Eff**I**cacy, mecha**N**isms and sa**F**ety of SGLT2 **IN**hibitors in k**I**dney **T**ransplant rec**I**pients:
The **INFINITI** study

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2. INTRODUCTION AND RATIONALE

2.1 Kidney transplantation: Treatment of choice for end-stage renal disease

Over 40,000 Canadians are currently living with end-stage renal disease (ESRD), and the prevalence continues to rise in Canada and worldwide[1]. The impact of ESRD on mortality is substantial, with an estimated 5-year survival of approximately 50% for patients on dialysis therapy[1]. Kidney transplantation is the renal replacement therapy of choice for patients with ESRD. It has been well established that kidney transplantation improves patient survival and quality of life, and results in significant savings to the health care system[2, 3]. Wolfe et al. demonstrated in a landmark study that kidney transplant recipients (KTR) gained an additional 10 years of life as compared to eligible transplant candidates who remained on chronic dialysis therapy[2]. These life-years gained were notable for all subgroups of the ESRD population, with even greater benefits for those with ESRD due to diabetic kidney disease (DKD), with up to 17 life-years gained in this subgroup[2].

DKD is the most common cause of ESRD in Canada, accounting for 36% of all incident cases of ESRD[1]. This group also comprises over a quarter of the 14,500 Canadians living with a functioning kidney transplant[1]. Furthermore, KTR without diabetes at the time of transplantation are at risk for developing post-transplant diabetes mellitus (PTDM). In addition to traditional risk factors, there are a number of transplant-specific risk factors such as the use of diabetogenic immunosuppressive medications, hypomagnesemia and post-transplant weight gain that predispose KTR to PTDM. The incidence of PTDM is variable based on the definition used and the transplant era, however a large US cohort study of 11,659 KTR demonstrated that the cumulative incidence of PTDM was 9.1% at 3 months, 16% at 12 months and 24% at 36 months post-transplantation[4].

2.2 Challenges in kidney transplantation: Mortality, cardiovascular disease and graft loss

Despite the survival benefit conferred by transplantation, KTR still face a number of challenges, especially in patients with diabetes. First, KTR still have a higher risk of mortality than their age-matched counterparts without kidney disease[5]. This mortality risk is even greater amongst KTR with diabetes. Ojo et al. showed that the risk of death with a functioning kidney transplant is increased by almost two-fold in those with diabetes as compared to those without[6]. Furthermore, mortality from cardiovascular disease (CVD) continues to be an important problem after transplantation. Large registry analyses from the Canada, the US and Europe have demonstrated that death accounts for up to 50% of graft losses, with CVD as the leading cause of death in KTR[6-8]. This is likely due to the high burden of cardiovascular risk factors in KTR such as hypercholesteremia and hypertension[9, 10]. Hypertension is reported to occur in up to 92% of KTR[10-12], and is often suboptimally controlled[9, 10]. An analysis of the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) study found that 69% of KTR did not meet blood pressure targets[9, 10]. The cause of hypertension in KTR is multifactorial, and includes transplant-

specific risk factors. For example, calcineurin inhibitors (CNIs) are used in virtually all KTR to decrease the risk of acute rejection and increase patient and graft survival[13, 14]. However, CNIs induce endothelial dysfunction, increase vascular and sympathetic tone and promote sodium reabsorption by activation of the renin-angiotensin-aldosterone system, thereby increasing the risk of hypertension[10, 15, 16]. The risk of hypertension in KTR is also increased by the use of glucocorticoids, by post-transplant weight gain and via the impact of ambient hyperglycemia in patients with diabetes.

Another major challenge faced by KTR is the continuing risk of developing graft failure over time. Although there has been substantial improvement in short-term graft survival over the last few decades, late graft failure (i.e., graft loss beyond 1 year after transplantation) continues to be an important problem in KTR. When graft failure occurs, the survival benefit offered by kidney transplantation is lost, and the transitional period between graft failure and re-initiation of dialysis is associated with a three-fold increase in the risk of death[17,18]. Furthermore, only 10 to 15% of those who experience graft failure eventually undergo re-transplantation[1, 19]. Unfortunately, in the subgroup of KTR with diabetes, the incidence of graft failure is 50% higher than the general kidney transplant recipient population, and recurrent DKD occurs in almost half of allografts after transplantation[20, 21]. The development of chronic kidney disease (CKD), a common precursor to graft failure, occurs due to a number of immunologic and non-immunologic risk factors. Examples of the latter include hypertension, dyslipidemia, obesity, recurrent disease (i.e., DKD) in the graft, and albuminuria[22]. CKD in KTR is common, affecting up to 90% of the population[23, 24, 25], and significantly increases the risk of death and graft loss[23, 25]. However, to date, effective strategies to mitigate the risk of graft dysfunction and slow the progression to graft failure are lacking. In light of the beneficial effects of angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) in reducing the risk of death and progression to ESRD in the non-transplant population with proteinuric CKD, a multi-centre randomized controlled trial comparing ramipril to placebo in KTR was conducted. Unfortunately, this trial showed that ramipril was not effective in reducing the risk of graft failure or cardiovascular death in KTR[26], albeit the study may have been underpowered. Current strategies in the management of graft dysfunction and CKD are therefore focused on optimizing immunosuppression and control of hypertension and dyslipidemia. Accordingly, there is an important unmet need for cardio- and renoprotective strategies to address premature death and graft loss in the KTR population.

2.3 SGLT2 inhibitors in the general (non-transplant) population

Sodium-glucose cotransporter 2 inhibitors (SGLT2i) are glucose lowering agents that are effective in the treatment of T2D, resulting not only in improved glycemic control, but also weight loss, blood pressure and albuminuria reduction[27, 28]. SGLT2i agents block glucose reabsorption in the proximal tubule epithelial cell of the kidney and increase urinary glucose excretion[28, 29]. This insulin-independent mechanism reduces blood glucose levels, without the risk of hypoglycaemia. Several clinical trials have shown significant benefits of SGLT2i on cardiovascular and renal

outcomes. The EMPA-REG OUTCOME study randomized patients with type 2 diabetes with established cardiovascular disease to empagliflozin vs. placebo[30]. This study showed significant reductions in major adverse cardiac events, cardiovascular mortality, all-cause mortality and hospitalization for heart failure with empagliflozin[30]. The CANVAS trial randomized patients with type 2 diabetes at high cardiovascular risk with and without established CVD and demonstrated a 22% reduction in cardiovascular death or hospitalization for heart failure with canagliflozin[31]. From a renal perspective, the CANVAS-R study showed a reduction in albuminuria progression, as well as a 40% reduction in ESRD, death and sustained eGFR reduction[31]. Similarly, a pre-specified analysis of the EMPA-REG OUTCOME trial also showed that empagliflozin was associated with slower progression of kidney disease and lower rates of renal events as compared to placebo[32]. CREDENCE was the first dedicated renal clinical trial, which aimed to randomize over 4000 patients with type 2 diabetes, stage 2 or 3 CKD and microalbuminuria treated with an ACEi/ARB, to canagliflozin vs. placebo[33]. The primary outcome of interest was a composite of ESRD, doubling of serum creatinine and renal or cardiovascular death. This trial was recently stopped early as it achieved the prespecified efficacy criteria for the primary composite endpoint[34]. Hence, in non-transplant patients with T2D, the use of SGLT2i is changing the way clinicians attenuate the progression of cardiorenal complications. Further evidence supporting the glycemic-independent benefits of SGLT2 inhibition has emerged from recent heart failure trials. The Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction (DAPA-HF) and Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure (EMPEROR-Reduced) trials were the first large trials to study SGLT2 inhibition in participants with heart failure and reduced ejection fraction (HFrEF) with and without T2D[35, 36]. The application of SGLT2i as dedicated kidney-protective agents continued to evolve with the publication of the DAPA-CKD trial, which extends their cardiorenal protection to patients with non-diabetic CKD[37]. DAPA-CKD enrolled 4304 participants with CKD, eGFR of 25-75 ml/min/1.73m^2 and UACR of 200 to 5000mg/g. About two-thirds of participants had T2D and almost all patients were on stable doses of ACE inhibitors/ARBs. Other than DKD, the cause of CKD was almost equally distributed between ischemic/hypertensive nephropathy and chronic GN with about 16% for each subgroup. The chronic GN subgroup was further divided into IgA nephropathy (IgAN) (6.3%), FSGS (2.7%) and membranous nephropathy (1%). Patients with kidney transplants, type 1 diabetes (T1D), polycystic kidney disease, lupus nephritis and ANCA vasculitis were excluded. Dapagliflozin was safe and significantly reduced the risk of the primary composite end point (≥50% eGFR decline, onset of ESKD, renal or CV death) by 39% versus placebo, with a number needed to treat of 19 to prevent one primary outcome event. Like DAPA-HF and EMPEROR-Reduced, this effect was consistent among patients with and without diabetes, with no evidence of hypoglycemia.

SGLT2i exert a number of glucose-independent effects, which have been linked to the beneficial cardiovascular and renoprotective effects observed in clinical trials. In the setting of diabetes, hyperglycemia results in increased tubular reabsorption of glucose and sodium, which activates tubuloglomerular feedback (TGF). This leads to a decrease in sodium delivery to the macula densa,

which in turn leads to vasodilation of the afferent arteriole and hyperfiltration[38]. SGLT2i increases delivery of sodium to the macula densa, restores TGF and results in vasoconstriction of the afferent arteriole, leading to reductions in intraglomerular hypertension, glomerular filtration rate (GFR) and albuminuria[39]. These mechanisms appear to be intact in non-DM populations as well. Bay and colleagues determined the effect of canagliflozin versus placebo on 376 non-diabetic obese subjects for 12 weeks [40]. Canagliflozin reduced weight and blood pressure over the study period. A decrease in eGFR over the 12-week period was observed with canagliflozin, consistent with SGLT2 inhibition-mediated reductions in glomerular pressure. Our group also published results of the DIAMOND trial where fifty-three participants with non-diabetic CKD, eGFR ≥25ml/min/1.73m^2, and proteinuria >500 to ≤3500mg/day were treated with dapagliflozin and placebo in a randomized, double-blind 6-week crossover trial. Dapagliflozin resulted in an acute and reversible decline in measured GFR while decreasing body weight and increasing markers of hemoconcentration, suggesting mechanisms of kidney function are intact irrespective of glycemic effects[41]. These mechanisms are shown in **Figure 1**. The natriuresis induced by SGLT2i is also one of the postulated mechanisms underlying reductions in the systolic and diastolic blood pressure and arterial stiffness observed with SGLT2i[42, 43, 28, 44]. Decreases in systemic and intraglomerular hypertension with SGLT2i may in turn be responsible for decreases in albuminuria and CKD progression in clinical trials of patients with and without T2D[28, 45]. Interestingly, although there is an attenuation of the glucose lowering effects of SGLT2i as GFR declines due to reduced glucosuria, natriuresis-related reductions in blood pressure and reductions in albuminuria persist even in the setting of reduced kidney function[46]. From a cardiac perspective, it is hypothesized that the natriuresis-induced decrease in intravascular volume and reduction in arterial stiffness that occurs with SGLT2i leads to an unloading of the myocardium and an improvement in cardiac function, although other factors may also play a role[39, 47].

