Protocol and Statical Analysis Plan

Title: Safety, tolerability, and feasibility of empagliflozin therapy in dialysis-dependent ESKD

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Safety, tolerability, and feasibility of empagliflozin therapy in dialysis-dependent ESKD

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1. INTRODUCTION

1.1 Background: Patients with end-stage kidney disease (ESKD) suffer extraordinarily high burden of cardiovascular (CV) disease, with a mortality rate that is 10- to 15-fold higher than the rate for those not receiving dialysis.[1] Most notably, sudden cardiac death accounts for 75-80% of all CV deaths in ESKD.[1] While the pathomechanisms underlying the potent susceptibility of dialysis patients to sudden death remain incompletely understood, metabolic derangements and inherent abnormalities of the uremic myocardium, such as left ventricular hypertrophy (LVH), fibrosis, and diastolic dysfunction, play a pivotal role in development of *heart failure (HF) and sudden death*.[2-5] Diagnosis of HF, one of the most powerful predictors of sudden death,[6] is particularly ominous in ESKD, with an 80% mortality risk within three years.[7] Despite the dire need for effective intervention in ESKD, there has been no recent advance to ameliorate the risk of CV mortality.

Sodium-glucose cotransporter 2 inhibitors (SGLT2is), a novel class of oral hypoglycemic medications that reduce glucose reabsorption in the renal proximal tubule, have in recent years demonstrated unprecedented CV and survival benefits in large randomized clinical trials in patients with[8-11] and *without* type 2 diabetes.[12,13] These benefits were driven by the reduction in *incident HF events* and *sudden death*, without significant impact on the atherosclerotic CV events.[14-16] Particularly noteworthy is the finding that the extraordinary benefits on HF hospitalization and CV mortality occur very early, within 1 to 3 months of the drug initiation and is maintained for several years,[11] suggesting that the underlying mechanisms are independent of the long-term secondary risk factors, such as effects on glucose and blood pressure control. Furthermore, the beneficial effects of SGLT2is are seen at similar proportions across patients with different levels of glycemic control, *including non-diabetics*,[12,13] and at *lower estimated glomerular filtration (eGFR) levels*.[9,12,17]

By promoting urinary glucose excretion, SGLT2is trigger multiple downstream effects, including optimization of ventricular loading conditions via reduction in preload (through natriuresis and osmotic diuresis[18]) and afterload (by decreasing blood pressure).[19] Induction of glycosuria by SGLT2is also leads to reduction in serum glucose and insulin levels, increase in glucagon, and a shift in whole-body fuel metabolism toward ketone production and fat oxidation.[20,21] As a result, SGLT2is cause significant reduction of fat mass,[22] marked improvement in insulin sensitivity,[23-25] and optimization of cellular bioenergetics and mitochondrial efficiency in multiple organ systems,[26-29] particularly in the heart.[30-32] An increasing number of studies demonstrate efficacy of SGLT2is to reverse LVH and improve diastolic function in both diabetic[33-35] and non-diabetic[36] experimental models and clinical studies of diabetic patients with CV diseases.[37-43] Given that the clinical benefits of SGLT2is are thought to be consequences of their primary action on the kidney (e.g., glycosuria and natriuresis), however, clinical trials have excluded those with advanced renal failure. Empagliflozin, a specific SGLT2i, is thus currently not approved by the Food and Drug Administration (FDA) for use in those with eGFR <30 ml/min/1.73m^2. There is compelling evidence, however, that some of the metabolic and cardiac effects of SGLT2is may be independent of renal SGLT2 inhibition (i.e., glycosuria, and natriuresis),[44,45] *raising a crucial question if SGLT2is may confer protection and improve survival in ESKD.*

1.2 The rationale for the SGLT2i therapy in ESKD: There has been no recent advance to address the metabolic and CV abnormalities underlying the profound mortality risk in ESKD, and SGLT2is represent a potentially unprecedented therapeutic opportunity for dialysis patients. Despite the widely held hypothesis that the benefits of SGLT2is are downstream consequences of glycosuria and natriuresis, there are compelling reasons to use SGLT2is in oliguric or anuric ESKD patients: (1) *In-vitro* studies suggest direct effects of SGLT2is on myocardium[46] and adipocytes,[26,29,47] suggesting that functioning kidney is not a prerequisite to receive benefits. Specifically, direct inhibition of myocardial NHE-1 by SGLT2is targets the pathways involved in development of LVH,[48,49] one of the most important pathogenic mechanisms underlying the increased sudden death risk in ESKD. The direct action on adipocytes may also result in mitigation of systemic insulin resistance, an important modifiable CV risk factor. (2) SGLT2is target and restore a fundamental metabolic defect in ESKD, the mitochondrial dysfunction and inefficient cellular bioenergetics in key organ systems, especially the heart.[30-32] (3) Glycosuria may not be critical to achieve benefits. Although the glucose-lowering efficacy of SGLT2is decreases at the lower eGFR range,[8,9] the CV benefits are remarkably preserved down to eGFR of 25 ml/min/1.73m^2.[9,12,17] (4) Therapy with SGLT2is is likely to be safe and well tolerated in ESKD. The major adverse effects of SGLT2is include volume depletion, mycotic genital infection, and rare instances of ketoacidosis.[50-52] Increased risk for fracture and leg amputation was reported with canagliflozin only [8,53] and no other SGLT2is. The complication of Fournier's gangrene remains very rare.[54] Given that these adverse events

are likely related to glycosuria, SGLT2is are *less likely* to cause these problems in oliguric or anuric dialysis patients. In addition, the risk of ketoacidosis may be fundamentally lower in dialysis patients with diabetes, given reduced insulin clearance and decreased renal reabsorption of ketones in ESKD.

1.3 Favorable safety profile in ESKD: Glucuronidation is the major metabolic pathway for empagliflozin, but no major metabolites of empagliflozin ($C_{23}H_{27}ClO_7$, molecular weight 450.9 g/mol) are detected in human plasma.[55] Approximately 11–19% of the administered dose is excreted unchanged in urine. A pharmacokinetic (PK) study including 8 non-diabetic hemodialysis patients indicated that renal failure only modestly increased systemic exposure of empagliflozin, and thus no dose adjustment of empagliflozin was required.[56] Plasma protein binding of empagliflozin was only slightly lower in subjects with renal impairment than in those with normal renal function (81.1% in ESKD vs. 85.1% in normal renal function).[56] It is thus unlikely that hemodialysis would remove the drug or alter plasma PK significantly. In addition, no clinically relevant alterations in PK were observed in mild to severe hepatic impairment.[57] Clinical studies also have not demonstrated any relevant drug–drug interactions with several other drugs commonly prescribed to diabetic patients, including warfarin.[58] One important caveat, however, is that the PK study in ESKD was performed following a *single* dose of empagliflozin only.

1.4 Knowledge gap and summary: Despite the plausible clinical benefit, no study has ever examined safety and tolerability of chronic administration of SGLT2is in ESKD. Interestingly, a recent meta-analysis suggested that the magnitude of CV benefit by SGLT2is was greatest on those with more severe baseline renal dysfunction with baseline eGFR as low as 30 ml/min/1.73m^2.[59] SGLT2is, through their potential direct effect on myocardium, adipocytes, and other possible unknown mechanisms, may improve cellular bioenergetics and survival in ESKD. Given the lack of effective therapies to mitigate profound CV and metabolic risk in ESKD, development of novel and safe therapeutic agents remains one of the most important priorities. In order to address this unmet need, we propose a pilot study to obtain preliminary data on safety, tolerability, and feasibility of implementing empagliflozin therapy in patients undergoing chronic hemodialysis. The information obtained from this pilot study will be instrumental to plan a future full-scale trial.

2. STUDY OBJECTIVES

2.1 OVERARCHING HYPOTHESIS: Empagliflozin provides CV and metabolic benefits independently of renal function and is safe and well tolerated by patients on chronic hemodialysis. The hypothesis on CV and metabolic benefits will *not* be tested in the current proposal. The **primary objective of this pilot study** is to test safety, tolerability, and feasibility to implement empagliflozin therapy in a Phase 2 or 3 clinical trial that will evaluate the efficacy of a SGLT2i to improve metabolic or CV outcomes in ESKD.

