute change from baseline for the short and longer-term safety electrolyte measures [continuous] (serum potassium, magnesium, corrected calcium);
- percentage change from baseline for the short and longer-term safety electrolyte measures [continuous] (serum potassium, magnesium, corrected calcium);
- occurrence of dialysis-associated hypotension- alternative definition (<80 mmHg) [binary] (short and longer-term safety blood pressure);
- occurrence of dialysis-associated hypotension- alternative definition (<90 mmHg) [binary] (short and longer-term safety blood pressure);
- value of lowest systolic BP during HD [continuous] (short and longer-term safety blood pressure);
- number (percentage) of patients withdrawing from the study [continuous] (short and longer-term acceptability);
- number (percentage) of patients not continuing into period 2 [continuous] (short and longer-term acceptability);
- value of serum furosemide levels [continuous; binary] (short and longer-term acceptability);
- value of urine furosemide levels [continuous; binary] (short and longer-term acceptability);
- absolute change from baseline for 24-hour creatinine clearance [continuous]
- percentage change from baseline for 24-hour creatinine clearance [continuous]
- absolute change from baseline in 24-hour fractional excretion of sodium [continuous]
- percentage change from baseline in 24-hour fractional excretion of sodium [continuous]
- absolute change from baseline in 24-hour fractional excretion of urea [continuous]
- percentage change from baseline in 24-hour fractional excretion of urea [continuous]
- value of pre-HD systolic BP [continuous]
- value of IDWG [continuous]
- value of UF volume [continuous]

- value of UF rate [continuous]
- occurrence of target weight achievement [binary]
- value of post-HD weight and target weight differential [continuous]
- occurrence of extra HD treatments [continuous]
- occurrence of hospitalizations [continuous]

Analogous to the primary analyses, percentage change measures, change measures, and value measures will be analyzed via one-way repeated measures of analysis of variance to determine the presence of absence of dose-related differences. In addition, we will use mixed effects modeling to test the effect of increased dose and duration with the fixed effect of time (dose), random subject effect, and an unstructured repeated covariance type. We will use generalized estimating equations to assess categorical outcomes. We will also consider binary outcomes as rates (events/time). Distribution of the exploratory parameters will be examined and appropriate transformation will be applied. These analyses are considered hypothesis-generating, and we will examine directionality and strength of relationships but will not assess via p-values.

6.3.6 Subgroup analyses

We will consider subgroups of incident vs. prevalent patients and subgroups of patients who were prescribed a diuretic (vs. not) prior to study start. Given the small sample, these analyses will be considered exploratory and models will be adjusted for baseline demographic and clinical factors.

6.3.7 Sensitivity analyses

We will conduct sensitivity analyses to test the robustness of our findings. Results of these analyses will be used to guide soundness of the main results. If the main results are demonstrated to be fragile, we will soften our conclusions around the main results as appropriate. Alternatively, if the main results are demonstrated to be robust, we will be confident in our main results conclusions. Specifically, we will conduct sensitivity analyses:

- excluding data from participants with endpoints drawn “off-schedule” (e.g. serum electrolytes, 24-hour urine, symptom assessment, etc.);
- excluding data with questionable values;
- testing for difference by clinic;
- evaluating the impact of variations on model assumptions and covariates;
- evaluating model residuals;
- testing for difference with transformations of scale and changes in the definitions of outcome variables

6.4 Sample Size and Power

We designed this pilot study to evaluate the safety, tolerability, and acceptability of furosemide use in HD patients and assess for furosemide efficacy and safety signals. We aim to enroll a sufficient number of participants to yield data supporting the efficacy, acceptability, tolerability, and safety of furosemide and to provide a basis for calculating the sample size of a future randomized trial. Sample size considerations were framed using standard study design parameters to ensure 80% power to detect pre-specified effect sizes utilizing intermediate outcomes.