A number of experimental studies have also uncovered other potential renoprotective effects of SGLT2i. In the setting of hyperglycemia, there is an increase in the reabsorption of sodium and glucose due to upregulation of the sodium-glucose cotransporter 2 in the proximal tubule[48]. These increases in metabolic demands predispose the proximal tubule epithelial cells (PTECs) to hypoxia, which in turn, stimulates a cascade of mediators leading to fibrosis and apoptosis[49-52]. The increase in glucose reabsorption that occurs in PTECs in the setting of diabetes also results in the generation of advanced glycosylation end-products. Advanced glycosylation end-products and the high glucose milieu have been shown trigger the generation reactive oxygen species as well as pro-fibrotic and pro-inflammatory pathways, ultimately leading to progressive DKD[53, 54]. Similarly, increased albumin reabsorption by the proximal tubule in the setting of diabetes is also toxic to the PTECs, promoting a similar cascade of cytokines and pro-inflammatory mediators contributing to tubulointerstitial injury[53-55]. In light of these known changes in DKD, SGLT2i have the potential to decrease the release of reactive oxygen species and attenuate pro-fibrotic and pro-inflammatory pathways by decreasing the metabolic demands of the PTECs. Although the results are conflicting, some experimental studies in mice have demonstrated a reduction in pro-

fibrotic and pro-inflammatory mediators, although this has yet to be shown in humans[56,39]. The various effects and mechanisms of SGLT2i are shown in **Figure 2**.

2.4 SGLT2 inhibitors in kidney transplant recipients: Special considerations

Given the glucose-dependent and independent effects of SGLT2i, as well as the accumulating evidence demonstrating cardiorenal protection in non-KTR, the use of these agents in KTR is attractive – especially since traditional renin-angiotensin-aldosterone system inhibitors are not effective. Moreover, the use of SGLT2i as a cardiorenal protective therapy may be of particular value in KTR given the high burden of comorbidities such as diabetes, CVD and hypertension, as well as the ongoing challenges of premature death and graft loss in this population. However, there are several unique aspects of KTR that are important to consider when using SGLT2i, including the immunosuppressed state of KTR, the impact of SGLT2i on denervated kidneys as well as the effectiveness of SGLT2i with concomitant use of CNIs. Given the susceptibility of KTR to infectious complications due to immunosuppression, there is the potential for concern regarding safety of these agents in this population. For example, urinary and mycotic infections are well described with SGLT2i use[57, 58], and urinary tract infections are one of the most common infectious complications in KTR[59, 60]. In addition, transplanted kidneys are denervated and therefore the autoregulation of renal blood flow that occurs in the setting of sympathetic nervous system activity, particularly when hypotension occurs, is attenuated[61]. SGLT2i can cause volume depletion and acute kidney injury (AKI), and it is unclear whether these adverse effects are magnified in KTR with denervated kidneys. Finally, CNIs are commonly used in almost all KTR, and are well known to affect the kidney[62]. In particular, CNIs can result in tubular toxicity through a variety of mechanisms including hypoxia of the tubulointersitium, apoptosis of tubular cells and alterations in tubular transport, as manifested by well-described electrolyte disturbances[15, 62]. In non-KTR, SGLT2i therapies are very safe over a wide range of kidney function and have even been shown to reduce the risk of AKI. Thus, an important aim of our study is to capture potential safety signals, including infectious complications and changes in renal function in the KTR population.

2.5 SGLT2i in kidney transplant recipients: Current state of knowledge

Currently, there are few published experiences on the use of SGLT2i in transplant recipients[63] (**Table 1**). To our knowledge, the use of SGLT2i has also been described in a few other immunosuppressed populations including heart transplant recipients. We studied 10 KTR and simultaneous pancreas-kidney transplant recipients and showed that the use of use of the SGLT2 inhibitor canagliflozin was associated with improvements in glycemic control (hemoglobin A1c (HbA1c) -0.84 ± 1.2 %), weight (-2.14 ± 2.8kg) and blood pressure (systolic -6.5 ± 10.8 mmHg and diastolic -4.8± 12 mmHg), which were similar in magnitude to effects reported in non-transplant cohorts. Furthermore, we did not observe any episodes of ketoacidosis, AKI, acute rejection or urinary/mycotic infections during treatment. One patient experienced hypoglycemia, and one patient developed cellulitis. Finally, there were no clinically significant changes in CNI

levels. However, this was a small cohort of patients with excellent kidney function (eGFR >60ml/min/1.73m^2) where canagliflozin was initiated several years after transplantation, with a limited follow-up of 80.5 person-months. In order to better understand the efficacy, safety and potential mechanisms of SGLT2i in this unique population with variable degrees of renal impairment, further comparative studies are needed in larger cohorts of KTR.

There are currently two clinical trials evaluating SGLT2i in KTR[64, 65], both of which do not include patients with pre-existing T2D, or patients without diabetes. These are summarized in **Table 1.** The first is a single-arm non-inferiority study of 16 patients with PTDM on insulin therapy, comparing the efficacy of empagliflozin to pre-existing insulin therapy, on glycemic control. The second is a randomized controlled trial of empagliflozin vs. placebo in a cohort of 50 KTR with PTDM. The primary outcome of this study is also glycemic control, as measured by a weighted mean glucose before and after SGLT2i therapy. However, based on the glucose-independent benefits of SGLT2i in the non-transplant cohorts with and without diabetes described above, we strongly believe that in KTR, the glucose-independent effects of SGLT2i are likely to be of greatest benefit given the high prevalence of CKD in the KTR population[23, 24]. In addition, in the setting of deceased donation, kidney transplant candidates with diabetes frequently receive kidneys from more 'marginal' donors given their high mortality on the transplant waiting list[1]. Although this strategy may be beneficial from a patient survival perspective (vs. chronic dialysis), KTR with diabetes are more likely to have kidney transplants with reduced kidney function[66]. It is known that HbA1c lowering is attenuated as the eGFR decreases, however, the glucose-independent effects, such as blood pressure reduction, persist across the range of eGFR irrespective of diabetes status[46]. In KTR, we believe that the most clinically important effect is likely the natriuresis induced by SGLT2i, resulting in a reduction in blood pressure as well as enhancement of other cardiovascular and renoprotective mechanisms beyond glycemic control.

3. OBJECTIVES

3.1 Primary

To evaluate the effect of 12 weeks of 10 mg daily oral dapagliflozin vs. placebo on systolic blood pressure in KTR with or without T2D or PTDM.

3.2 Secondary

1. To determine the safety of 12 weeks of 10 mg daily oral dapagliflozin therapy vs. placebo in 52 KTR with or without T2D or PTDM.

2. To elucidate the potential mechanisms responsible for blood pressure lowering, as well as the potential cardio- and renoprotective effects of 12 weeks of 10 mg daily oral dapagliflozin therapy vs. placebo in 52 KTR with or without T2D or PTDM. In order to

elucidate the mechanisms underpinning the potential blood pressure lowering and cardiovascular effects of SGLT2i in KTR, we have incorporated comprehensive measurements of natriuresis, arterial stiffness and sympathetic nervous system activity, as well as changes in weight and lipid profile. Furthermore, to elucidate potential renoprotective effects, we have incorporated detailed study of renal function, neurohormone biomarker measurements, as well as markers of oxidative stress and changes in allograft hypoxia using novel photoacoustic ultrasonography, as a measure of renal metabolic demands. Serum, plasma and urine will be collected and stored for potential future analyses for exploratory biomarkers to assess relationships with disease activity and effects of study drug.

3. To determine the glucose lowering effects of 12 weeks of 10 mg daily oral dapagliflozin therapy vs. placebo in 52 KTR with or without T2D or PTDM.

3.3 Anticipated Outcomes

1. We hypothesize that dapagliflozin will result in a significant reduction in systolic and diastolic blood pressure in a cohort of KTR with or without T2D and PTDM.

2. We hypothesize that dapagliflozin will be well tolerated in KTR with or without T2D and PTDM, with adverse events similar to that of non-transplant populations. We also hypothesize that there will be no clinically significant changes in CNI levels or episodes of acute rejection in KTR treated with dapagliflozin vs. placebo during the study.

3. We hypothesize that dapagliflozin will result in a significant natriuresis, as well as reductions in arterial stiffness, albuminuria, allograft hypoxia and markers of oxidative stress, while having neutral effects on cardiac and renal function over the course of the 12-week study period.

4. We hypothesize that the glucose-lowering effects of dapagliflozin will be modest in KTR with T2D or PTDM due the high prevalence of CKD in this population. In participants without T2D or PTDM, we do not expect any glucose lowering.

4. STUDY DESIGN

This study will be a randomized, double-blind, placebo-controlled clinical trial comparing the SGLT2 inhibitor dapagliflozin to placebo in 52 KTR with or without pre-existing T2D or PTDM. The primary outcome of the trial is to determine if dapagliflozin is superior to placebo in reduction of blood pressure in KTR.

We will include incident and prevalent KTR at least 6 months after their transplant date We selected a period of at least 6 months after kidney transplantation prior to study enrolment for safety reasons. The risk of infection as well as acute rejection is highest in the early post-transplant period[67, 68]. Furthermore, fluctuations in kidney function as well as adjustments to immunosuppressive medications are also common. Thus, a period of 6 months was selected to capture stable KTR for study inclusion.

We have selected patients with and without diabetes (including T2D and PTDM) to be included in the study for the following reasons: 1) As described in detail in the sections above, the mechanisms hypothesized to confer cardiorenal benefit are independent of improvements in glycemic control and are intact in patients with and without diabetes. Furthermore, recent large clinical trials such DAPA-HF, EMPEROR-Reduced and DAPA-CKD demonstrate that SGLT2 inhibition is safe and highly effective in non-transplant populations without diabetes, leading to approval for clinical use in multiple health jurisdictions; 2) It is still important to include KTR with T2D and PTDM as both conditions are common in the KTR population, with T2D as the cause of ESRD in approximately 30 to 40% of KTR, and PTDM occurring in approximately 25% of KTR[4]. Additionally, both conditions are associated with a high prevalence of hypertension in KTR, as well as an increased risk of cardiovascular disease, death and graft loss[69] with existing preliminary data from our centre suggesting the potential efficacy and safety of SGLT2i in a selected group of KTR with both T2D and PTDM[63].