2.2 SPECIFIC AIMS

1. To evaluate safety and tolerability of empagliflozin in chronic hemodialysis patients through (a) safety and tolerability questionnaire and clinical observations; and (b) a pharmacokinetic sub-study of two different single oral doses (10 mg and 25 mg) of empagliflozin.

   Hypotheses: *(a) Both low and high doses of empagliflozin are safe and well tolerated. (b) ESKD only modestly increases systemic exposure of empagliflozin, and removal of the drug by hemodialysis is modest.*

2. To evaluate the feasibility of performing a full-scale clinical trial of empagliflozin in chronic hemodialysis patients by obtaining rates of recruitment, retention, and adherence to study procedures.

   Hypotheses: *Majority of the participants will complete the intervention and adhere to the study procedures.*

2.3 STUDY OUTCOMES

2.3.1 The primary safety and tolerability endpoint for Aim 1:
The proportion of participants in each intervention group who remain in follow-up and adhere to the full randomized dose of empagliflozin at the end of the 12-week intervention period.
Rationale: The evaluation of the primary endpoint in the two active treatment groups will provide ***aggregate measures*** of the overall feasibility to implement either (10 mg) or both (10 mg and 25 mg) empagliflozin doses

in a subsequent Phase 2 or 3 trial, by jointly incorporating the key factors such as safety (hypoglycemia, hypotension), intolerance (allergic reaction, pill burden), and participant drop-out or loss to follow-up (due to patient choice, transplant, or death). All of these factors can lead to dose reduction or discontinuation of the drug, negatively affecting the primary endpoint. The proportions of participants who meet the primary endpoint *within* each of the active treatment groups will thus directly inform the feasibility of implementing the empagliflozin intervention in a future full-scale trial, and accordingly will serve as our primary measure for evaluating feasibility. We will also provide comparisons of the proportions of participants who meet the primary endpoint *between* each of the two active intervention groups and the placebo group in secondary analyses. Subject to constraints on statistical power in a feasibility study with 25 participants per group (see Section VIII), these secondary between-group comparisons will inform the extent to which treatment with empagliflozin increases non-adherence beyond the background level observed under a placebo.

In addition, although compliance will also be evaluated by pill counts, pill count compliance will not be considered in the primary endpoint assessments because (i) poor compliance will not trigger a reduction in the prescribed dose unless there is intolerability or an adverse safety event, and (ii) obtaining pill counts from participants has many limitations.[60,61] Thus, pill count compliance will be evaluated as a secondary outcome.

The minimum benchmarks for proceeding to a Phase III trial (modeled after the BASE pilot study[62]): Subject to an adequate safety profile (see Section VI.B.), the 10-mg intervention will be regarded as suitable for evaluation in a future Phase 3 trial if at least 68% of the participants in that dose group adhere to the assigned empagliflozin dose at the end of the 12-week intervention period. The 25-mg intervention will be regarded as suitable for evaluation in a future Phase 3 trial if (a) at least 17 of 25 (68%) participants in that dose group adhere to at least a 10-mg empagliflozin dose and (b) at least 14 of 25 (56%) adhere to the full 25-mg dose at the end of the 12-week intervention period.

The general rationales for the minimum benchmarks are (i) a potential loss to follow-up and drop-out rate of 10% in the active treatment arm and (ii) that the 25-mg dose intervention may be viable for consideration in a full-scale trial even if some proportion of participants reduce their dose to 10 mg. We chose the precise values of the benchmarks to obtain favorable performance characteristics for the Go/NoGo rule, as displayed in the statistical power section (Section VIII). The 68% threshold would allow, for example, for up to 22% of patients to fail to tolerate 10 mg empagliflozin assuming 10% loss-to-follow-up. This percentage is comparable to the proportion of patients with premature discontinuation of empagliflozin in the EMPA-REG study (23.4%).[11]

If the pilot study shows that both 10-mg and 25-mg doses of empagliflozin are well tolerated and exhibit safe PK profile, a future trial could evaluate if the higher dose confers superior protection against HF and sudden death compared to the 10-mg dose, as suggested by the EMPA-REG study.[11] If only the low dose is tolerated, our data will support implementation of the 10-mg dose in a larger clinical trial.

2.3.2 The secondary safety and tolerability endpoints for Aim 1. Additional safety and tolerability endpoints and definitions of prespecified adverse events (AEs) of interest are described in Table 1.

Table 1. The secondary safety and tolerability outcome parameters

Main secondary endpoints: Proportions of participants in each group who (a) reduce and/or discontinue treatment for safety (i.e., AEs), (b) reduce and/or discontinue treatment because of intolerance (i.e., allergy, nausea) or other reasons unrelated to safety (i.e., pill burden, study withdrawal due to transplant), and (c) have ≥ 80% pill count compliance.
Safety outcomes (to be assessed in each treatment group): 1. Proportions of participants with any of the following *prespecified AEs of interest* (see Section VI for details): (i) Severe hypoglycemia[1]; (ii) Ketoacidosis[2]; (iii) Hypotension[3]; (iv) Genital infection; (v) Hepatic injury[4] 2. Composite of the individual components of the prespecified AEs of interest (i) through (v) 3. Occurrence of any hospitalization and/or visit to Emergency Department
[1] Severe hypoglycemia is defined by severe cognitive impairment requiring external assistance for recovery and serum glucose level of <54 mg/dL; [2] Ketoacidosis is defined in Table 4; [3] The definition of hypotension: (a) intradialytic hypotension (systolic blood pressure, SBP <90 mm Hg) or (b) predialysis SBP <110 mm Hg; [4] Hepatic injury is defined as transaminases (1 or both) ≥5x upper limit of normal (ULN) or the combination of transaminases ≥3x ULN with bilirubin ≥2x ULN.

Rationale for the secondary safety and tolerability outcome parameters: Given the low rates of adverse events in published clinical trials of empagliflozin, particularly of ketoacidosis (0.2 to 0.6 per 1,000 patient-years),[63] we expect that the AEs will be infrequent in our pilot study. Nonetheless, our study will provide preliminary safety and tolerability signal associated with empagliflozin in a new patient population by differentiating the frequency of drug discontinuation or dose reduction from safety issues from other issues unrelated to safety (i.e., pill burden). The prespecified AEs of interest were chosen *because of their possible increased risk with SGLT2i therapy and the potential to impact clinical practice.* Although hepatic injury has not been commonly observed in clinical trials of SGLT2is,[64] it is important to confirm the result in ESKD.

2.3.3 Pharmacokinetic endpoints will include evaluation of the following: (1) PK parameters: maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), clearance (CL), volume of distribution (V_d) and area under the curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) and from time zero to 72 hours (AUC_{0-72}); (2) Dialytic clearance of empagliflozin; and (3) Long-term accumulation of the drug.

Rationale for PK endpoints: While our PK study procedure is based on the previous PK report of empagliflozin in patients with CKD and ESKD,[56] our proposal is the first dedicated ESKD study to evaluate for *systemic* exposure and clearance of empagliflozin at *steady state* following *chronic daily administration*, rather than after a single dose. It will also provide novel information regarding the following unknown parameters: (1) the steady-state PK data of 10-mg and 25-mg doses of empagliflozin in ESKD, (2) dialytic removal of empagliflozin by measuring drug concentration in paired samples from pre- and post-dialyzer at 2 & 4 hours after hemodialysis initiation, and (3) the systemic accumulation of empagliflozin over time in ESKD, by measuring predialysis plasma drug concentration at weeks 2 and12. The data obtained in our PK study will be critical to assess long-term safety of 10-mg and 25-mg doses of empagliflozin in ESKD.

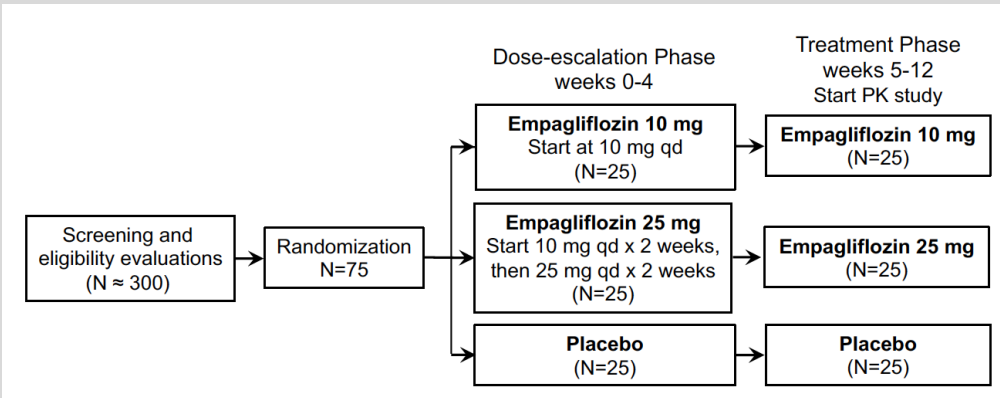
2.3.4 Feasibility endpoints for Aim 2. The feasibility of performing future Phase 2 or 3 trials will be evaluated first by the pre-specified benchmark for the primary safety and tolerability endpoint. In addition, the feasibility assessment will include the rates of recruitment/screen failure, dropout, loss to follow-up, and protocol adherence via pill counts.