With a sample size of 36 patients, we have 83% power to detect a mean change in urine volume from baseline of at least 5 ml/24-h, assuming a change S.D. = 10 ml/24-h[17] and a 2-sided $\alpha = 0.05$ (>99% power for urine volume change of 10 mL/24-h). With 30 patients, we have 99% power to detect a mean change in urine volume from baseline of at least 10 mL/24-h. Sample size calculation estimates were based on existing data about expected response to furosemide and S.D.s observed in other studies of urine volume in HD patients.[17]

Our goal sample size is 36 participants. We will account for dropout by enrolling up to 40 participants. If fewer than 30 participants are eligible for period 2 at the end of the period 1 dose titration period (i.e. participants who did not

tolerate or accept furosemide in period 1), we will recruit additional participants to maintain a sample size over 30 participants for the follow-up period.

6.5 Interim Analysis

No interim analysis is planned. See ***Section 7*** for safety management plans.

7 SAFETY MANAGEMENT

7.1. 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

7.1.1. 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.

7.1.2. Serious Adverse Event

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

- fatal or results in death
- life-threatening
- requires inpatient hospitalization (>24 hours) or prolongs existing 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.

7.1.3. Unanticipated Problems Involving Risk to Participants or Others

(See also ***Section 7.3.4***)

An Unanticipated Problem is any incident, experience, or outcome that meets **all** 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.

7.2. Adverse Event Tracking Period

The study period during which AEs 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 2 weeks following the last dose of study drug.

7.2.1. Post-study Adverse Event

All unresolved adverse AEs will be followed by the investigator until the events are resolved, the participant is lost to follow-up, or the AE 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 study monitor of any death or AE 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.

7.3. Recording of Adverse Events

At each contact with the participant, the investigator or site designee will seek information on AEs by specific questioning and, as appropriate, by examination. Information on AEs will be recorded in the source document, and also on the AE 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.

7.3.1. Adverse Events of Interest

The following AEs are anticipated in patients treated with furosemide and will be recorded on the appropriate AE of Interest CRF. See ***Section 7.3.2*** for definitions.

- Hypokalemia
- Hypocalcemia
- Hypomagnesemia
- Hypotension
- Hearing change
- Tinnitus
- Rash

If the event meets the definition of serious as defined in ***Section 7.1.2***, it will be recorded on an SAE form in addition to the appropriate AE of special interest CRF. Participants will be assessed for the occurrence of AEs of Interest at study visits (weekly during dose escalation and every other week during follow-up). 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.

7.3.2. Trial-Defined Adverse Events

The HD population undergoes frequent laboratory testing and has a high rate of peri-dialytic hypotensive events requiring changes in the dialysis prescription, adjustment of estimated dry (target) weight, or change in dialysis-related medications. Due to the unique nature of this population, the following events are considered routine aspects of maintenance HD therapy and will not be recorded as an AE except as noted:

- Anemia – will be reported when hemoglobin is <7.5 mg/dL
- Hypokalemia – will be reported when potassium level <3.2 mEq/L
- Hyperkalemia – will be reported when potassium level ≥6.6 mEq/L
- Hypomagnesemia – will be reported when magnesium level <0.8 mEq/L
- Hyperphosphatemia – will be reported when phosphorous >9.5 mg/dL

- Hypocalcemia – will be reported when serum corrected calcium <7.0 mg/dL
- Hypercalcemia – will be reported when serum corrected calcium >11.0 mg/dL
- Hyperparathyroidism – will be reported when PTH >1200 pg/mL
- Hypotension – will be reported when it meets the criteria for serious dialysis-associated hypotension (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))
- Hearing change – will be reported if Inner EAR score decreases by 10 points from baseline
- Tinnitus- will be reported at level of severe or very severe patient-reported tinnitus
- Rash- will be reported at level of severe or very severe patient-reported rash

7.3.3. Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE 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 7.3.2.***

7.3.4. Anticipated Adverse Events

The following AEs are anticipated in the HD 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 7.1.3***.

- Death
- Coronary Ischemia including:
    - o Unstable angina
    - o Acute MI
    - o Coronary revascularization
- Heart failure hospitalization or exacerbation
- Cardiac arrest
- Cardiac arrhythmia (ventricular or atrial)
- Peripheral vascular revascularization
- Amputation
- Vascular Access Events Including:
    - o Catheter exchange, removal or declotting
    - o Arteriovenous graft or fistula complications
    - o Clotting
    - o Stenosis
    - o Revascularization
    - o Infection
- Infections Including:
    - o Pneumonia
    - o Vascular access infection
    - o Bacteremia
    - o Clostridium difficile infection

7.4. Reporting of Serious Adverse Events and Unanticipated Problems

The investigator will report SAEs and Unanticipated Problems to the safety monitor 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 sent to the safety monitor. The safety monitor will facilitate the timely medical review and reporting of the event in accordance with 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
- Participant identifier
- 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 AEs should be provided promptly to the safety monitor.