In patients with diabetes, we have imposed an upper limit on HbA1c of <12.0% to avoid starting the drug in participants with poorly controlled diabetes who may be pre-disposed to ketoacidosis due to relative hypoinsulinemia. There is minimal experience with SGLT2 inhibition in patients with stage 4 or 5 CKD; therefore an eGFR of ≥30 ml/min/1.73m^2 is required for inclusion. The BMI criteria is reflective of the current experience to date using SGLT2i in non-transplant populations.

5. STUDY POPULATION

5.1 Inclusion Criteria

1. Male or females >18 years old ≥ 6months after kidney transplantation;
2. In patients with T2D or PTDM, HbA1c <12.0%;
3. eGFR ≥30 ml/min/1.73m^2 (as per the CKD-EPI equation[70]);
4. BMI ≤45kg/m^2;
5. Blood pressure ≤160/90 and ≥90/60 at screening.

5.2 Exclusion Criteria

1. Diagnosis of type 1 diabetes;

2. Presence of severe peripheral vascular disease (i.e. prior amputation, gangrene, non-healing ulcer or ischemic rest pain);
3. Presence of acute coronary syndrome, stroke or transient ischemic attack in the 3 months prior to screening;
4. Prior episode of graft pyelonephritis in the 1 month prior to screening;
5. Episode of acute graft rejection in the 3 months prior to screening;
6. Initiation of a new immunosuppressive agent or discontinuation of an immunosuppressive agent in the 1 month prior to screening;
7. Untreated urinary or genital tract infection;
8. Severe hypoglycemia within 3 months of screening, or hypoglycemia unawareness;
9. Pre-menopausal women who are nursing, pregnant, or of child-bearing potential and not practicing an acceptable method of birth control;
10. Participation in another trial with an investigational drug within 30 days of informed consent;
11. Alcohol or drug abuse within 3 months prior to informed consent that would interfere with trial participation;
12. Any ongoing clinical condition that would jeopardize subject safety or study compliance based on investigator judgement.
13. Patients currently using antipsychotic medications.
14. Use of SGLT2 inhibitors within 1 month of starting the study.

5.3 Sample Size

The sample size is based on the primary endpoint of blood pressure. Currently, there is only one publication from our group with information available on the effect of SGLT2i on blood pressure reduction in KTR with diabetes. In this observational study of 10 KTR and simultaneous pancreas-kidney transplant recipients treated with canagliflozin, we reported a SBP decrease of 6.5 ± 10 mmHg[63]. Given the small sample size in our observational study, we believe the standard deviation would be smaller in a larger cohort. Blood pressure reduction with SGLT2 inhibition appear to be similar between patients with and without diabetes in non-transplant studies. We, therefore, estimated the sample size based on a SBP change of 6.5 mmHg with a standard deviation of 8 mmHg. Under the hypothesis that SGLT2i decreases SBP by 6.5 ± 8 mmHg compared to placebo with a two-sided alpha of 0.05 and 80% power, we calculate a sample size of n=48, with 24 randomized to dapagliflozin and 24 randomized to placebo. Assuming a 10% attrition rate, the final sample size required is 52 participants. This sample size is similar to those successfully used in previous studies[71], and sufficient to measure secondary renal endpoints related to renal hemodynamic function[71], natriuresis and arterial stiffness[72-74]. Based on previous studies, we anticipate an even sex distribution in each group.

6. TREATMENT OF SUBJECTS

6.1 Investigational Product/Treatment

Dapagliflozin and matching placebo will be provided in 35 cc bottles, and the sponsor will be responsible for labelling. Each bottle of IP will be identified with a unique Composite ID in order to maintain the blind. By way of an Unblinding List, Pharmacy will provide treatment allocations of each Composite ID. The Unblinding List will be maintained within a secure location in the Pharmacy. Blinded personnel will not have access to this list. The blind must not be broken during the course of the study unless breaking the blind is required to provide medical care to the subject in the event of a medical emergency. The decision to break the blind is the responsibility of the Investigator. The Investigator may obtain treatment assignment directly from the unblinded Pharmacist. The Unblinding List will be used to confirm treatment assignment in the event of a medical emergency. The reason for breaking the blind must be clearly recorded by the Investigator in the subject's source documentation. Patients will take 10 mg dapagliflozin (1 tablet 10mg once daily) or matching placebo according to randomised treatment scheme.

Study participants will be allocated to receive either 10 mg daily of dapagliflozin or a matching placebo for 12 weeks in a 1:1 ratio. Participants with diabetes will continue on existing medical therapy for glycemic control as recommended by Diabetes Canada[75]. The study design is shown in **Figure 3.** The study investigator can titrate dapagliflozin down to 5 mg in the event that dapagliflozin 10 mg dose is not well tolerated by the participant. However, in the event of hypoglycemia, a decrease in dose of other glucose lowering therapies (including insulin), will be first initiated, if applicable, by the study investigator prior to down-titration of dapagliflozin. Every effort will be made to keep the dose of dapagliflozin constant at 10mg daily.

There are no known or reported drug interactions with dapagliflozin and immunosuppressive therapies commonly used in KTR including CNIs (tacrolimus, cyclosporine), mammalian target of rapamycin inhibitors (sirolimus, everolimus), antiproliferative medications (mycophenolic acid, mycophenolate mofetil, azathioprine) and prednisone. SGLT2i are primarily metabolized via UDP-glucoronosyltransferase, with minimal effects on cytochrome P450 enzymes. CNIs are substrates of these enzymes, however the interaction does not appear to be clinically relevant[63]. CNI levels will be monitored for the duration of this trial and will be reported to the participants' primary transplant nephrologist within 24 hours for CNI dose adjustments, as needed. There will be no change required to the general management of KTR as a result of participation in the study.

Study medication is received at the study site by a designated person, handled and stored safely and properly, and kept in a secured location. The study medication will be stored according to the instructions specified on the drug labels. Storage conditions are adequately monitored. Subjects are asked to return all unused study drug and packaging at the end of the study or at the time of study drug discontinuation or in every visit to the outpatient clinic. Appropriate documentation of

the subject specific dispensing process is maintained. Unused drugs are destroyed by the pharmacy department at the end of the study.

6.2 Use of co-interventions (if applicable)

Use of the following treatments will be strongly discouraged starting 4 weeks before the start of the study and during the study as these medications may interfere with the evaluation of safety, tolerability and/or efficacy.

- Non-steroidal anti-inflammatory drugs (NSAIDs)

The dose of diuretics and vitamin-D analogues should be stable at least 4 weeks prior to enrolment. Dose adjustments of diuretics or vitamin-D analogues are strongly discouraged during the trial.

In subjects receiving oral medications containing NSAIDs, ephedrine, phenylephrine, pseudoephedrine, or phenylpropanolamine, administration of such medications is recommended to be discontinued at least 48 hours before iohexol injection and should not be resumed until completion of the GFR measurement procedure.

Drugs which lower seizure threshold, especially phenothiazide derivatives including those used for their antihistaminic properties, are not recommended for use with iohexol. Others include monoamine oxidase (MAO) inhibitors, tricyclic antidepressants, CNS stimulants, psychoactive drugs described as analeptics, major tranquilizers, or antiphychotic drugs. The dose of such medications, if applicable, are recommended to be discontinued at least 48 hours before iohexol injection.

KTR are typically treated with multiple medications including immunosuppression, anti-hypertensive and glucose-lowering therapies. This may predispose participants to side effects of SGLT2i given their known blood pressure and glucose lowering properties. To mitigate this, we will implement the following strategies:

1. At the time of study drug initiation, if participants are on a loop diuretic, the dose will be reduced by 50% by the study investigators to avoid volume contraction given the natriuretic effect of SGLT2i. The loop diuretic dose will be titrated back to the full dose by study investigators as needed, over a period of 7 days after drug initiation;
2. Our study endocrinologist, Dr. Bruce Perkins will be responsible for glycemic management in participants with diabetes during the study through study and telephone visits[71]. All anti-diabetes medications will be allowed including insulin, sulfonylureas, DPP4 inhibitors, and GLP-1 receptor agonists. SGLT2 inhibitors may not be used within 1 month of starting the study. No minimum time on anti-diabetes medications will be required prior to randomization. Adjustment of insulin and other glucose-lowering medications will be

made as necessary by Dr. Perkins utilizing an individualized approach based on the patients' specific risk of hypoglycemia and/or DKA. Changes in glycemic management will always made in conjunction with the patients' own endocrinologist/family doctor. Major hypoglycemic events will be captured as an adverse event of interest.

3. Given the lack of definitive data supporting the use of ACEi/ARB as a renoprotective strategy, the use of an ACEi/ARB is variable in KTR. However, these drugs are prescribed to KTR based on physician preference, and due to non-renal indications such as post-transplant erythrocytosis, hypertension or cardioprotection. The use of ACEi/ARB with SGLT2i will be permitted during the entre duration of the trial and we have incorporated frequent safety monitoring in our study to allow for the early detection of any potential adverse events that may occur with concomitant renin-angiotensin-aldosterone system blockade and SGLT2i.

7. INVESTIGATIONAL PRODUCT

7.1 Name and description of investigational product(s)

Drug name: dapagliflozin (Forxiga, AstraZeneca, RPF-EU-12-MB102-016);

Chemical structure: (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6 (hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol;

Product Description and Dosage Form	**Dosage form and strength**	**Manufacturer**
Dapagliflozin 10mg Tablets	10 mg Yellow, biconvex, round, film coated tablets with "10" engraved on one side and "1428" engraved on the other side (orally)	Astra Zeneca
Dapagliflozin 5mg Tablets	5 mg Yellow, biconvex, round, film coated tablets with "5" engraved on one side and "1427" engraved on the other side (orally)	Astra Zeneca
Placebo Matching Dapagliflozin Tablet	Yellow, plain, film coated tablet. Does not contain active ingredient	Astra Zeneca

7.2 Summary of findings from non-clinical studies

Available data from non-clinical study in animals is provided in the Investigator's Brochure.