3. STUDY DESIGN

3.1. Overview

We propose a randomized, double-blind, placebo-controlled, three-arm pilot study of 75 chronic hemodialysis patients at University of Utah. Diabetic and non-diabetic participants on chronic hemodialysis will be randomized to two different doses (10 mg or 25 mg) of empagliflozin or placebo at 1:1:1 ratio (N=25 per group, Figure 1). The study will be conducted in two phases, a dose-escalation phase (week 0-4) and a treatment phase (week 5-12). Those randomized to 25 mg daily dose will be treated with 10 mg for the first 2 weeks before escalating the dose to 25 mg in week 3, based on tolerability (see Section V for methods on blinding). Participants will be evaluated for safety and tolerability weekly during the dose-escalation phase and every 2 weeks during the treatment phase. All study visits will occur during regular dialysis sessions. At the end of the dose-escalation phase, participants will continue the randomized assignment for additional 8 weeks (5-12 weeks, treatment phase), for a total of 12 weeks. The dose of empagliflozin will be decreased during the dose-escalation and treatment phases for unexplained or persistent hypotension, unexplained hypoglycemia, or other side effects (see Section V for the strategy to maintain blinding with dose titration). As a part of the safety evaluation, a detailed PK study will be performed in 10 participants from each dose group (N=20 total), starting in week 5, after all participants have had a minimum of 2 weeks of treatment with the assigned dose (Figure 1).

Figure 1. The Study Design



3.1.1 Rationale for the double-blind, placebo-controlled study design: Although it is possible to evaluate safety and tolerability without direct comparison to a placebo group, there are important reasons to include a placebo group with double blinding for this pilot study. (1) A double-blind study design will eliminate performance and detection biases and significantly strengthen the reliability of the safety/tolerability results. (2) Subject to limitations of statistical power in a trial with 25 participants per group, inclusion of a placebo arm will provide enhanced ability to distinguish adverse effects caused by empagliflozin from those resulting from underlying disease (i.e., ESKD and/or dialysis procedure). This is particularly important given that the potential adverse effects of SGLT2is, such as hypoglycemia and hypotension, commonly occur in dialysis patients.

Alternative study design: While a cross-over design would improve efficiency of the study and allow for estimation of treatment effects with greater precision, the effects of empagliflozin may be carried over and alter the response to the subsequent treatment, thus hampering the valid estimation of treatment effects.

3.1.2 Rationale for evaluating 2 doses: In the EMPA-REG study, the higher dose of 25 mg was associated with a consistent trend for greater CV benefit. Compared to 10 mg of empagliflozin, the higher dose caused 25% greater reduction in sudden death risk and 43% greater reduction in worsening HF.[11] Although these differences were not statistically significant, they raise an important question if the higher dose may confer superior protection in ESKD patients who have the greatest CV mortality risk, particularly of sudden death.[1] The counter-argument of using the higher dose is the possibility, albeit unlikely on a theoretical basis, that there is retention of the drug or its metabolite as a result of kidney failure. Our study will thus investigate safety and tolerability of both lower and higher doses in ESKD.

In order to determine optimal lower and higher doses of empagliflozin in ESKD, we first developed and validated a physiologically-based pharmacokinetic (PBPK) model of empagliflozin in humans with normal kidney function using published clinical PK studies data.[65] We used PK-Sim®, Version 9, Bayer GmbH software for PBPK modeling and simulations. We then created virtual populations of patients with ESKD (N=100; 50% female; age 18–80 years, GFR <30 ml/min/1.73m^2) and simulated the exposure of empagliflozin at doses 1, 5, 10, and 25 mg by calculating respective Area Under the Curve (AUC) parameters. The simulation data showed that the AUC with 1 or 5 mg was lower than the AUC for doses showing therapeutic benefit (10 and 25 mg) as reported in the empagliflozin FDA label.[66] Therefore, we decided not to use lower doses of empagliflozin but will instead investigate 10-mg and 25-mg doses for our study.

3.1.3 Rationale for the 12-week treatment duration: The median time to onset of most of the adverse effects of SGLT2is is within 3 months of drug initiation. The hemodynamic effects related to osmotic diuresis and natriuresis occur within hours to days,[67] but this is less likely to occur in oliguric or anuric dialysis patients. The majority of genital infections occur within the first 12 weeks, mostly as a single occurrence.[68] The median time to onset of ketoacidosis, the more serious yet uncommon complication of SGLT2is, is 43 days (range of 1 to 365 days).[69] The 12-week treatment duration thus allows reasonable time for preliminary assessment of safety.

3.1.4 Sample size

Seventy-five (75) participants will be randomized. Twenty-five (25) will be assigned to three groups, placebo, 10-mg dose, and 25-mg dose of empagliflozin.

3.2 STUDY PARTICIPANTS

3.2.1 Inclusion. The study will include diabetic and non-diabetic adults with age ≥18 years and dialysis treatment history of ≥3 months.

Rationale for the inclusion criteria: Given our focus to evaluate safety and tolerability of empagliflozin in chronic dialysis patients, we will exclude incident dialysis patients with dialysis history <3 months, as some of the incident dialysis patients may have reversible acute renal injury.

3.2.2 Exclusion. Patients will be excluded if they have type 1 diabetes, ongoing intravenous antibiotic therapy for infectious disease, active treatment for malignancy, unhealed lower extremity skin ulceration, or history of Fournier's gangrene, diabetic ketoacidosis, severe hypoglycemia (requiring external assistance within the past one year), allergy to empagliflozin, or pregnancy.

> **Rationale for the exclusion criteria:** SGLT2is pose greater risk for ketoacidosis in type 1 diabetes[70,71] and are not approved by the FDA to treat patients with type 1 diabetes. Patients with serious infectious diseases may also be at higher risk for ketoacidosis. Due to the potential concern for increased risk of amputation, those with chronic non-healing ulcers will be excluded. Pregnancy and active cancer will be excluded for safety concerns. Patients with history of severe hypoglycemia and ketoacidosis will be excluded because such patients are likely to have future events irrespective of the addition of SGLT2i.

3.3 Identification of participants for screening

Patients will be screened from the Davita dialysis clinics associated with the University of Utah. The study personnel will review the medical record to identify patients who meet key entry criteria such age and medical diagnoses.

3.4 Informed consent procedures

Prospective participants will be approached at the time of their routine dialysis session. This will occur as follows. After obtaining permission from the nephrologist, study coordinators will approach potential participants at the time of the routine dialysis visit and inform them of the nature and purpose of the trial during their dialysis procedure. Patients expressing interest in participation will be told about the entry criteria and given ample time to ask questions about the study, risks, benefits, and study-related procedures. Patients will be informed that research participation is voluntary and the refusal to participate will in no way affect their medical care. Those who meet the entry criteria and are interested in participating will sign the informed consent document and will be provided with a copy of the form.

3.4.1 Consent and withdrawal of consent for the biosample repository

Participants may consent or withdraw consent for the repository at any time during the EMPA-ESKD Study. If a participant consents to the biosample repository, his or her samples will be stored at the biosample repository indefinitely via Utah Clinical & Translational Science Institute (CTSI). A participant may withdraw consent for the repository during the study by notifying the principal investigator at any time. The PI will then notify CTSI to destroy and discard the samples with the corresponding code, as the samples will not have any identifying information. The PI will follow through to make sure the samples have been destroyed.