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.

7.4.1. Investigator Reporting to the IRB

Investigators will report SAEs and Unanticipated problems to the IRB in accordance with the Office of Human Research Protections (OHRP) guidelines. OHRP recommends that:

1) Unanticipated problems that are SAEs 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.

<u>Reporting Process</u>

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 study files.

<u>Other Reportable events:</u>

• Any adverse experience that, even without detailed analysis, represents a serious unexpected AE that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).

• Any AE 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.

7.4 Reporting of Pregnancy

Pregnancies (including a positive pregnancy test regardless of age or disease state) of a female participant occurring while the participant is enrolled will result in study cessation for the pregnant participant. The female participant will be referred to an obstetrician-gynecologist. Pregnant dialysis patients require more intensive dialysis therapy and are not appropriate for ongoing study participation. The Investigator will follow the female participant until completion of the pregnancy and must document the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

7.5 Stopping Rules

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

7.6. Data Safety and Monitoring Plan

7.6.1 Overall Framework

There are three different acceptable approaches to data and safety monitoring plans. Monitoring can be conducted by the research team, an independent (external) safety monitor or group, or an independent data safety monitoring board. This study will enroll up to 40 patients and will last for 18 weeks (6 weeks of dose escalation and 12 weeks of subsequent follow-up per participant). Given the small number of participants, relatively short duration of the study, the frequency of study visits (weekly in the first 6-weeks and then every 2 weeks in the latter 12-weeks), the participants' regular contact with medical professionals (three times a week at a dialysis clinic), the lack of blinding, that all activities will take place at a single site (University of North Carolina at Chapel Hill), and that the potential AEs are likely to be mild in nature and detected by study personnel and/or dialysis clinic personnel, we will ensure participant safety through regular review by the research team. This will include review of study progress, listings of AEs, and AE logs. We will also use an independent safety monitor to provide an additional layer of protection.

7.6.2 Monitoring and Frequency

Monitoring by the research team: The PI and study coordinator will maintain non-serious AE and SAE logs and case report forms throughout the study. The research team (study investigators, other significant contributors and research coordinator) will review study records, AEs, and SAEs for all participants on an ongoing basis. The UNC research team includes 1 board-certified nephrologist and a dialysis-trained pharmacist, both with the necessary expertise to assess study AEs. The Principal Investigator (PI) will review all such events every 2 weeks. All accumulated AEs of interest, SAEs,

and data on overall AE rates will be presented at the monthly project team meetings to the broader research team. Review of the data will uncover patterns that might suggest a need for modification of study practices to ensure patient safety. If the research team uncovers such patterns, the safety monitor will be asked to review the events.

The PI and/or research coordinator will report all SAEs to the research team and external safety monitor within 24 hours of becoming aware of the event. The PI and/or research coordinator will report SAEs that are unanticipated and related to the research to the research team, external safety monitor, and institutional review board (IRB) within 24 hours of becoming aware of the event. The PI will personally review all study results and notify participants of any abnormal results, including those present at baseline unrelated to the study intervention to ensure participants are able to seek appropriate medical care.

***Monitoring by an external safety monitor*:** Additional monitoring will be provided by a board-certified adult nephrologist not associated with the study. This external monitor will review all AEs and SAEs in detail including the time of onset, description, severity, time course, duration, outcome, relationship of the AE/SAE to the study drug, and any action(s) taken. The external safety monitor will review AEs and SAEs every 6 months (3 times over the ~18-month study), and SAEs that are unanticipated and related to the research as soon as the research team becomes aware. The monitor will help the research team determine whether further safety guards should be instituted and whether the benefit of the study continues to outweigh potential risks.

Information to be monitored: The research team and external safety monitor will monitor AEs including hypotension, allergic or toxicity-related symptoms (rash, tinnitus, or hearing change), objective hearing change, and electrolyte abnormalities as well as SAEs.