7.3 Summary of findings from clinical studies

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Despite the presence of hyperglycaemia in type 2 diabetes, reabsorption of filtered glucose continues. Dapagliflozin improves both fasting and post-prandial plasma glucose levels by reducing renal glucose reabsorption leading to urinary glucose excretion. This glucose excretion (glycosuric effect) is observed after the first dose, is continuous over the 24-hour dosing interval and is sustained for the duration of treatment. The amount of glucose removed by the kidney through this mechanism is dependent upon the blood glucose concentration and GFR. In patients with low plasma glucose or low eGFR dapagliflozin's efficacy to lower HbA1c is low and so the risk of hypoglycemia is low as well. Effects on blood pressure, body mass index, albuminuria, hematocrit appear to be independent of GFR.

Dapagliflozin does not impair normal endogenous glucose production in response to hypoglycaemia. Dapagliflozin acts independently of insulin secretion and insulin action rendering the risk of hypoglycemia low. Increases in the amount of glucose excreted in the urine were observed in healthy subjects and in subjects with type 2 diabetes mellitus following the administration of dapagliflozin. Approximately 70 g of glucose was excreted in the urine per day (corresponding to 280 kcal/day) at a dapagliflozin dose of 10 mg/day in subjects with type 2 diabetes for 12 weeks. Evidence of sustained glucose excretion was seen in subjects with type 2 diabetes given dapagliflozin 10 mg/day for up to 2 years.

Twelve double-blind, randomised, controlled clinical trials were conducted with 6,144 subjects with type 2 diabetes to evaluate the efficacy and safety of Forxiga; 4,164 subjects in these studies were treated with dapagliflozin. Two double-blind, randomized, controlled clinical trials were conducted in 9,048 with and without T2D to evaluate the efficacy and safety of Forxiga; 4,520 subjects in these studies were treated with dapagliflozin. In DAPA CKD study, dapagliflozin was safe and significantly reduced the risk of the primary composite end point (≥50% eGFR decline, onset of ESKD, renal or CV death) by 39% versus placebo, with a number needed to treat of 19 to prevent one primary outcome event. Moreover, all the secondary end points including the composite kidney outcome (sustained 50% eGFR decline, ESKD or renal death), the composite CV outcome (hospitalization for heart failure or CV death) or all-cause mortality were significantly lower in the dapagliflozin group. Examining the hazard ratios of primary and secondary outcomes across subgroups of CKD etiology, it is apparent that the long-term kidney benefits of dapagliflozin in non-diabetic participants are similar to those with T2D.

7.4 Monotherapy

A double-blind, placebo-controlled study of 24-week duration (with an additional extension period) was conducted to evaluate the safety and efficacy of monotherapy with dapagliflozin in

subjects with inadequately controlled type 2 diabetes. Once-daily treatment with dapagliflozin resulted in statistically significant ($p<0.0001$) reductions in HbA1c compared to placebo.

7.5 Combination Therapy

In a 52-week, active-controlled non-inferiority study (with a 52-week extension period), dapagliflozin was evaluated as add-on therapy to metformin compared with a sulphonylurea (glipizide) as add-on therapy to metformin in subjects with inadequate glycaemic control (HbA1c > 6.5% and ≤ 10%). The results showed a similar mean reduction in HbA1c from baseline to Week 52, compared to glipizide, thus demonstrating non-inferiority. At Week 104, adjusted mean change from baseline in HbA1c was -0.32% or dapagliflozin and -0.14% for glipizide. At 52 and 104 weeks, a significantly lower proportion of subjects in the group treated with dapagliflozin (3.5% and 4.3%, respectively) experienced at least one event of hypoglycaemia compared to the group reated with glipizide (40.8% and 47.0%, respectively). In a study of 808 patients with type 2 diabetes who had inadequate glycemic control and received on average 30 units insulin, dapagliflozin 10 mg/day caused a 0.57% reduction in HbA1c relative to placebo. Dapagliflozin did not increase insulin requirements but these requirements increased progressively in the placebo group.[76]

7.6 Summary of known potential risks and benefits

Dapagliflozin has global market approval and based on global cumulative sale figures is estimated that dapagliflozin has been administered during >1000000 patient years. Details regarding potential risks associated with administration of dapagliflozin once daily are provided in the Investigator's Brochure (IB).

7.6.1 Potential risks

The potential risks for the treatment with dapagliflozin and other SGLT2 inhibitors are described in the IB. Due to its mode of action resulting in increased urinary glucose excretion an increased risk of urinary tract infections (slightly higher compared to placebo in the phase Ill studies) and genital infections has been seen.

Based on the mechanism of action of dapagliflozin there may be a potential risk for this compound to cause hypovolaemia or electrolyte imbalance. As a precaution, patients who, in the judgment of the Investigator may be at risk for dehydration or volume depletion due to co-existing conditions or concomitant medications, such as loop diuretics, should have careful monitoring of their volume status. In patients already receiving dapagliflozin who develop conditions that may cause hypovolaemia or electrolyte imbalance, decisions to interrupt or discontinue dapagliflozin therapy and management of patients should be based on clinical judgment.

Higher proportions of patients with marked laboratory abnormalities of hyperphosphatemia were reported in dapagliflozin vs placebo. The magnitude and clinical significance of this in patients with CKD is unclear.

After the introduction of dapagliflozin and other SGLT2 inhibitors there have been post marketing reports of ketoacidosis, including diabetic ketoacidosis (DKA), in patients with type 1 diabetes (T1D) and T2D, although a causal relationship has not been established. Patients presenting signs and symptoms consistent with ketoacidosis, including nausea, vomiting, abdominal pain, malaise, and shortness of breath, should be assessed for ketoacidosis, irrespective of blood glucose levels. If ketoacidosis is suspected, discontinuation or temporary interruption of dapagliflozin should be considered and the patient should be promptly evaluated.

Predisposing factors to ketoacidosis include a low beta-cell function reserve resulting from pancreatic disorders (e.g., T1D, history of pancreatitis, or pancreatic surgery), insulin dose reduction, reduced caloric intake, or increased insulin requirements due to infections, illness or surgery and alcohol abuse. Dapagliflozin should be used with caution in these patients.

There is no reason to believe that dapagliflozin poses an undue risk of hypoglycemia in non-diabetic patients. The amount of glucose excreted in the urine depends on the total filtered glucose load (blood glucose concentration x GFR). Thus, at blood glucose levels in the low normal range, the amount of glucose excreted into the urine is not sufficient to induce hypoglycemia [77]. In Clinical Pharmacology studies in healthy subjects' single doses up to 500 mg and multiple oral doses of 2.5 to 100 mg up to 14 days have been evaluated and have shown that dapagliflozin does not induce even a single case of hypoglycemia in non-diabetic subjects. Additionally, in a recent clinical trial in 50 non- diabetes obese individuals treated with the combination of dapagliflozin and exenetide, none of the patients experienced a hypoglycemic event [78].

7.6.2 Protection against risks

This study has been designed with appropriate measures in place to monitor and minimize any of the potential health risks to participating patients. Safety signal detection will include the integration of all available sources of safety information, including clinical study data, adverse events (AE) reports, pre-clinical data, epidemiological studies and literature reports, to identify and characterize unrecognized safety risks or changes in those which are currently expected Adverse Drug Reactions. Any information that may affect the benefit-risk profile of dapagliflozin will be immediately communicated to relevant Health Authorities and appropriate actions will be taken regarding the clinical study as needed. Thus real-time, active safety surveillance will be conducted during the entire duration of this study.

In addition, if deemed necessary by the treating physician temporary and if necessary permanent discontinuation of dapagliflozin in individual patients in whom a potential health risk or a laboratory abnormality of clinical concern has been identified.

7.6.3 Potential benefits to patients

In this study, the dose of dapagliflozin 10 mg was chosen based on previous clinical experience. This mechanistic study is non-therapeutic; therefore, it has limited or no direct clinical benefit for the subjects. In studies of longer duration, patients randomized to active drug, dapagliflozin is expected to reduce progression of renal failure and reduce CV mortality. Dapagliflozin is known to decrease body weight (or prevent weight gain) as well as lower blood pressure and albuminuria in patients with and without type 2 diabetes.

Patients are also expected to receive some benefit in the form of increased medical care/attention when participating in study procedures.

7.6.4 Informed consent and alternatives to participation

All prospective participants will be fully informed of the possible risks and benefits associated with this study, and their consent will be obtained prior to performing any study-specific activity. Should a prospective participant elect to not participate in the study or to withdraw from the study, other medications are available to treat their CKD, and other possible concomitant diseases according to the discretion of their treating physician, and the patient will not be disadvantaged in any way.

7.6.5 Conclusion

Considering the pre-clinical and clinical experience with dapagliflozin and the precautions included in the study protocol, participation in this study presents a minimal and thus acceptable risk to patients who meet the inclusion/exclusion criteria and consent to take part in the study.

7.7 Description and justification of route of administration and dosage

Dapagliflozin is absorbed from the digestive tract and can therefore be orally administered.

7.8 Dosages, dosage modifications and method of administration

Dapagliflozin will be administered in a dose of 10 mg/day.

7.9 Preparation and labelling of Investigational Medicinal Product

Study medication (dapagliflozin and matching placebos) will be provided by AstraZeneca. The pharmacy department of the University Health Network will be responsible for labelling and distribution of study medication to the participating sites. Study medication will be stored by the pharmacy unit in accordance with relevant guidelines.

7.10 Drug Accountability

All study medications will be stored at room temperature (<30°C) in a secure location. Study medication will be collected from the pharmacy department by the principle investigator. Unused and partially used study drug will be returned by the subject to the site for drug accountability. Returned and unused study drug will be returned to the pharmacy department where it will be destroyed.

7.11 Handling and Dispensing

The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects and only from official study sites by authorized personnel, as dictated by local regulations.

The investigator is responsible for ensuring that the investigational product is stored under the appropriate environmental conditions (temperature, light, and humidity). If concerns regarding the quality or appearance of the investigational product arise, the investigational product will not be dispensed and AstraZeneca will be contacted immediately.

7.12 Drug Ordering

Initial Orders and Re-Supply

Contact the protocol manager at the UHN (vesta.lai@uhn.ca) for information.

When assessing need for resupply, institutions should keep in mind that shipments may take 10-15 business days as of receipt of request. Be sure to check existing investigational stock to assure optimal use of drug on hand.

8. NON-INVESTIGATIONAL PRODUCT

8.1 Name and description of non-investigational product(s)

Iohexol (Omnipaque), lithium carbonate (300 mg one day prior to each physiological study day i.e. on three occasions).