3.5 Privacy and security

The study coordinators and PI will store participant information in a secure manner, and HIPAA privacy rules will be followed. For purposes of this study, participants will be identified only by an assigned alphanumeric code. Only the PI and study coordinators with direct contact with participants will have access to the key for the code. The study database will be password protected. No individual identifiers (participant name, social security number, or device serial number) will be used to store serum samples or data. Data will be entered with strong encryption.

4. SCREENING & BASELINE PHASES

4.1 Screening

Trained study coordinators will review the electronic medical records to assess eligibility of patients treated at Davita outpatient dialysis clinics affiliated with University of Utah and contact the primary nephrologist to further assess eligibility and obtain permission to contact the patient. Once preliminary eligibility has been confirmed, a screening visit will be scheduled to discuss the details of the protocol and obtain informed consent. The screening visit will occur at the next routine dialysis session during the week (i.e. Mondays, Wednesdays, Fridays for those who get dialyzed on these days of the week or Tuesdays or Thursday for the rest). If prospective participants require more time to decide, the study coordinator will follow up with him or her at subsequent dialysis sessions. During the screening visit, demographic information, medical and dialysis care history (including dialysis access, dialysis vintage, and dialysis adequacy data), medications, blood pressure, weight, and the most recent laboratory data will be collected.

4.2 Randomization
The Principal Investigator will review the screening data and confirm that the participant is eligible and should be randomized. Once a participant has been randomized, efforts will be made to conduct all evaluation and data collection irrespective of whether the participant starts the study treatment regimen, how long the participant continues on the study treatment regimen, and how well the participant complies with the study treatment regimen. Routine data collection will end when a participant receives a pre-emptive kidney transplant, dies, or is deemed completely lost to follow-up.

If a participant withdraws consent for some types of data collection, other data will continue to be collected. All efforts will be made to continue data collection through the end of study follow-up even if a participant's medication never begins, the participant is not compliant, or the participant must stop randomized medications for a safety-related reason or any other reason.

4.2.1 Method for randomization. The randomization to two doses of empagliflozin and placebo will be performed with an allocation ratio of 1:1:1 using QuickCalcs® software (GraphPad Software Inc.). Randomization will be constrained to assure ~40% of all participants will have type 2 diabetes in accordance with the dialysis population at the University of Utah. In addition, randomization will be stratified by diabetic status to assure this predictor of adverse events is balanced between the treatment groups. Random permuted blocks will be used to balance the baseline characteristics between treatment arms within each stratum.

4.3 Recruitment pool, study timeline, and retention. Participants will be recruited from the pool of 486 patients treated at one of the 9 dialysis clinics affiliated with University of Utah that are located within 50 miles of the University campus. We expect that we can recruit up to 15-20% (75 to 100 out of 486). Accounting for screen failure rate (either due to medical reasons or patient choice) of 75%, we will screen 300 patients to randomize 75 participants. Assuming 10% attrition rate over the 12-week treatment period, we expect that 67 participants will contribute to the final analyses. We plan to screen a minimum of 20 patients monthly, with an enrollment rate of 4-5 participants per month and completion of recruitment by 22 months. The data collection should be completed by 26 months, allowing 10 months for data analyses and manuscript preparation. In order to encourage participation and maximize retention, participants will be contacted frequently via telephone calls or visit during their dialysis sessions and reimbursed for time.

4.3.1 How recruitment will be monitored
The principal investigator will meet with the study coordinators weekly to review recruitment status and to discuss progress on weekly goals, identify barriers and problems, and revise recruitment plans as necessary.

5. TREATMENT ARMS

5.1 Initiation of intervention
Participants who have met eligibility criteria and provided informed consent will be randomized to one of 3 groups at 1:1:1 ratio (N=25 per group, Figure 1). Empagliflozin will be taken in the morning.

1. Lower-dose (10-mg) oral empaliglozin
2. Higher-dose (25-mg) oral empagliflozin
3. oral placebo

The study will be conducted in **two phases, a *dose-escalation phase* (week 0-4) and a *treatment phase* (week 5-12).** Those randomized to 25 mg daily dose will be treated with 10 mg for the first 2 weeks before escalating the dose to 25 mg in week 3, based on tolerability. Participants will be evaluated for safety and tolerability weekly during the dose-escalation phase and every 2 weeks during the treatment phase. All study visits will occur during regular dialysis sessions. At the end of the dose-escalation phase, participants will continue the randomized assignment for additional 8 weeks (5-12 weeks, treatment phase), for a total of 12 weeks. The dose of empagliflozin will be decreased during the dose-escalation and treatment phases for unexplained or persistent hypotension, unexplained hypoglycemia, or other side effects

Safety and tolerability assessment visit during the dose-escalation phase (0-4 weeks, on a weekly basis) and treatment phase (5-12 weeks, every 2 weeks, through week 14) will include the following:

- Pill count
- Completion of the safety and tolerability questionnaire
- Review of blood pressure during the hemodialysis session
    - Frequency of intradialytic hypotension (systolic blood pressure, SBP <90 mm Hg) or (b) predialysis SBP <110 mm Hg
- Pre-dialysis weight
- Frequency of symptomatic hypoglycemia
- Any visit to Emergency Department or hospitalization
- Review of laboratory test values

If the participant is tolerating the study medication well without specific concerns, the those randomized to 25 mg will have dose titration to 25 mg at the beginning of week 3. This dose will be continued for the remainder of the treatment phase. If any of these adverse events are observed at the week 2 visit, the adverse event will be managed as described in Section 8 regarding SAFETY ASSESSMENT.

5.2 Source of empagliflozin and placebo
Study drug and matching placebo will be prepared and dispensed by the University of Utah Investigational Drug Service (IDS). Study drug will be prepared using gelatin encapsulation such that all drugs, including placebo and dose, are identical in appearance. IDS will bottle the capsules, label the bottles with code numbers to maintain the study's double blind, and supply the study team every 4 weeks.

5.3 Initial distribution and resupply to participants
Each participant's initial supply (provided during the Baseline Visit at week 0) and subsequent resupplies will be initiated by the site study coordinator. The study coordinator will dispense a 2-week supply at the Baseline Visit (2eek 0) and at week 2, then 4-week supply bottles starting at week 4.

5.4 Blinding. Study drug and matching placebo will be prepared and dispensed by the University of Utah Investigational Drug Service (IDS). Study drug will be prepared using gelatin encapsulation such that all drugs, including placebo and dose, are identical in appearance. In order to maintain blinding, all study participants, regardless of randomized assignment, will receive a new study drug supply every 2 weeks during the dose escalation phase (weeks 1-4), then every 4 weeks for the treatment phase. Only the IDS and the PK personnel, who have no direct contact with the participants, will have access to the randomization code.

5.4.1 Maintenance of blinding with dose titration. Unless there is a contraindication to a dose increase (e.g., hypoglycemia or hypotension), the investigator will request IDS for dose escalation. If up-titration to the next dose is contraindicated, the investigator will request IDS to maintain the current dose of empagliflozin or placebo. If down-titration of the study drug dose is required during the dose-escalation or the treatment phase, a supply of blinded study medication using the next lower dose of empagliflozin or placebo will be provided (Table 2). As only 2 doses of empagliflozin are specified, study medication will be discontinued in the event that a second down titration of dose is required. For participants already on placebo, a new supply of placebo will be provided in order to maintain blinding. **Unblinding** to preserve patient safety will be considered for serious adverse events of interest determined by the investigator to be important for patient management.

5.4.2 Blinding for the pharmacokinetic (PK) study. In order to maintain the blinding for the PK study, blood samples will be collected from the first 30 participants in the order of recruitment. Given that the randomization is at 1:1:1 ratio, we expect that this will result in ~20 participants allocated to empagliflozin treatment. We will

Table 2. Study drug down-titration scheme to maintain blinding

Randomized assignment	**Placebo**	**Empagliflozin (10 mg)**	**Empagliflozin (25 mg)**
1st down titration	Placebo	Placebo	10 mg
2nd down titration	Discontinue	Discontinue	Discontinue

follow the following procedures to minimize any risk of accidental unblinding during the study. (1) Only pre-identified designated personnel will have access to the treatment information. This includes Dr. Venkata Yellepeddi, who will develop the randomization sequence and perform the PK data analyses and Dr. Christopher Reilly and his laboratory staff, who will perform empagliflozin drug assay for the PK study. These

individuals will not have any direct patient contact or participate in evaluation of the other study outcomes. (2) Once 30 participants have completed the blood draw for the PK study, Dr. Yellepeddi will unblind the collected blood samples so that only samples from the participants allocated to empagliflozin (N≈20) are assayed. If there are insufficient number of participants assigned to empagliflozin, enrollment to the PK study will continue until 10 participants from each dose group has completed the blood collection.