7.7 Medical Emergency Procedures

7.1.1 Hypokalemia management

Serum potassium will be measured weekly during dose escalation and monthly during follow-up. Treating clinicians will manage the dialysate potassium according to clinic standards. Study staff will monitor potassium values and contact treating clinicians in the event of hypokalemia to ensure that hypokalemia is being actively managed. Treating clinicians will be free to manage hypokalemia according to local standards, including changes to the dialysate potassium concentration. However, study drug dose reductions or discontinuation will be directly managed by study investigators. Occurrence of a serum potassium <3.2 mEq/L will end dose titration, and the participant will return to the prior tolerated furosemide dose. If the hypokalemia occurs at the lowest administered dose, furosemide will be stopped and not restarted. Serum potassium will be rechecked within one week when potassium is <3.2 mEq/L. Results of serum potassium will be conveyed to treating clinicians and the dialysis unit nursing staff.

7.1.2 Hypomagnesemia management

Serum magnesium will be measured weekly during dose escalation and monthly during follow-up. Treating clinicians will manage the dialysate magnesium according to clinic standards. Study staff will monitor magnesium values and contact treating clinicians in the event of hypomagnesemia to ensure that hypomagnesemia is being actively managed. Treating clinicians will be free to manage hypomagnesemia according to local standards, including changes to the dialysate magnesium concentration. However, study drug dose reductions or discontinuation will be directly managed by study investigators. Occurrence of a serum magnesium <0.8 mEq/L will end dose titration, and the participant will return to the prior tolerated furosemide dose. If the hypomagnesemia occurs at the lowest administered dose, furosemide will be stopped and not restarted. Serum magnesium will be rechecked within one week when magnesium is <0.8 mEq/L. Results of serum magnesium will be conveyed to treating clinicians and the dialysis unit nursing staff.

7.1.3 Hypocalcemia management

Serum corrected calcium will be measured weekly during dose escalation and monthly during follow-up. Treating clinicians will manage the dialysate calcium according to clinic standards. Study staff will monitor corrected calcium values and contact treating clinicians in the event of hypocalcemia to ensure that hypocalcemia is being actively managed. Treating clinicians will be free to manage hypocalcemia according to local standards, including changes to the dialysate calcium concentration. However, study drug dose reductions or discontinuation will be directly managed by study investigators. Occurrence of a corrected serum corrected calcium <7.0 mg/dL will end dose titration, and the participant will return to the prior tolerated furosemide dose. If the hypocalcemia occurs at the lowest administered dose, furosemide will be stopped and not restarted. Serum corrected calcium will be rechecked within one week when corrected calcium is <7.0 mg/dL. Results of serum corrected calcium will be conveyed to treating clinicians and the dialysis unit nursing staff.

7.1.4 Hypotension management

In the event of serious dialysis-associated hypotension (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)) study investigators will review the participant's non-study anti-hypertensive medications, estimated dry (target) weight, and volume status. The participant's nephrologist 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 prescribed dry (target) weight if there is no evidence of peripheral or pulmonary edema
3) Reduce UF rate by increasing dialysis session duration
4) Reduce dose of study drug if 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.

7.1.5 Ototoxicity management

Change in hearing will be monitored by patient self-report (on symptom assessment) weekly during dose titration and every other week during follow-up and measured with the Inner EAR instrument every other week during dose titration and monthly during follow-up. A decrease by ≥10 points from baseline will constitute clinically significant hearing loss. We will refer participants with self-reported hearing loss (any severity) or ≥10-point decline in Inner EAR scores after furosemide initiation and/or dose titration for audiometry testing.

8 DATA COLLECTION AND MANAGMENT

8.1 Source Documents and Access to Source Data/Documents

Each participating clinic will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants. As part of participating in a NIH IC-sponsored study, each clinic will permit authorized representatives of the NIH IC and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity. Study staff will have access to study records.