8.2 Summary of findings from non-clinical studies

Iohexol has very low acute intravenous toxicities in mice and rats. Animal studies have shown that these agents have a very low protein binding and are well tolerated by the kidneys. The cardiovascular and neurotoxicity are low.

8.3 Summary of findings from clinical studies

Close to 100 per cent of the intravenously injected Iohexol is excreted unchanged through the kidneys within 24-hours in patients with normal renal function. The elimination half-life is

approximately 2 hours in patients with normal renal function. The protein binding of these agents is so low (<2%) that it has no clinical relevance and can therefore be neglected. In the doses used in this set of studies, lithium does not reach steady state and has no known risk.

8.4 Summary of known potential risks and benefits

Lithium will not be used in patients with a known intolerance to this medication. Iohexol will not be administered in patients with known allergic reactions to this substance or to iodine based solutions. Iohexol has a very low risk of causing allergic reactions, and anyone with an allergy will be treated appropriately by the study team and will not receive it again on future study visits.

8.5 Description and justification of route of administration and dosage

Iohexol will be intravenously administered according to established UHN protocols through an injection catheter. Lithium will be administered orally on three separate occasions to measure tubular sodium handling.

8.6 Drug accountability

Iohexol will be stored at room temperature at each site, and will be collected along with lithium from the pharmacy department by the study nurse at each participating center in the morning of the study visit.

9. METHODS

9.1 Study parameters/endpoints

9.1.1 Main study parameter/endpoint

The primary outcome of this study is blood pressure (SBP) reduction. Blood pressure will be measured using a calibrated automated office oscillometric device with a proper sized cuff, as per the Hypertension Canada Guidelines[79]. Patients will be seated comfortably for 5 minutes. After this period of rest, blood pressure will be obtained on 3 occasions with at least 1 minute between measurements. The first measurement will be discarded, and an average of the subsequent two measurements will be recorded. Similar methodology for blood pressure measurement has been used in previous clinical trials in KTR[26]. Furthermore, patients with CKD have a higher prevalence of peripheral vascular disease and increased arterial stiffness as compared to the general population. As a result, the first SBP measurement may be less accurate as compared to subsequent measurements in patients with CKD[80]. Blood pressure will be measured at the following time points during the study: Day 0 (baseline), day 7 (1 week), day 42 (6 weeks), day 84 (12 weeks) and day 91 (13 weeks).

9.1.2 Secondary study parameters/endpoints

The secondary outcomes of this study include metabolic, vascular, renal and transplant-specific measures. These outcomes have been included to elucidate the potential mechanisms responsible for blood pressure lowering, and putative cardio- and renoprotective effects in KTR. Safety outcomes (described further below) will also be assessed. The time points and specific measurements of secondary outcomes are shown in **Table 2**.

Metabolic outcomes: Glycemic control will be measured using the following methods: Fasting blood glucose, HbA1c and home glucose monitoring. Home glucose monitoring will be performed in patients with T2D/PTDM during 2 discrete 14 day periods during the study using the participants' usual glucose monitoring device. These two discrete 14 day periods are as follows: 1) Starting 7 days prior to drug administration and stopping 7 days after drug administration (week -1 to 1); and 2) Starting 7 days prior to drug discontinuation, and stopping 7 days after during drug discontinuation (week 11 to 13). The rationale for home glucose monitoring is to study the impact of SGLT2i on blood glucose dynamics in this unique population with variable degrees of renal impairment, concomitantly treated with diabetogenic immunosuppressive medications such as glucocorticoids and CNIs. Although the impact of CNIs on the expression of SGLT2 in human kidney allografts is not known, changes to the proximal tubular epithelial cell have been well documented with use of CNIs in KTR[62]. Urinary glucose excretion will also be measured using a 24-hour urine collection for glucose. Lastly, height, weight, waist circumference and fasting lipid profile will also be measured.

Vascular outcomes: Arterial stiffness will be assessed by measuring right radial artery waveforms by high-fidelity micromanometer (SPC-301; Millar Instruments) and central aortic pressure waveforms (SphygmoCor, AtCor Medical Systems, Sydney, Australia) after a euglycemic clamp. Systemic arterial stiffness will be determined by the augmentation index (AIx), which is calculated as the difference between the second systolic peak and inflection point, expressed as a percentage of the central pulse pressure and corrected to an average heart rate of 75 beats/min. Aortic pulse wave velocity will be measured by sequentially recording ECG-gated right carotid and radial artery waveforms. Our group has extensively used this methodology in prior studies of participants with diabetes[81]. Sympathetic nervous system activity will be measured by heart rate variability using methods previously described by our group (SphygmoCor, Atcor Medical Systems Inc., Sydney, Australia). Cardiac output, stroke volume and total peripheral resistance will measured using the Non-Invasive Cardiac Output Monitoring (NICOM® system Cheetah Medical Inc.).

Renal outcomes: Renal function will be assessed using serum creatinine in order to calculate the estimated GFR (CKD-EPI equation). GFR will also be measured in the supine position using the gold-standard iohexol clearance technique, standardized per 1.73m^2 body surface area. Both eGFR and measured GFR are included in this study as the eGFR is the renal parameter that is utilized in

clinical practice, and provides information on what eGFR change could be expected with dapagliflozin therapy clinically. In addition, the eGFR is also a safety measure that is followed in clinical practice.

Markers of oxidative stress, including 8-hydroxydeoxyguanosine and 8-isoprostane will be measured in plasma and urine using an ELISA assay (Eve technologies, Calgary, Alberta). Albuminuria will be assessed by measuring urine albumin excretion from a 24-hour urine collection. Natriuresis will be assessed with a 24-hour urine collection for sodium excretion. In addition, fractional lithium excretion (FELi), fractional sodium excretion (FENa) and renal segmental tubular Na+/Li+ clearances will also be measured to study the SGLT2i-related natriuresis mechanisms and localization of sodium excretion in the renal tubule. This methodology has been described in detail elsewhere in our previous work[82, 83].

Transplant-specific outcomes: CNI levels will be measured during the trial. All results of CNI monitoring will be communicated within 24 hours to the primary transplant nephrologist, in the event that dose adjustments to CNIs are required. In light of the data in native kidneys suggesting that tubulointerstitial hypoxia plays an important role in the development of DKD[50], all participants will be offered the opportunity to undergo a renal ultrasound (photoacoustic ultrasonography) at baseline (between day -7 and 0) prior to drug administration, and at the end of the study (between day 77 and 84) prior to drug discontinuation. This novel methodology uses spectroscopic photoacoustics to assess kidney ischemic damage using non-invasive ultrasonography (Vevo System, FujiFilm, VisualSonics Inc., Toronto). This tool determines the degree of tissue hypoxia, as measured by oxygen saturation, and has been shown to correlate with areas of renal tissue ischemia[84, 85, 86]. In addition, the use of this methodology is ideal in KTR due to the anatomic proximity of the transplanted kidney, which is traditionally placed in the anterior abdomen (iliac fossa). This methodology has been previously used by Dr. Darren Yuen (co-applicant). Photoacoustic renal ultrasounds will be performed at SMH and incorporated into a study visit when possible, given the short distance (~ 2 kilometers) between TGH and SMH. This is an optional ultrasound for participants of the study.

Safety outcomes: The side effects of SGLT2i have been well described and will be assessed at each study visit. Adverse events include AKI, volume depletion, hyperkalemia, urinary and mycotic infections, ketoacidosis, amputations and allergic reactions. Episodes of biopsy-proven acute rejection (as defined by the Banff criteria), death-censored graft failure (defined as the need for initiation of chronic dialysis or re-transplantation) or death with graft function (defined as death with a functioning allograft) will also be collected. Kidney transplantation assures complete denervation of the transplanted kidney and the renal vasoconstrictive response in the setting of intravascular volume depletion is diminished in KTR. Therefore, frequent monitoring for adverse events has been integrated in our study design, occurring on 7 separate occasions, to capture these adverse events should they occur. Adverse event monitoring is shown in **Table 2** and will occur

in person on study day 7 (1 week), day 42 (6 weeks), day 77 (11 weeks), day 84 (12 weeks) and day 91 (1 week after drug discontinuation) and by telephone/e-mail on study day 21 (3 weeks) and day 63 (9 weeks). All adverse events will be recorded on case report forms.

9.2 Randomization, blinding and treatment allocation

The randomization scheme will be computer-generated by an independent biostatistician. To achieve a balance of participants in each arm, permuted block randomization will be used with variable blocks of 4 and 6. Randomization will be stratified based on T2D and PTDM status, as the effects of dapagliflozin may differ between these two groups. Participants will be randomized only after screening and informed consent has been obtained. All participants, nurses, physicians, study coordinators and investigators will be blinded to the treatment allocation. In addition, all participants, study personnel and study sponsor will be blinded until the end of the trial to all the laboratory and test results obtained during the trial. Dapagliflozin and placebo tablets, as well as drug packaging will be identical in appearance. Adherence to the intervention will be determined using pill counts and questioning of participants at each study visit[87]. Reasons for non-adherence and drug discontinuation will be ascertained.

9.3 Study procedures

Leading up to week 0, 1 and 12, participants will be placed on a 7-day diet targeting 150 mmol/day Na^+ and ≤1.5 g/kg/day protein, verified with a 24-hour urine sample to measure Na^+ and urea excretion prior to all experiments. This moderate, fixed dose Na^+ and moderate protein diet avoids the confounding effect of circulating volume contraction and renin-angiotensin-aldosterone system activation due to low Na^+ diets, and the renal hyperfiltration effect of high protein intake[88]. An electrocardiogram will also be performed at the screening visit. At 22:00 on the night prior to the study day, all participants will take 300 mg of lithium carbonate (Li^+CO_3) to derive renal segmental tubular Na+/Li+ clearances the following day, as described previously[82, 83]. Week 0, 1 and 12 studies will start at 0745 hours, and participants will fast for the duration of the day. All studies will be performed under clamped euglycemic conditions (capillary blood glucose 4 to 6 mmol/L) to avoid confounding effects of hyperglycemia on blood pressure and renal function. After clamped euglycemia is achieved for approximately 2-4 hours, blood samples will be collected for makers of oxidative stress (8-isoprostane and 8-hydroxydeoxyguanosine). Sympathetic nervous system activation will be assessed using heart rate variability[89, 90]. Following blood and urine tests, blood pressure will be assessed using an automated vital signs monitor (Connex Vital Signs Monitor, Welch Allyn) at 60-minute intervals. Baseline arterial stiffness (SphygmoCor, Atcor Medical Systems Inc., Sydney, Australia) will be measured non-invasively using applanation tonometry using standard techniques that we have used previously[72, 73]. Cardiac output, stroke volume and total peripheral resistance will be derived from stroke volume and heart rate that is measured using the Non-Invasive Cardiac Output Monitoring (NICOM® system Cheetah Medical Inc.) that we have also used previously[91]. GFR (per 1.73 m^2) will be measured in

the supine position using iohexol clearance[71]. Participants will then be randomized to dapagliflozin 10 mg PO daily or a placebo. Identical studies will be performed at study weeks 0, 1 and 12, with the exception of NICOM, which will be performed on study weeks 0 and 12 only. All procedures will be supervised by a study physician.