5.4.3 Unblinding

In the circumstance that the investigator feels that unblinding would be helpful or influence participant management, the investigator will formally request unblinding.

6. STUDY VISITS & PROCEDURES

6.1 Overview. A schedule of study visits and procedures is outlined in Table 3. All study visits will occur during regular hemodialysis sessions to minimize burden on the participants. During the screening visit, demographic information, medical and dialysis care history (including dialysis access, dialysis vintage, and dialysis adequacy data), medications, blood pressure, weight, and the most recent laboratory data will be collected. A baseline visit will be scheduled within 28 days of screening. Randomization will occur prior to the baseline visit.

In-person follow-up visits will occur weekly for the first 4 weeks, then every 2 weeks for the remainder of the study period. Additional follow-up visits will be held at the discretion of the site investigator. Telephone calls, in place of in-person follow-up visits, will occur at the discretion of the investigator and/or study personnel for some of the follow-up visits beyond first 4 weeks (between week 6-12).

Table 3. Schedule of visits and procedures

Study Procedure	Visit 1 Screening	Visit 2 Baseline	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
Study week	-1 to -4	0	1 (+/- 3 d)	2 (+/- 3 d)	3 (+/- 3 d)	4 (+/- 3 d)	6 (+/- 7 d)	8 (+/- 7 d)	10 (+/- 7 d)	12 (+/- 7 d)	14 (+/- 7 d)
Consent	X										
Screen for inclusion/exclusion	X										
Demographics/Medical history	X										
Medication review[1]	X	X	X	X	X	X	X	X	X	X	X
Hemodialysis data review[2]	X	X	X	X	X	X		X		X	X
Vitals, weight, physical exam	X	X	X	X	X	X		X		X	X
Laboratory tests for safety (ketone, chemistry panel, glucose[3], liver function test)		X		X		X	X	X		X	
Biorepository blood samples		X								X	
Study drug dispensing/ Pill accountability		X		X		X		X		X	
Safety/tolerability questionnaire		X	X	X	X	X	X	X	X	X	X
Pharmacokinetic study	Begin in week 5										

[1] At each safety visit, any changes to medications (including dose, new, or stopped drugs) will be reviewed, especially blood pressure medications, erythropoietin-stimulating agents, and intravenous iron. [2] Hemodialysis data include pre-, intra-, and post-dialysis blood pressure readings, pre- and post-dialysis weights, and goal dry weight. [3] Fasting glucose levels will be drawn from participants in the morning dialysis shift. For those in the afternoon shift, random glucose levels will be drawn to reduce the risk of hypoglycemia from a prolonged fasting period.

6.2 Data elements collected during in-person visits

At each study visit as specified in Table 3, study coordinators will review any changes in medications, vitals, weight, and hemodialysis-related data (pre-, intra-, and post-dialysis blood pressure readings, pre- and post-dialysis weights, and goal dry weight), and review safety questionnaire. On study weeks 0, 2, 4, 8, and 12, the participants will undergo blood draw for the laboratory tests (ketone, chemistry panel, glucose, liver function test).

6.3 Participant Adherence Monitoring.
The study drug will be prepared for dispensing by the Investigational Drug Service Pharmacy at University of Utah. Study drug containers will be returned to the study coordinator at study visits per schedule (weeks 2, 4, 8, and 12) as outlined in Table 3. Pill counts will be performed to assess participant adherence, although we recognize the shortcomings of this method. Barriers and strategies to promote compliance will be explored and individualized.[72] Participants will be counseled about the importance of taking the assigned treatment and asked to think of ways they can improve compliance, including use of filling pillboxes. Study coordinators will assist participants in obtaining and filling pillboxes if this seems advisable. Additional follow-up phone calls or visits during the hemodialysis sessions will be performed as needed to reinforce compliance.

6.4 Participants who undergo kidney transplantation, or become pregnant
Participants who undergo kidney transplantation during the study will be censored, given the uncertainty of safety of SGLT2i in this setting. Participants who receive a pre-emptive kidney transplant during the study will also be censored because of unknown safety in the peri-transplant and post-transplant settings. Participants who become pregnant will also be censored.

7. MEASUREMENT TECHNIQUES

7.1 Blood collection and processing. Because of wide fluctuations in intravascular volume status in relation to hemodialysis schedule, all blood samples for the study will be drawn consistently predialysis on mid-week days by a dialysis staff from the dialysis tubing. Serum samples for safety assessment (Na+, K+, Cl-, HCO3-, BUN, creatinine, glucose, Ca2+, liver function test (transaminases and bilirubin), and ß-hydroxybutyrate at weeks 0, 2, 4, 6, 8, and 12, as noted in Table 3) will be sent to ARUP Laboratory, a national reference laboratory associated with University of Utah, for further processing and assays. The plasma samples for the PK study will be separated by centrifugation, aliquoted, and stored at -80°C.

Because dialysis patients have usual monthly laboratory tests that include chemistry and Complete Blood Count (CBC), we will use those results whenever possible to reduce the amount of phlebotomy on the dialysis patients. We will use CBC results from the routine Davita Care for out study to minimize phlebotomy. All blood samples will be drawn via dialysis access via the Davita dialysis personnel. The study participants will not undergo separate venipuncture for the study-related serum or plasma sample collection.

7.2 Physical examination measurements
Height will be measured at baseline. Pre-dialysis weight will be measured at each visit using standard clinic equipment per protocol at each Davita dialysis clinic. Blood pressure measurements will be taken per usual practice before and during hemodialysis procedure by the Davita dialysis clinic staff.

7.3 Safety and tolerability questionnaire
Participants will be asked to complete a symptoms questionnaire weekly for the first 4 weeks then every other week for the remainder of the study period (weeks 6, 8, 10, 12, and 14) to assess the tolerability of the intervention. Briefly, participants will be asked to rate symptoms of hypoglycemia, abdominal symptoms, genital infection, orthostasis, on a scale of 1-5.

7.4 Collection of serum samples for biorepository
Whole blood samples (10 mL) will be collected at week 0 and 12, centrifuged at room temperature, aliquoted, and the serum will be stored at -80°C for future biomarker assays.

7.5 Pregnancy test
Serum or urine pregnancy tests, if required, will be performed using the laboratory's standard technique. We expect that most women will be beyond their reproductive years and thus the chance of pregnancy during the study is expected to be very low.

8. SAFETY ASSESSMENT
Using a standardized safety and tolerability questionnaire based on the comprehensive review of the risk associated with SGLT2is,[73] study personnel will assess each participant for any AEs on a *weekly* basis for the first 4 weeks then every 2 weeks. The last safety evaluation will be 2 weeks following the last dose (Visit 11), in

order to extend safety monitoring through the drug elimination phase. The prespecified AEs of interest are described in Table 1. All AEs will be coded to preferred terms and system organ classes using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) for data summary purposes. The participants' primary nephrologists will be requested to report any subsequent events that might reasonably be related to participation in this study. All acute medical issues will be immediately assessed in conjunction with the primary nephrologist and managed according to standard medical practice.

8.1 Adverse events (AEs)
The study coordinators will prompt questioning of participants about the occurrence of relevant AEs in accordance with the protocol at each study visit. The research coordinator should obtain details of the AE from the participant and enter these directly into *Data management system (REDCap).* Serious AEs (SAEs) can be entered either at or in-between clinic visits.

8.2 Non-serious adverse event
A Non-Serious Adverse Event (NSAE) is defined as any untoward medical occurrence in a study participant that does not fulfil the definition of "serious". Such an event is not necessarily thought to have a causal relationship with the study treatment. The study coordinators will be prompted to ask about and enter details of any NSAEs during the study visits.

These events include the following:

- Any event that leads to discontinuation of study treatment, or
- one of the following:
  - Bone fracture (with additional information recorded about fracture site and etiology [i.e. distinguishing those resulting from high and low impact])
  - Symptomatic dehydration (defined as whether or not a participant has experienced symptoms they attribute to dehydration, such as feeling faint or fainting);
  - An Adverse Event of Special Interest (see below)
  - Events that could lead to amputation (which include diagnosis or treatment for peripheral arterial disease, peripheral neuropathy, diabetic foot ulcer, and lower limb infection or gangrene).