Data (BPs, weights, dialysis treatment data) will be extracted from EHR and stored in REDCap databases housed on a secure Department of Medicine server. Symptoms, Inner EAR responses, and acceptance report will be recorded on study data collection forms. All electronic information will be stored on a password-protected UNC server (with secure,

password and firewall-protected Department of Medicine server back-up) and accessed via a UNC password-protected computer and will be identifiably by study identification number only. Information linking the study identification number to the patient will be kept in a separate, password-protected database on the server. Study charts will be maintained in a locked room of the dialysis clinic. Study charts will be identified by study IDs. Signed consent forms will be kept in a locked filing cabinet in the PI's locked office. Initial paper records of symptoms, Inner EAR responses, and acceptance report will be stored in the study chart and, after study completion, will be transferred to a locked filing cabinet in the locked office of the PI. Paper records will be destroyed (shredded and disposed in locked, shred bins) after verification of electronic data entry. Only the PI and research coordinator will have access to patient information with patient identifiers.

8.2 Quality Assurance and Quality Control

Quality control procedures will be implemented beginning with data entry system and data quality control checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. Following written SOPs, the monitors will verify that the clinical trial is conducted and data generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. The investigational site will provide direct access to all trial-related sites, sources data/ documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The PI will be responsible for quality assurance and quality control and will provide oversight of all research team members to assure adherence to the protocol. Except for in an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

8.3 Data Handling and Record Keeping

8.3.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring accuracy, completeness, legibility and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Research material for this study will consist of baseline patient data including demographics, co-morbidities, biochemical data, and HD treatment data (dialysate, dialysis time, target weight, UF volume, and medications) collected via patient interview and EHR review. Patient data will be identified with a study identification number. Patient-signed study informed consent forms will contain patient names and signatures. These consent forms will be stored separately from other study data in a locked filing cabinet in the PI's locked office. Only the PI and research coordinators will have access to consent forms.

The collected study data will not contain participants' names, medical record numbers, social security numbers, or contact information (address, telephone number), such that these data cannot be linked back to the source patients. Data collected about patients will be labeled with a study identification number. A database linking patient names and study identification numbers will be maintained separate from the study data. This database will be password-protected and will be maintained on a password-protected UNC Department of Medicine server that is accessed via a UNC password-protected computer with anti-virus and UNC firewall protection. The study statistician (to-be-named) will have access to the de-identified electronic data.

The PI will be the primary responsible party for the oversight and management of the study data. The study research coordinator will assist with data management.

8.3.2 Study Records Retention

Study documentation includes all case report forms, data correction forms or queries, source documents, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases (as applicable to this study), study documents should be kept on file until three years after the completion and final study report of this investigational study.

8.3.3 Protocol Deviations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs:
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants
- Has damaged the scientific integrity of the data collected for the study
- Results from willful or knowing misconduct on the part of the investigator(s)
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs, the below guidelines will be followed:

Protocol Deviations**:** UNC personnel will record the deviation in a Case Report Form and report to IRB or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to the IRB at the time of continuing review.

Protocol Violations**:** Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

Unanticipated Problems**:** Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

9 RECRUITMENT STRATEGY

9.1 Strategies for Recruitment and Retention

Recruitment

Our goal sample size is 36 participants in period 1 with the goal of >30 participants continuing on to period 2. We will recruit up to 40 participants to account for dropout and will recruit additional participants if fewer than 30 participants are eligible for period 2. All participants will be recruited from the University of North Carolina (UNC)-owned Siler City, Pittsboro, Sanford, Lee County, and Carrboro Carolina Dialysis HD clinics. IRB-approved flyers providing brief study information (including background and rationale) and research team contact information will be placed in the

participating dialysis clinics prior to participant recruitment. Nephrologists at the participating dialysis clinics will identify eligible patients from the dialysis clinics' patient rosters. A care team member will then briefly introduce the study to the patient and obtain the patient's permission to be contacted by the study team for further screening with study inclusion and exclusion criteria. The PI will approach eligible patients who have expressed interest in participating in the study and provide them with detailed information about the study and answer their questions. If the patient is willing to participate in the research study, informed consent will be obtained by the PI, a licensed physician. Recruitment scripts and informed consents will be available in both English and Spanish. Interpreters will be used as needed.

Based on the composition of the patient populations at the participating UNC sites, the expected sex breakdown is 50% male and 50% female and the expected enrollment of Hispanic or Latino participants is 10%. No exclusion by sex or by ethnic/ racial background will be made. There are no expected differences in the response to furosemide across sex or racial/ ethnic groups. Children (individuals < 18 years) and vulnerable participants are not included in the study.