Serum, plasma and urine will be collected and stored on study weeks 0,1, and 12 for potential future analyses for exploratory biomarkers to assess relationships with disease activity and effects of study drug. After completion of the study, samples will be stored into a secure central storage facility at Toronto General Hospital. Those results may be pooled with biomarker data from other similar studies to generate hypotheses to be tested in future research. Potential biomarkers to be measured include but are not limited to electrolytes, natriuretic peptides, markers of inflammation/fibrosis, metabolomics and urinary vesicles.

9.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

9.5 Reasons for possible drug discontinuation

1. Serious adverse events or an intolerable adverse effect such as a persistent allergy or rash;
2. Clinically significant persistent changes from baseline based on laboratory safety assessments;
3. Pregnancy or breastfeeding;
4. Withdrawal of consent;
5. Participant unable to adequately complete study visits or procedures.

9.6 Replacement of individual subjects after withdrawal

Patients that meet inclusion criteria will be invited for participation in the study. When patient agrees to participate in the study, informed consent is signed. When patient decides to withdraw after start of the treatment period, new patients can be admitted to the study to reach the required sample size.

9.7 Follow-up of subjects withdrawn from treatment

Subjects who withdraw from the study will be followed up according to the routine terms of patient care at the outpatient clinic. Subjects will be followed up until resolution of the inciting adverse event responsible for drug withdrawal. If withdrawal was unrelated to an AE, then subjects will be followed up approximately 10 days after drug discontinuation for an end of treatment visit with

repeat bloodwork from the time of screening. In case of withdrawal of consent, participants will not be contacted nor followed by the study team.

9.8 Premature termination of the study

There are no predefined criteria for premature termination of the study. If, however, during the conductance of the study information becomes available showing that continuation of the study would result in a significant safety risk for the patients, the principal investigator and project leader will decide to terminate the study.

10. SAFETY REPORTING

10.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited medical ethics committee if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited medical ethics committee, except insofar as suspension would jeopardize the subjects' health.

The investigator will take care that all subjects are kept informed.

10.2 AEs and SAEs

10.2.1 Adverse events (AEs)

An adverse event is any untoward medical occurrence in a patient administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product. (ICH E2D).

10.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- results in death;
- is life threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe;
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;

- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

AEs will be recorded in the electronic case report form (eCRF).

10.2.3 Adverse events of interest

Urinary tract infections

Genital infections

Diabetic ketoacidosis (DKA)

Volume depletion
(Eg, dehydration, hypovolemia, or hypotension)

Fractures

Major hypoglycaemic event
A major hypoglycaemic event is defined as an event that requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the corrective actions is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

Acute Kidney Injury
Acute kidney injury is defined as an increase of 40% in serum creatinine from the previous visit or hospitalization due to acute kidney injury.

Amputation:
Amputations will be recorded in the eCRF. We will report the underlying cause of amputation as the AE and include the information regarding the actual amputation in the AE description rather than reporting the amputation procedure as a separate AE.

10.3 Recording of Adverse Events

- *Time Period for Collection of Adverse Events*

  For this study, SAEs, discontinuations due to AEs (DAEs), AEs of special interest and other AEs will be collected from the time of signing the informed consent and throughout the study, including the run-in and wash-out periods. Information about all adverse events (serious or not) will be recorded in source documents according to good clinical practice, and retained at the investigative sites.

- *Follow-up of Unresolved Adverse Events*

  Any SAEs, DAEs, AE of special interest, or other AEs will be followed by the investigator until resolution or until a clinically stable outcome is reached, or until further follow-up is no longer considered by the investigator to provide clinically meaningful information.

- *Variables*

  The following variables will be collected for each SAE/DAE/AE:
  AE (verbatim); The date and time when the AE started and stopped; Intensity; Whether the AE is serious or not; Investigator causality rating against the study drug (yes or no).
  Action taken with regard to study drug; AE caused patient's withdrawal from study (yes or no); Outcome.

  In addition, the following variables will be collected for SAEs:
  Date AE met criteria for a serious AE; Date Investigator became aware of the SAE.
  SAE Variables to be collected:

  i. Date of hospitalisation (if applicable).
  ii. Date of discharge (if applicable).
  iii. Probable cause of death (if applicable).
  iv. Date of death (if applicable).
  v. Autopsy performed (if applicable).
  vi. Causality assessment in relation to Study procedure(s).
  vii. Causality assessment in relation to other medication (e.g., concomitant medication, background therapy).

  Description of intensity

  The intensity of the reported SAEs/DAEs/AE will be assessed according to the following scale:
  - Mild (awareness of sign or symptom, but easily tolerated).
  - Moderate (discomfort sufficient to cause interference with normal activities).
  - Severe (incapacitating, with inability to perform normal activities).

  It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria above. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered

severe nausea, but not a SAE unless it meets the criteria shown in above. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE when it satisfies the above criteria.

- *Causality Collection*
  The Investigator will assess causal relationship between Investigational Product and each SAE/DAE, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

- *Adverse Events Based on Signs and Symptoms*
  All SAEs/DAEs/AE spontaneously reported by the patient or reported in response to the open question from the study personnel: 'Have you had any health problems since the previous visit/you were last asked?' or revealed by observation will be collected and recorded in the eCRF. When collecting SAEs/DAEs/AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

- *Adverse Events Based on Examinations and Tests*
  The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, or vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria, are the reason for discontinuation of study treatment, or fulfil criteria of AEs of special interest.
  If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an SAE/DAE or AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (e.g., anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).
  Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

10.4 Serious Adverse Event Collecting and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs will be collected that occur during the screening period and within 30 days of discontinuing dosing. If applicable, SAEs must be collected that relate to any later protocol-specific procedure (such as follow-up skin biopsy).

If an SAE occurs after the 30 days of drug discontinuation, it should be reported. If an SAE occurs after the occurrence of a protocol-specific procedure, it should also be reported. If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form. SAEs that result in death or are life threatening should be reported expedited to the competent authorities. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report. Concurrently, the sponsor's principal investigator and project leader will report SAEs accompanied by a cover page mentioning the study code, patient number, country, investigator, seriousness to AstraZeneca via the product safety e-mail box AEMailboxClinicalTrialTCS@AstraZeneca.com within the same timelines.

10.5 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious;
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorised medicinal product;
    - Investigator's Brochure for an unauthorised medicinal product.

Health Authority and METC Reporting

SUSARs will be reported to the accredited ethics committee and recorded in an overview list. When needed, they will be also reported expedited to the appropriate health authority (Health Canada).

In an emergency, unblinding can be performed to identify the treatment given to that subject. Unblinding is not to be performed for any reason, other than an emergency where unblinding is required. When the Investigator unblinds the subject he/she must note the date, time and reason for removing it and record this information in the Comments section of the CRF and in the source data. He/she must also immediately inform the project leader/principal investigator about the unblinding of the subject treatment. Even though the subject has been unblinded, any blood samples for safety or pharmacodynamic assessments will continue to be drawn, for at least 24hr following the last dose as long as doing so will not compromise subject welfare. It is the responsibility of the Principal Investigator to ensure the investigator site staff are appropriately

trained on the unblinding procedure in case of emergency. Study drug must be discontinued after unblinding but the subject will be followed until resolution of the adverse event. It is the intent that subjects who discontinue treatment with the study drug will continue in the study according to the visit schedule. At the conclusion of the study, the occurrence of any unblinding can be reported.

10.6 Pregnancy

All pregnancies and outcomes of pregnancy will be reported to the sponsor and AstraZeneca, after obtaining separate consent from the study participant/participant partner. If a patient becomes pregnant during the course of the study IP should be discontinued immediately. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IP under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy) should be reported. If any pregnancy occurs in the course of the study, then the Investigator or other site personnel informs the sponsor's representatives within 1 day of when he/she became aware of it.

10.7 Overdose

Dapagliflozin has been well tolerated at doses of up to 500 mg/day in single dose testing in healthy volunteers and up to 100 mg/day in repeat dose testing for 14 days in healthy volunteers and patients with T2D. Overdose defined as at least twice the intended dose within the therapeutic interval, whether accidental or intentional, suspected by the Investigator or spontaneously notified by the patient, will be recorded in the eCRF overdose module. If the overdose is symptomatic, it will also be recorded as an adverse event. If an overdose is suspected, monitoring of vital functions as well as treatment should be performed as appropriate.

An overdose with associated AEs is recorded as the AE diagnosis/symptoms on the relevant AE modules in the eCRF and on the Overdose eCRF module. An overdose without associated symptoms is only reported on the Overdose eCRF module.

10.8 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States. This safety report consists of:

- a list of all suspected (unexpected or expected) adverse reactions and serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;

- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation. All safety reports should be simultaneously communicated to Astra Zeneca.

11. STATISTICAL ANALYSIS

The analytical plan mirrors that operationalized in previous work[92, 93]. Data will be analyzed using Stata/SE 15. The primary outcome is the difference in blood pressure reduction in dapagliflozin vs. placebo treated participants at 12 weeks. Secondary outcomes will consist of within and between group changes metabolic, vascular, renal and transplant outcomes. Frequency of adverse events, non-adherence and drug discontinuation will be tabulated (no comparisons will be made). Within group comparisons will be analyzed using repeated measures ANOVA (baseline value will be included in these ANOVA models). As in previous work, between group comparisons will also be made using a 2-way ANOVA with treatment as the dependent variable[71, 92, 94-96]. This clinical trial is not sufficiently powered to detect sex- or gender-based differences. However, data analyses will be stratified by sex and gender to detect any similarities or differences. These will be exploratory analyses and hypothesis generating, which may be informative in the design of future studies. Other exploratory analyses will include stratification by GFR (measured iohexol GFR of <45 ml/min/1.73m^2 vs. >45 ml/min/1.73m^2), as well as diabetes type (T2D and PTDM). An intention-to-treat (ITT) approach will be used. Adherence will be measured (see '**Randomization**' in the proposal), and non-adherent patients will also be included in the ITT analysis.