8.3 Assessment for AEs of interest: While participants will be closely observed for all AEs, special attention will be paid to detect occurrences of the following prespecified AEs of interest (Table 1), as discussed below:

8.3.1 Severe hypoglycemia (defined by severe cognitive impairment requiring external assistance for recovery and serum glucose level of <54 mg/dL, based on the American Diabetes Association and the European Association[74]): The overall impact of SGLT2i on glycemia is expected to be minimal. The addition of empagliflozin to pre-existing insulin or sulfonylurea therapy has been associated with slight increase in incidence of hypoglycemia (20.9 events/100 patient-years) compared to placebo (20.4 events/100 patient-years) in patients with type 2 diabetes.[64] Of note, SGLT2is have not been shown to cause hypoglycemia in patients without diabetes.[12,13] Under the guidance of an endocrinologist, therefore, the need for adjusting doses of insulin and/or sulfonylurea will be carefully reviewed for diabetic participants prior to randomization. Symptoms or history of hypoglycemic episodes will be inquired at each study visit. In addition, fasting glucose levels will be monitored every 2 weeks for the first 4 weeks, then monthly in those with hemodialysis sessions in the morning. Random glucose levels will be drawn in those with afternoon dialysis sessions to minimize the risk of hypoglycemia from prolonged fasting. Recurrent episodes of symptomatic hypoglycemia despite clinical optimization, including adjustment of doses of concomitant sulfonylurea and/or insulin therapy, will require drug dose reduction or discontinuation.

8.3.2 Ketoacidosis and serum ketone (ß-hydroxybutyrate)/anion gap monitoring: In order to minimize the risk of ketoacidosis, participants will be advised to hold empagliflozin during any acute illness or prolonged period of fasting or decreased fluid intake. At each safety visit, participants will be monitored for any suggestive history that may cause ketoacidosis (insulin pump failure, insulin dose omission, acute illness, infection) and new symptoms suggestive of ketoacidosis (confusion, drowsiness, lethargy, dehydration, nausea/vomiting, abdominal pain, Kussmaul breathing). Participants will be instructed to seek medical attention immediately should these symptoms occur. Serum ß-hydroxybutyrate, electrolytes, and anion gap [calculated as sodium - (chloride + bicarbonate)] ≥15 mEq/L will be frequently monitored as specified in Table 3. Table 4 describes the

definition and categorization of ketoacidosis, which was created based on a composite of diagnostic criteria in accordance with the American Diabetic Association and published work.[75,76]

For any participants with potential or certain ketoacidosis, the study drug will be stopped immediately, with immediate medical evaluation and treatment in consultation with endocrinology service.[77]

Table 4. Definition and categorization of ketoacidosis	
Certain ketoacidosis	Confirmed ketosis (ß-hydroxybutyrate >1.5 mmol/L) *and* confirmed acidosis with blood pH ≤7.3[1]
Potential ketoacidosis	(a) Confirmed ketosis (ß-hydroxybutyrate >1.5 mmol/L)[2] *and* suggestive history[3] or symptoms[4] or (b) confirmed acidosis with blood pH ≤7.3 *and* suggestive history[3] or symptoms[4]
Unlikely ketoacidosis but ketosis	Blood ß-hydroxybutyrate >1.5 to <3.8 mmol/L *and* one of the following: (1) Blood pH >7.3 or serum bicarbonate >18 mEq/L; (2) No history[3] or symptoms[4] reported
Unlikely ketoacidosis	Blood ß-hydroxybutyrate ≤ 1.5 mmol/L *and/or* one of the following: (1) Blood pH >7.3 or (2) Serum bicarbonate >18 mEq/L or absence of anion gap
Unclassifiable	If only one of the following is available: (1) pH ≤7.3; (2) Bicarbonate ≤18 or anion gap >15 mEq/L; or (3) Suggestive history[3] or symptoms[4]

[1] Blood pH will be drawn from arteriovenous fistula. [2] For potential ketoacidosis, a blood ß-hydroxybutyrate value ≥3.8 mmol/L will be confirmed by an additional measurement ≥3.8 mmol/L within 24 hours. A single ß-hydroxybutyrate value ≥3.8 mmol/L without symptoms/suggestive history will be regarded as unlikely ketoacidosis but ketosis. [3] Suggestive history includes insulin pump failure, insulin dose omission, acute illness. [4] Typical ketoacidosis symptoms include neurological (confusion, drowsiness, loss of consciousness) and non-neurological symptoms (dehydration, nausea/vomiting, abdominal pain, Kussmaul breathing).

8.3.3 Hypotension and blood pressure (BP) management: In non-ESKD patients, SGLT2is cause a small decrease in systolic BP of ~3 mm Hg, and it is unlikely that SGLT2is will cause significant hypotension in ESKD. There is no generally accepted definition for predialysis hypotension, owing to their high variability and poor reproducibility.[78] We will thus follow the most recent expert consensus to define hypotension during hemodialysis (intradialytic hypotension) as a nadir intradialytic systolic BP (SBP) <90 mm Hg, as this threshold is most associated with mortality.[79,80] We will also define hypotension outside of hemodialysis procedure as predialysis SBP <110 mm Hg, based on its association with increased mortality.[81]

BP will be managed by the primary nephrologist per routine clinical practice, including, but not limited to, the adjustment of anti-hypertensive medications and dry weight. If the standard approach does not improve BP, the study drug dose will be reduced or discontinued. It is recognized that, since factors regulating BP are complex in chronic hemodialysis patients and the effect of SGLT2 inhibition on BP is probably modest, the ability to definitively identify an effect of empagliflozin on BP in this study is likely to be limited. Instead, the goal here is to observe if an obvious safety signal exists with empagliflozin.

8.3.4 Genital infection*:* Participants will be monitored for any symptoms suggestive of genital infection (localized itching, discharge, erythema, odor, or dysuria) at each safety visit. Suspected cases will be promptly treated with an anti-fungal agent and followed for resolution of symptoms and referred to the General Medicine or Gynecology service as appropriate.

8.3.5 Hepatic injury*:* Using the standardized guidance, hepatic injury will be defined as increase in alanine aminotransferase and/or aspartate aminotransferase ≥5x upper limit of normal (ULN) or the combination of alanine aminotransferase and/or aspartate aminotransferase ≥3x ULN with bilirubin ≥2x ULN.[82] The study drug will be discontinued if the liver function parameters increase beyond these thresholds. For less severe changes, the decision for drug discontinuation will be made based on the rate and severity of change.

8.3.6 Other laboratory monitoring: As erythrocytosis has been reported with SGLT2i therapy,[83,84] changes in hemoglobin levels and the doses of intravenous iron and erythropoietin-stimulating agents will also be monitored as specified in Table 3.

8.4 Serious adverse event (SAE)

An AE is defined as serious if it:

- Results in death
- is life-threatening

- requires in-participant hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect; or
- is an important medical event in the opinion of the responsible investigator, i.e. any event that is not immediately life-threatening and does not result in death or hospitalization but which may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

8.5 Data safety Monitoring Board (DSMB).
The study will be monitored by an independent DSMB to ensure participant safety. The DSMB will consist of at least three members and will review all AEs and serious AEs (SAEs, which represent any AEs which may result in death, complications that require urgent interventions, significant disability, or prolonged hospitalization), make recommendations, and perform the usual DSMB functions, as outlined in a DSMB charter to be developed prior to initiating the study.

8.6 Recording and reporting of SAEs
When SAEs occur that are unanticipated (i.e., not listed in the Data and Safety Monitoring Plan) and that are likely or probably related to the intervention, they will be reported to the IRB and the DSMB as early as possible but no later than 10 days of study's knowledge of SAE.

8.7 Stopping study treatment
It is hoped that the number of participants stopping study treatment will be low. If a participant does stop study treatments, however, this will be recorded at the clinic visit with the date on which study medication was last taken. The study coordinator will explain to the participant the great value of continued follow-up for the trial and encourage the participant to continue to participate in the study follow up. Stopping study treatment does not mean that the participant has withdrawn from the trial.

8.8 Strategy for the management of adverse events, including reducing or terminating the assigned intervention
The strategies to optimize safety are discussed in the above sections discussing severe hypoglycemia, ketoacidosis, and hypotension.