Retention

Retention of research participants in clinical trials is always a concern. However, the activities of the proposed study are not especially burdensome. Participants will be taking a twice a day medication that is unlikely to have substantial side effects. The duration of the entire study is relatively short (18 weeks). The dose escalation period, the first 6 weeks of the study, requires blood draws at weekly intervals during participants' routine HD treatments and two urine collections. Clinic personnel will collect blood samples via the vascular access at the start of dialysis treatment. Therefore, study blood collections will not necessitate additional needle sticks. Participants will return urine collection containers to the study team at routine HD treatments. In the subsequent 12-week follow-up period, blood draws occur every 4 weeks during participants' routine HD treatments and urine will be collected twice. All study visits will occur at the time of regularly scheduled dialysis treatments at the participant's dialysis clinic. Participants will receive up to $199 remuneration for study participation. We will encourage adherence to study testing by 1) timing remuneration provision with urine sample collections and 2) providing larger remuneration amounts to participants who return their 24-hour urine collection samples to the study team compared to participants who do not return their 24-hour urine samples. The research team will remain in close contact with participants through weekly study visits in the first 6 weeks of the study and then every other week visits in the final 12 weeks of the study. In addition, the research team will provide participants with the research team's contact information (telephone and email) and encourage participants to contact the team at any time. The research team will provide participants with updates on study progress at pre-specified time points. At the conclusion of the study, results will be shared with participants who expressed interest in receiving them via follow-up letters written at a 5th grade reading level.

9.2 Participant Withdrawal of Termination

9.2.1 Reasons for Withdrawal of Termination

- Active (voluntary) withdrawal of consent by the study participant
- Transfer of care to a non-participating dialysis clinic
- An investigator may terminate participation in the study if:
    - Non-adherence to study data collection procedures (e.g. refusal of blood draw or symptom monitoring)
    - Violation of the inclusion/ exclusion criteria determined after the fact
    - Any clinical AE, laboratory abnormality or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant. Such events are specified in ***Section 1.3.1.***

9.2.2 Handling of Participant Withdrawals or Termination

While participants will be encouraged to complete the study, they may withdraw from the study at any time and for any reason. Every effort will be made to determine why any participant withdraws from the study prematurely. This information will be recorded.

9.3 Premature Termination or Suspension of Study

If the investigators become aware of conditions or events that suggest a possible hazard to participants if the clinical study continues, the clinical study may be terminated after appropriate consultation between the involved parties. Conditions that may warrant termination of the clinical study include but are not limited to the discovery of an unexpected, relevant or unacceptable risk to the participants enrolled in the clinical study or failure to enroll participants at the required rate.

10 ETHICS/ PROTECTION OF HUMAN SUBJECTS

10.1 Ethical Standard

The investigators will adhere to NIH Human Research Protections Program policies and procedures.

10.2 Institutional Review Board

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The protocol, informed consent, recruitment materials and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented. The investigators will adhere to NIH Human Research Protections Program policies and procedures.

10.3 Informed Consent Process

10.3.1 Consent and Other Informational Documents Provided to Participants

An IRB-approved consent form and IRB-approved study informational page will be provided to potential participants.

10.3.2 Consent Procedures and Documentation

In obtaining and documenting informed consent, the investigators will comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) Good Laboratory Practice (GLP) and to ethical principles that have their origin in the Declaration of Helsinki. The PI will approach eligible patients and offer then the opportunity to participate in the study. If the patient does desire to participate in the research study, informed consent will be obtained by the PI. The purpose of the informed consent procedure is to assist the patient in understanding the nature and purpose of the study and the risks and benefits of the study, to offer answers to questions about any details of the study, and to obtain the patient's signature on the IRB-approved consent form. All patients will be reminded that participation in the study is voluntary and that their medical care will not be affected if they choose not to participate. The patients will be informed that they will be free to terminate participation at any time during the study. The rights and welfare of participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in the study. The PI will also ask the patients to sign HIPAA waivers allowing the PI to review the individual's EHR for clinical data.