12. ETHICAL CONSIDERATIONS

12.1 Regulation statement

The trial will be carried out in compliance with the protocol, the ethical principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonized Tripartite Guideline for GCP, relevant Standard Operating Procedures (SOPs), and relevant regulations.

Standard medical care (prophylactic, diagnostic and therapeutic procedures) remains in the responsibility of the treating physician of the patient.

12.2 Recruitment and consent

Patients will be enrolled to the trial from our center in Canada. Identification of patients eligible for trial participation will occur at two sites, Toronto General Hospital (TGH) and St. Michael's Hospital (SMH) in Toronto, Ontario. Both sites have large, well-established kidney transplant programs (total of 300 to 350 kidney transplants per year and 4000 to 5000 prevalent KTR across both centres). After identification of a patient potentially eligible for the trial, all screening activities and informed consent will take place at TGH. Prior to their visit to the outpatient clinic,

patients will be invited to participate in the study by verbal invitation when they attend the clinic or by sending an invitation letter. In this letter, patients will find a full explanation of the study, advantages and disadvantages of participating, and contact information of the research team members working on this study. Moreover, the letter contains contact information of an independent physician, to whom subjects can address questions about the research before, during and after a study. The patients will be provided with as much time as required to consider their decision and will also be provided with an informed consent form. Participants will be required to sign their written informed consent before they take part in the study.

12.3 Objection by minors or inanticipated subjects (if applicable)

No minors or incapacitated adults will be included in this study.

12.4 Benefits and risks assessment, group relatedness

There are no direct benefits for the patients to be included. Participation in the study is on a free-will base. Patients will receive restitution of all costs of transportation. Patients will not receive priority for treatment of other diseases in the clinic during this study. Participation in the proposed study is accompanied with only minor risks. The blood samples will be drawn by means of venipuncture that will be performed during the visit to the outpatient clinic. All further performed measurements are non-invasive and therefore only minor risks are associated with participation.

12.5 Compensation for injury

The sponsor has an insurance which is in accordance with the legal requirements in Canada. This insurance provides cover for damage to research subjects through injury or death caused by the study.

12.6 Incentives (if applicable)

Participation of patients in the study is a free-will decision. Patients will receive restitution of all costs for transportation for participating. Patients do not receive priority for treatment of other diseases in the clinic during this clinical trial.

13. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

13.1 Handling and storage of data and documents

A subject identification code list will be made to link the data to the subject in order to be able to trace data to an individual subject. This code will not be based on the patient initials and birth-

date. The key to the code will be safeguarded by the investigator since the data will be kept for a period of 25 years. The handling of personal data will comply with privacy laws, legislation, codes and/or guidelines that apply in the applicable jurisdictions the study is conducted.

13.2 Monitoring and Quality Assurance

The investigator will permit trial-related monitoring, audits, IRB/IEC review, and regulator inspection(s), providing direct access to source data/documents.

13.3 Amendments

A 'substantial amendment' is defined as an amendment to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the local competent authority.
Non-substantial amendments will be reported to Health Canada as a CTA-Notification, or to the local competent health authority as appropriate as per the jurisdiction.

Non-substantial amendments will not be notified to the competent authority, but will be recorded and filed by the sponsor.

13.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the REB as part of the annual renewal process. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

13.5 Public disclosure and publication policy

The study will be registered at clinicaltrials.gov or equivalent trial registries. Publication policy is in agreement with international regulations. Nor the sponsors, nor the principal investigator has a right of veto regarding the way of publishing the results.

13.6 Data Safety Monitoring Board (DSMB)/Safety Committee

The institution of a DSMB is not a prerequisite to improve the safety of the patients as the safety profile of dapagliflozin has been well characterized and investigated in completed clinical trials that involved >6000 patients with type 2 diabetes. Dapagliflozin is registered for clinical use in Europe.

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15. Tables and Figures.

Figure 1: Effects of SGLT2 inhibition on the nephron.
Figure reproduced from: van Bommel EJ. Et al. SGLT2 Inhibition in the Diabetic Kidney-From Mechanisms to Clinical Outcome. *Clin J Am Soc Nephrol*. 2017;12:700-710.

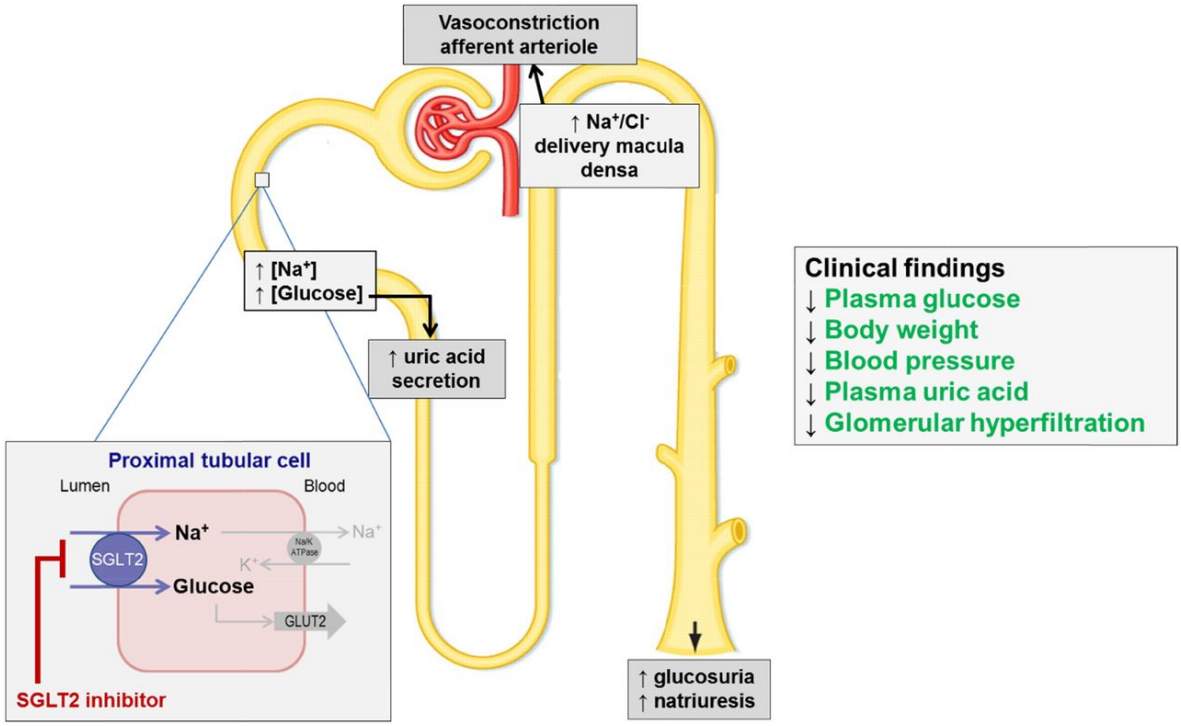

Figure 2: Potential cardio- and renoprotective mechanisms of SGLT2 inhibition.
Figure reproduced from: Heerspink HJ. et al. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes Mellitus: Cardiovascular and Kidney Effects, Potential Mechanisms, and Clinical Applications. *Circulation*. 2016;134:752-72.