8.8.1 Reducing and/or stopping the randomized intervention for safety reasons
In general, the anticipated side effects of empagliflozin can be medically managed by adjusting hypoglycemic agents, antihypertensive and/or diuretic agents (for those who have sufficient residual renal function), and holding the dose during acute illness. If medical management is ineffective, doses of the assigned intervention will be reduced to the lower dose or placebo, or by discontinuation of therapy if the side effect persists (see Table 2).

The assigned intervention will be discontinued for the following reasons as well, however these events will be considered censoring events.
- Kidney transplant
- Pregnancy

8.9 Restarting the intervention and/or increasing reduced doses back to the full randomized dose
The decision to increase the dose back to the randomized dose or to restart therapy will be at the discretion of the site investigator and will consider whether the reason(s) for reducing or stopping treatment have resolved as well as the participant's willingness to restart or escalate the assigned treatment.

9. PHARMACOKINETIC (PK) STUDY

Given that the half-life of empagliflozin in ESKD is ~22 hours[56] and it takes 5 half-lives to reach steady state, the PK study will start in week 5, when all participants have taken at least 2 weeks of the assigned dose of empagliflozin, to assess PK at steady state. On the day of the PK study, participants will take the assigned study drug immediately prior to the hemodialysis procedure. They will hold further doses until the completion of the 5-day PK study period and restart daily dosing on the 6th day. Blood samples (5 mL) will be collected from

the arterial (pre-filter) side of the dialyzer at 0, 20, 40, 60 and 90 min, 2, 4, 48, 52, 96, and 100 hours after oral administration of the study drug. These time-points were selected based on the previous report on the PK of empagliflozin in patients with CKD and ESKD.[56]

To assess for removal of drug by hemodialysis, plasma samples will be drawn from the post-filter side of the dialysis access, paired with the pre-filter draw, at 2 and 4 hours after the dose. To evaluate for accumulation of the drug, we will also determine the predialysis plasma drug concentration at week 2 and week 12. Plasma will be separated by centrifugation and stored at -80°C until assay.

Concentrations of empagliflozin will be determined using a validated liquid chromatography-mass spectrometric method at the Center for Human Toxicology (CHT) at University of Utah as previously described,[85] under the direction of Dr. Christopher Reilly. The CHT is a bioanalytical laboratory with >30 years of history of specializing in the development, validation, and application of quantitative methods for xenobiotics in biological matrices. The PK parameters of empagliflozin will be evaluated by noncompartmental analysis using Phoenix® WinNonLin® software. The following parameters will be evaluated: maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), clearance (CL), volume of distribution (V_d) and area under the curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) and from time zero to 72 hours (AUC_{0-72}). The PK data from noncompartmental analysis will determine if sufficient plasma levels of empagliflozin are reached in dialysis patients with our dosing regimens. Furthermore, a population PK (PopPK) model for empagliflozin will be developed from the data using NONMEM®, Icon plc software to identify the covariates (age, sex, dialysis vintage, diabetes, co-medications, etc.) that can impact PK parameters clearance (CL) and volume of distribution (Vd) of empagliflozin in chronic dialysis patients. This information on covariates that impact PK variability of empagliflozin will be used to develop a general guidance on empagliflozin dosing in ESKD.

10. STATISTICAL METHODS

Aim 1: Safety and tolerability of empagliflozin through (A) clinical observations and (B) PK study.

10.1 The primary safety and tolerability endpoint for Aim 1: The primary analyses will be performed in the full randomized study population. We will provide point estimates of the proportions of participants in each treatment group who meet the primary endpoint along with exact 90% confidence bounds based on binomial enumeration within each treatment group. The dose prescribed for each participant at the last On-Treatment visit (week 12) will be used. The premise of the primary endpoint is that safety and tolerability concerns during the follow-up period would have resulted in a reduction or discontinuation of the prescribed dose per protocol, regardless of the participant's compliance. This will also take into consideration any protocol-mandated reductions that should have occurred at the last On-Treatment visit had the pilot study continued.

The primary analysis (safety and tolerability) will be regarded as supporting the 10-mg empagliflozin intervention in a future Phase 3 trial if at least 68% of the participants in that dose group adhere to the randomized dose at the end of the 12-week intervention period. The 25-mg intervention will be regarded as suitable for evaluation in a future Phase 3 trial if at least 17 of 25 (68%) participants in that dose group adhere to at least a 10-mg empagliflozin dose and at least 14 of 25 (56%) adhere to the full 25-mg dose at 12 weeks. In secondary analyses, exact 90% confidence intervals will be constructed for the differences between the proportions reaching the primary endpoint between each active dose group vs. the placebo group. Selection of the dose(s) to be used in the future Phase 3 trial will also partly depend on the results of the PK study and other safety analyses.

10.2 Secondary safety/tolerability analyses:

10.2.1 Main Secondary Analyses. Exact 90% binomial confidence intervals will be constructed for the proportions of individuals in each treatment group who (a) reduce and/or discontinue treatment for safety reasons (i.e., AEs), (b) reduce and/or discontinue treatment because of intolerance or other reasons unrelated to safety (i.e., allergic reaction, pill burden), and (c) have ≥80% pill-count compliance. These endpoints will respectively address safety, tolerance or other reasons for dose reduction/discontinuation, and compliance for each dose group during the 12-week intervention period. In contrast to the primary endpoint, these endpoints

will be analyzed in participants who receive at least one dose of study medication and will be treated as missing if they cannot be ascertained due to loss-to-follow-up or missing data. Exact 90% binomial confidence intervals will also be constructed for the differences between the proportions of participants reaching these endpoints in the active-dose groups and the proportion who reach these endpoints in the placebo group.

10.2.2 Additional Safety Analyses. We will summarize the previously defined specific AEs of interest. In addition, a comprehensive tabulation of all AEs and SAEs will be provided in participants who receive at least one dose of the study medication by MedDRA-preferred terms, severity, and perceived relationship to the study drug. In the event of multiple occurrences of the same AE in the same participant, the AE will be counted only once. For each AE or SAE category, these tabulations will be provided for the frequencies and proportions of participants who are required to (a) reduce the dose of the study drug or (b) permanently discontinue the study drug. Each of these frequencies and percentages will be provided for the overall study and by treatment group. The tabulations of AEs will be primarily descriptive. To help guide interpretation, however, we will use binomial enumeration to provide exact 90% binomial confidence limits for the proportions of participants experiencing the designated AEs, overall and by treatment group. Exact p-values will be calculated using the exact Barnard test[86] to compare the proportions of participants with AEs between the two active-dose groups. Results of these analyses will be interpreted with awareness of the increasing probability of obtaining at least one nominally significant result with the number of comparisons performed.

10.2.3 Additional analyses of pill counts. Pill counts will be expressed as a percent of the number of pills prescribed for a particular participant and summarized by treatment group at each follow-up visit using box plots and histograms. Ninety % confidence bounds will be constructed for the average pill count as a percent of prescribed pills as well as for the proportion of participants whose counts meet a target of ≥80% compliance for each treatment group and each follow-up visit during the 12-week intervention period.

10.2.4 Assessment of continuous safety-related endpoints. Box plots will be constructed to display the distributions of predialysis systolic and diastolic blood pressure, predialysis weight, and the serum measures of ß-hydroxybutyrate, bicarbonate, anion gap, fasting glucose, and hemoglobin by treatment group at baseline and each follow-up visit during the 12-week intervention period. Mixed-effect analyses will also be used to compare the mean blood pressures, predialysis weight, and blood laboratory measures between the treatment and placebo groups at each of the follow-up visits, while assuming equal mean levels for each randomized group at baseline to increase the statistical power of the treatment comparisons. The covariance structure of the mixed models will be determined based on likelihood ratio tests comparing the fit of nested covariance models and by comparisons of the Bayes Information Criterion for non-nested models.