We are recruiting English-speaking and Spanish-speaking individuals for this study. Each participating dialysis clinic has in-clinic non-English interpretation coverage by a professional interpreter. The interpreter will interpret for Spanish-speaking patients while the PI or other study staff consent patients for the study. If Spanish-speaking participants have questions outside of study visit times, the interpreter will provide language interpretation over the phone. In addition, one of our research assistants is Spanish speaking.

10.4 Participant and Data Confidentiality

Study procedures and data collection will be performed after informed consent is obtained via patient signature on the IRB-approved consent form. All electronic information will be stored on a password-protected UNC computer (with secure, password and firewall-protected Department of Medicine server back-up) in the locked office of the study PI and will be identifiably by study identification number only. Information linking the study identification number to the patient will be kept in a separate, password-protected database on the computer. Signed consent forms will be kept in a locked filing cabinet in the PI's locked office. Initial paper records of study HD treatment data will be stored in a locked filing cabinet in the locked office of the PI. Paper records will be destroyed (shredded and disposed in locked, shred bins) after verification of electronic data entry. Only the PI and research assistant will have access to patient information with patient identifiers. No documents containing patient information with patient identifiers will be downloaded from the secure Department of Medicine server onto personal computers.

10.4.1 Research Use of Stored Human Samples, Specimens or Data

One vial of stored blood and one aliquot of urine will be maintained in UNC Kidney Center freezers (5th floor Burnett-Womack) until 12 months after study completion. Access to stored samples will be limited to study staff. Samples and data will be stored using codes assigned by the investigators. Data will be kept on password-protected secure Department of Medicine servers. Only study staff will have access to the samples and data. Study participants who request destruction of the sample prior to the 12 months after study completion date will be notified of compliance with such request and all supporting details will be maintained for tracking.

10.5 Future Use of Stored Specimens

With the participant's approval and as approved by the IRB, de-identified biological samples (blood and urine) will be stored in the UNC Kidney Center freezers (5th floor Burnett-Womack). After the study is completed, the de-identified, archived blood and urine samples will be maintained for 12 months under the supervision of the PI, Dr. Flythe. There will be no transfer of the specimens for use outside of the study. Potential additional laboratory testing relevant to this study may be performed. No genetic testing will be performed. The potential for additional laboratory testing on stored specimens will be listed in the IRB-approved consent form. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future use. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

11 PLANS FOR PUBLICATION AND DATA SHARING

This study will comply with NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts to the digital archive PubMed Central upon acceptance for publication. Specifically, the investigative team intends to publish a manuscript describing the study protocol and, separately, a manuscript reporting the study results, both in peer-reviewed journals. The study will be registered at clinicaltrials.gov, sponsored by the National Library of Medicine.

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13 APPENDICES

13.1 Symptom Assessment

Items

During your dialysis treatments over the [last week (for dose titration period) and two weeks (for follow-up period)] did you have…?

1. Cramping
2. Dizziness or lightheadedness
3. Unusual tiredness
4. Unusual weakness
5. Chest pain
6. New rash
7. Nausea
8. Vomiting
9. Diarrhea
10. Tinnitus or ear ringing
11. Hearing change
12. Other (patient self-report)

Response Options**:** 0=did not have.

- If present, how severe was it? 1=mild, 2=moderate, 3=severe, 4=very severe

13.2 Inner EAR Instrument

Items

1. Ability to understand with a family and close friends are saying.
2. Ability to understand speech in a quiet room.
3. Ability to hear what is said by those at your table in a crowded restaurant.
4. Ability to hear what you want and filter out unwanted noises.
5. Ability to hear telephone conversations.
6. Ability to hear in different listening situations.
7. Ability to hear soft household sounds (e.g. car turn signal, clock ticking).
8. Mood based on your ability to hear.
9. Bothered by having to ask people to repeat.
10. Bothered by having to restrict activities because of your hearing.
11. Please rate your overall hearing.

Response Options

Questions 1-8: 5-point Likert scale- 1=poor, 2=fair, 3=good, 4=very good, 5=excellent

Questions 9-10: 4-point Likert scale- 1=very bothered, 2=somewhat bothered, 3=a little bothered, 4=not bothered

Question 11: 11-point scale (select a number between 0 and 11 with the following markers- 0=hate, 5=it's OK, 10=love it