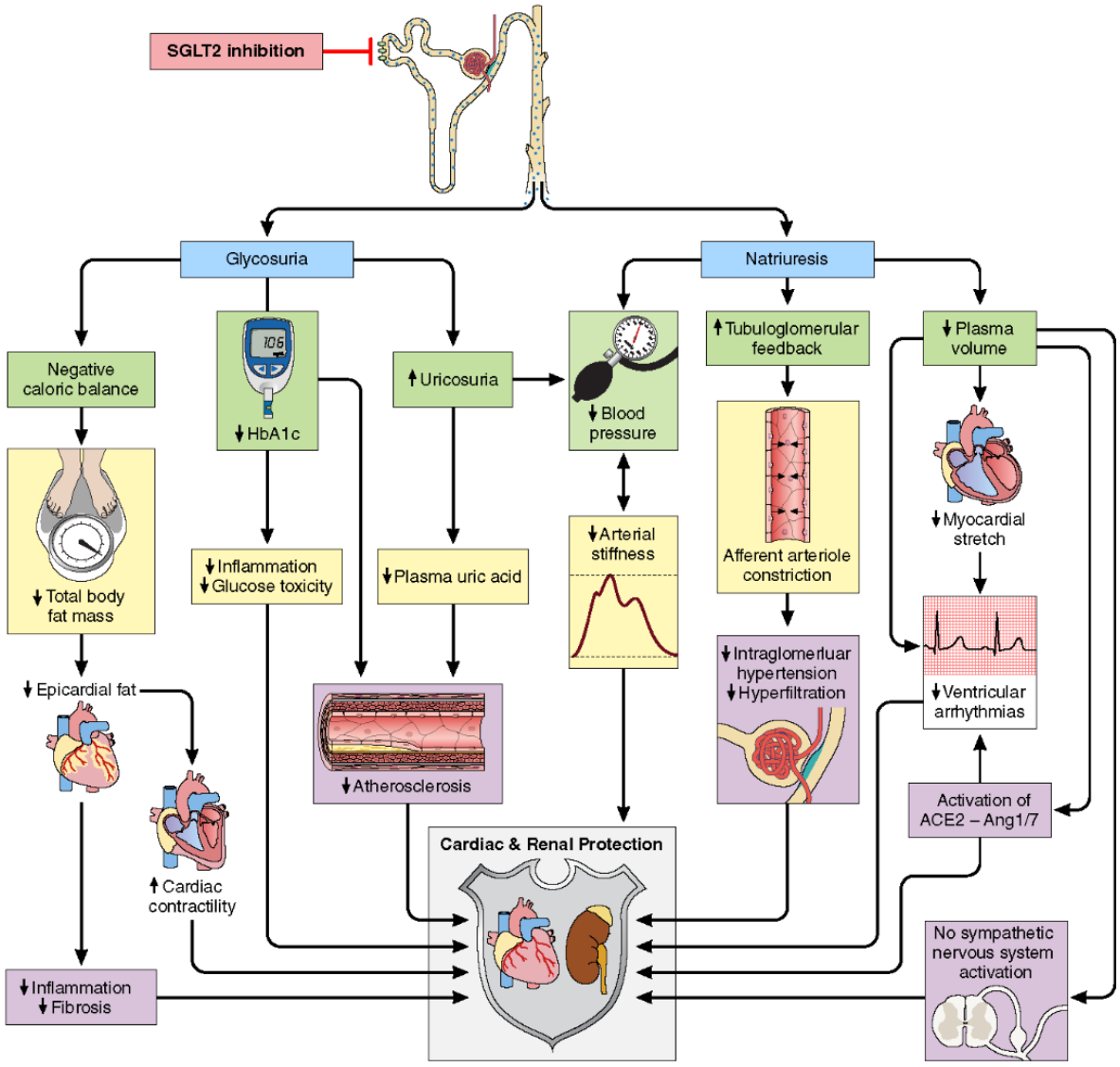

Figure 3: Study design

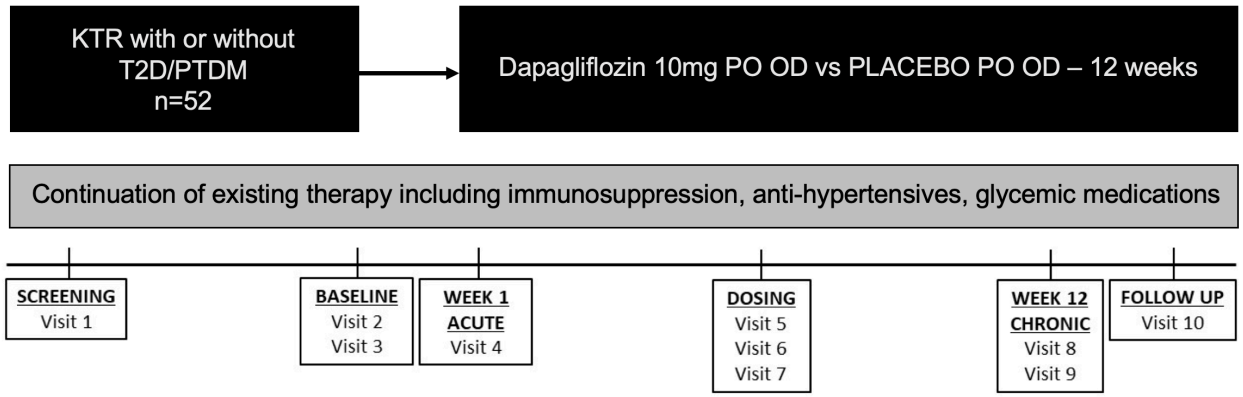

Table 1: Summary of studies of SGLT2i in kidney transplant recipients.

Observational studies				
Author, Year	**Participants**	**Design**	**Treatment**	**Results**
Rajasekeran et al. *Diabetes Care* 2017[1]	N=10 KTR & SPKTR with PTDM or T2D	Observational cohort study, 80.5 person-months follow-up	canagliflozin	*Weight*, kg: -2.14 (SD 2.8) *Systolic blood pressure*, mmHg: -6.5 (SD 10.8) *Diastolic blood pressure*, mmHg: -4.8 (SD 12) *Serum creatinine*, mmol/L: 9.7 (SD 14.6); eGFR, mL/min/1.73^2 : -4.3 (SD 12.2) No urinary/mycotic infections, AKI or acute rejection
Mahling et al. *Kidney Blood Press Res* 2019[2]	N=10 KTR with PTDM or T2D	Observational study, 6.3 person-years	empagliflozin	*Weight*, kg: -1.9 (-1.9 to 0.1) *Systolic blood pressure*, mmHg: -2.5 (-36.3 to 0.8) *Diastolic blood pressure*, mmHg: -0.5 (-9.5 to 7.5) 2 episodes of UTI, 1 episode of AKI
Alkindi et al. *Transplant Proc* 2020[3]	N=8 KTR with PTDM or T2D	Retrospective chart review	empagliflozin (6 patients), dapagliflozin (2 patients)	*BMI,* kg/m^2, 32.7±7.2 to 27.4±4.2 after 12 months ($p<0.05$) *Systolic blood pressure*, mmHg: 135±9.6 to 126.4±11.5 after 12 months ($p>0.05$) *Diastolic blood pressure*, mmHg: 80.6±10 to 74.5±7.3 after 12 months ($p>0.05$) *eGFR,* no significant change 1 episode of UTI, no AKI, ketoacidosis, acute rejection
Schwaiger et al. *AJT* 2018[4]	N=14 KTR with PTDM, 6 months post-transplant, eGFR ≥ 30ml/min/1.73m^2, stable on insulin therapy	Open-label, single-arm, non-inferiority study Single-centre study	Treatment with empagliflozin 10mg after discontinuation of insulin therapy, 4 weeks	*Change in baseline to 4 week OGTT* - fasting and 2-hour glucose levels increased to 144 ± 45 mg/dL (P = .005) and 273 ± 116 mg/dL (P = .06), respectively *Avg body weight*: -1.6kg; *Bioimpedance volume status*: fluid overload decreased from 2.7 ± 2.1 (baseline) to 1.8 ± 1.8L (P = .006) 5 episodes of urinary tract infection
Randomized Control Trial				
Author, Year	**Participants**	**Design**	**Treatment**	**Results**

Halden et al. *Diabetes Care*[5]	N=49 KTR with PTDM, 1 year post transplant, eGFR ≥30m/min/1.73m^2	Double-blind RCT Single centre study	1:1 empagliflozin 10mg vs. placebo, 24 weeks	**Secondary Outcomes** *Change in median HbA1c* (%): -0.2 (-0.6 to -0.1) with empagliflozin vs +0.1 (-0.1 to 0.4); p=0.025 *Body Weight*: -2.5 kg (-4.0 to -0.05) with empagliflozin vs +1.0 kg (0.0 to 2.0); p=0.014 *24hr blood pressure* – no difference in blood pressure between groups *Safety Outcomes* – no significant between group differences

KTR: Kidney transplant recipients; SPKTR: Simultaneous pancreas-kidney transplant recipients; T2D: Type 2 diabetes; PTDM: Post-transplant diabetes mellitus; eGFR: Estimated glomerular filtration rate; SD: Standard deviation. OGTT: Oral Glucose Tolerance Test

1. Rajasekeran H, Kim SJ, Cardella CJ, et al. Use of canagliflozin in kidney transplant recipients for the treatment of type 2 diabetes: A case series. *Diabetes Care.* 2017;40(7): e75-e76.
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Table 2: Study activities

	Screening	**Baseline**		**Acute 1 week Drug/Placebo**	**Dosing**			**Chronic 12 week Drug/Placebo**		**Follow-up**
Visit*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10/EEOS**
Week	**-2**	**-1**	**0**	**1**	**3**	**6**	**9**	**11**	**12**	**13**
Day	**-14**	**-7**	**0**	**7**	**21**	**42**	**63**	**77**	**82**	**91**
Clinic Visit	**X**		**X**	**X**		**X**			**X**	**X**
Phone/email visit		**X**			**X**		**X**	**X**		
Fasting visit			**X**	**X**					**X**	
Demographics, medical Hx & ECG	X									
Physical exam	X		X	X		X			X	X
BMI and waist measurement	X		X	X		X			X	
Pregnancy test	X		X	X					X	
Start modified diet[a]		X	Until V4	X				X		
Modified euglycemic clamp			X	X					X	
Randomization			X							
Take study medication				X	X	X	X	X	X	
Phone call					X		X			
Adverse events query				X	X	X	X	X	X	X
Metabolic parameters										
Start HGM[b]		X						X		
Stop HGM[b]				X						X
Vascular parameters										
Vital signs	X		X	X		X			X	X
Arterial stiffness			X	X					X	
NICOM			X						X	
Renal parameters										
Renal ultrasound[c]			X						X	
Lithium tablet intake[c]			X	X					X	
Blood and urine collection										
24 hour urine collection			X	X					X	
Blood and urine samples	X		X	X		X			X	X
Biobanking samples			X	X					X	
Reactive oxygen species collection			X						X	

[a] The diet includes a target of 140 mmol of sodium per day to avoid salt depletion and consequence activation of the renin-angiotensin-aldosterone system which could confound sodium handling and neurohormonal assessments. In addition, we target a moderate protein diet of approximately 1.5gram/kg/day to avoid the confounding effect of high protein diets on GFR (induces

hyperfiltration). This has also been part of previous mechanistic studies in the Cherney Laboratory in patients with diabetes. [b]In patients with T2D/PTDM [c] Renal ultrasound will be performed between visits 2 and 3 and between visits 8 and 9. [c] Lithium tablet will be taken at 10pm the night before Visits 3,4 and 9. *Study visits are subject to PI's discretion based on participants' condition/safety/lab results. HGM; home glucose monitoring; NICOM; Non-invasive cardiac output monitoring; EEOS; early end of study

Table 3: Study variables included in data collection

Category	Demographic & medical history	Imaging	Physical examination	Adverse events	Laboratory (Blood)	Laboratory (Urine)
Variables	Date of birth, age, race/ethnicity, sex, gender, cause of ERSD, modality and duration of RRT prior to transplant, date of transplant, type of transplant, induction immuno-suppression, maintenance immuno-suppression, delayed graft function, acute rejection (date, type and treatment), history of urinary tract infection or mycotic infection (date, organism, and treatment), medication names, doses, allergy history	Tissue hypoxia ultrasound of allograft	Blood pressure, respiratory rate, heart rate, temperature, height, weight, abdominal circum-ference	Hypotension, volume depletion, Hyper-kalemia, acute kidney injury, urinary infections, mycotic infections, amputation, allergic reaction, hypo-glycaemia, ketoacidosis	Fasting blood glucose, flash glucose monitoring, hemoglobin A1C, complete blood count, sodium, potassium, chloride, bicar-bonate, calcium, phosphate, albumin ALT, ALP, AST, total bilirubin, amylase, ketones, urea, creatinine, fasting cholesterol profile, CNI level, beta-HCG (if applicable), neurohomone – Aldosterone, Renin, Ang II, ANP, and catecholamines	24 hour and/or spot urine collection for sodium, potassium, protein, urate, urea, creatinine, glucose, albumin, fractional lithium excretion (FE Li), fractional sodium excretion (FE Na)

					Biomarker biobanking	
			Heart rate variability	Death-censored graft failure, death with graft function, biopsy-proven acute rejection	GFR (iohexol), 8-hydroxy-deoxyguanosine, 8-isoprostane	8-hydroxy-deoxyguanosine, 8-isoprostane, urine adenosine, biomarker biobanking
			Arterial stiff-ness, aortic pulse wave velocity, cardiac output, stroke volume, total peripheral resistance			

ESRD: End-stage renal disease; RRT: Renal replacement therapy; GFR: Glomerular filtration rate.