10.3 Missing data: The primary outcome will be coded as failures if participants are lost to follow-up prior to the 12-week assessment or the 12-week outcomes are otherwise missing. This strategy is intended to reflect the global nature of these outcomes in representing achievement of complete follow-up as well as tolerability and safety. Both the numerators and denominators of the three main secondary safety outcomes, which pertain to specific outcome domains, will be restricted to participants who complete follow-up and provide the 12-week assessments. Other safety outcomes will be tabulated for all participants who receive at least one dose of the randomized medication. The analyses of continuous safety-related endpoints will use all available follow-up data, and will provide valid statistical inference as long as any missing measurements follow a missing-at- random (MAR) pattern.[87]

10.4 PK analysis: For noncompartmental PK analysis, plasma empagliflozin concentrations that are below the lower limit of quantitation (BLQ) will be treated as zero. The plasma concentration-versus-time data will be evaluated for presence of any outliers using Dixon's Q test. Any outlier from the study conduct or the assay will be excluded from interpretation. The covariate analysis for PopPK modeling will be performed using a stepwise covariate modeling approach involving forward inclusion and backward elimination of covariates. The goodness-of-fit between hierarchical models will be measured by the difference in the objective function value (OFV) as produced by NONMEM®. An OFV is -2 times the minimum log of the likelihood of the data (-2LL). The difference in OFV values (Δ) is approximately chi-squared distributed with n degrees of freedom (n=difference in number of parameters between models).

10.4.1 Dialyzer clearance of drug. On the first day of the PK study, participants will take the assigned empagliflozin dose immediately prior to hemodialysis and hold further doses for the duration of the PK study (5 days). We will distinguish dialytic and non-dialytic clearance by the following methods: (1) The plasma empagliflozin concentration will be determined in blood samples obtained simultaneously from pre- and post-filter tubings at 2 and 4 hours post dose in order to quantify dialytic clearance. The dialyzer clearance at each participant's routine dialyzer blood flow rate (Q_b) and dialysate flow rate (Q_d) will be calculated from the plasma concentrations in pre-filter and post-filter tubings and will be added to the clearance pathways in the population PK model of empagliflozin.[88,89] (2) Additional PK samples will be collected before and after dialysis at 0, 4, 48, 52, 96, and 100 hours after the oral dose. Because no additional doses will be given during the 5-day PK sampling period, non-dialytic clearance will be calculated using the concentration-time slopes between dialysis sessions. These results will be used to develop a general guidance on empagliflozin dosing in ESKD.

10.4.2 Chronic accumulation of drug. Predialysis plasma drug concentration will be measured at week 2 and week 12 to determine if empagliflozin accumulates over time in the 20 PK sub-study participants. The accumulation of empagliflozin in plasma will be calculated by comparing the $AUC_{0-\infty}$ values of empagliflozin on day 42 with that on day 1. The cumulative accumulation of empagliflozin will be reported as fold-increase of $AUC_{0-\infty}$ from day 1 to day 42. The AUC0-∞ values will be calculated by noncompartmental analysis using Phoenix® WinNonlin®, Certara, USA.

10.5 Feasibility data regarding rates of recruitment, retention, and adherence to study procedures.
The feasibility of performing future Phase 2 or 3 trials will be evaluated first by the pre-specified benchmark for the primary safety and tolerability endpoint, as discussed in Section IV.A.1. We will also report point estimates and exact 90% confidence intervals for the following parameters based on analysis of the full data set: (i) the overall recruitment rate, (ii) the screen failure rate and the reasons, (iii) drop-out and drop-in rates, (iv) time to and reasons for drop-out, and (v) loss to follow-up. We will report these parameters for the overall study and by treatment group as appropriate.

11. SAMPLE SIZE AND POWER

11.1 Statistical Power for Aim 1 Analyses: Primary analysis of achievement of benchmarks.
The performance characteristics of the primary Go/NoGo criteria are provided below.

As illustrated in Table 5, the risk of inappropriately reaching a Go decision in the 10-mg dose group is no greater than 0.054 if the true proportion of participants who remain in follow-up and adhere to the 10-mg dose is ≤ 0.50, and the power for correctly making a Go decision is at least 0.953 if the true proportion is ≥ 0.80. In addition, the risk of inappropriately reaching a Go decision in the 25-mg dose group is no greater than 0.051 if the true proportion of participants who remain in follow-up and adhere to the 10-mg dose is ≤ 0.50. The power for correctly making a Go decision in the 25-mg dose group is at least 0.838 if the true proportion who remain in follow-up and adhere to a ≥ 10-mg dose is ≥ 0.80 and the conditional probability of adhering to a 25-mg dose assuming adherence to a ≥ 10-mg dose is at least 0.80.

Table 5. Probability of reaching the benchmarks for the 2 doses of empagliflozin

Probability of Reaching a Go Decision for 10-mg Dose Group							
Probability of remaining in follow-up and adhering to the 10 mg dose	0.40	0.50	0.60	0.70	0.80	0.90	0.95
Probability of Reaching a Go Decision	0.004	0.054	0.274	0.677	0.953	0.999	>0.999

Probability of Reaching a Go Decision for 25-mg Dose Group*							
Conditional probability of remaining in follow-up and adhering to a 25 mg dose assuming adherence to a ≥10 mg dose	Probability of remaining in follow-up and adhering to a ≥ 10 mg dose						
	0.40	0.50	0.60	0.70	0.80	0.90	0.95
0.50	0.000	0.001	0.006	0.025	0.077	0.181	0.262
0.80	0.003	0.034	0.188	0.518	0.838	0.973	0.992
0.90	0.004	0.051	0.258	0.657	0.943	0.999	<0.999

*Calculations assume the number of participants adhering to a 25-mg dose among those adhering to a ≥ 10 mg dose follows a binomial distribution with probability of adherence to the full 25-mg dose given by the entry in the first column.

11.2 Precision of 90% confidence limits. The expected margin of error (expressed as ½ the width of a 90% confidence interval) for estimates of a proportion of participants meeting a binary endpoint in a particular treatment group (with n=25) will be approximately 0.13 if the true proportion is 0.20, 0.165 if the true proportion is 0.50, and 0.13 if the true proportion is 0.80. The maximum margin of error based on a 90% confidence interval for comparisons of the proportion meeting a binary endpoint between an active treatment group (with n=25) and the placebo group (also with n=25) is 0.23.

11.3 Power analysis to justify the number of participants for pharmacokinetic study: The PK study will have 80% power to target a 95% confidence interval within 60-140% of the geometric mean clearance and volume of distribution estimates. Based on available literature, we estimate that the coefficient of variation will not exceed 50% for empagliflozin PK parameters (clearance, AUC, volume of distribution). Therefore, 10 study participants for each dose will be sufficient to achieve 90% power.

12. STRENGTHS, LIMITATIONS, ANTICIPANTED RESULTS, AND FUTURE DIRECTIONS

12.1 Strengths. (i) Our study addresses a profound unmet clinical need to develop effective interventions to improve CV outcomes and survival in ESKD patients. SGLT2is have been shown to have powerful cardioprotective effects in non-ESKD (including CKD) populations, and the literature suggests a strong plausibility of direct effects on the heart. (ii) The study employs a double-blind, placebo-controlled design. (iii) The study recognizes the importance of safety, tolerability, and feasibility as main outcomes for planning of a large definitive trial. (iv) The study design minimizes participant burden by conducting all study procedures during their regular dialysis sessions, hence maximizing pharmaco-adherence and data collection. (v) The chosen doses of empagliflozin are based on PK modeling to balance safety and efficacy. (vi) We have a strong team of clinical trialists and statisticians with solid track records as well as adequate patient pool and strong infrastructures.

12.2 Limitations. (i) The relatively small sample size and short intervention duration of 12 weeks cannot provide definitive evidence for safety. (ii) We are unable to include intermediate efficacy endpoints, such as cardiac MRI, due to budgetary constraints. On the other hand, we recognize the caveats associated with intermediate efficacy endpoints.

12.3 Anticipated results. We anticipate that 90% of the participants will complete the 14-week study. Based on the published literature and our PK modeling, we expect that both doses of empagliflozin will be safe and well tolerated in this study.

12.4 Future directions. The combination of safety, tolerability, feasibility, and PK results from this study will greatly facilitate the decision and design of a larger trial of empagliflozin in the ESKD population to evaluate its effects on hard clinical endpoints. If our study suggests that both low and high doses of empagliflozin are safe and well tolerated, it will encourage larger clinical trials to evaluate if the 25 mg-dose may provide greater protection against HF and sudden death in chronic dialysis patients. Positive efficacy results in a large outcome trial will not only provide a powerful armamentarium for CV protection in the ESKD population, but also promote further insights into and provide opportunities to examine the mechanisms for the beneficial effects of SGLT2is.

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