ERtugliflozin triAl in DIabetes with preserved or reduced ejeCtion FrAcTion mEchanistic evaluation in Heart Failure: "ERADICATE-HF"

EudraCT 2017-001840-37

PROTOCOL TITLE: ERtugliflozin triAl in DIabetes with preserved or reduced ejeCtion FrAcTion mEchanistic evaluation in Heart Failure: "ERADICATE-HF"

Protocol ID **EUDRA-CT**	**XXXXXXXXXXX** 2017-001840-37
Short title	**Effect of ertugliflozin in heart failure**
EudraCT number	2017-001090-16
Version	**6.0**
Date	**July 9, 2018**
Coordinating investigator/project leader	Prof. D. Cherney Division of Nephrology University Health Network, University of Toronto Toronto Tel: 416 340 4151 E-mail: david.cherney@uhn.ca
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	Prof. D. Cherney Division of Nephrology University Health Network, University of Toronto Toronto Tel: 416 340 4151 E-mail: david.cherney@uhn.ca Prof. H.J. Lambers Heerspink Department of Clinical Pharmacology University Medical Center Groningen Groningen Tel: 050-3617859 E-mail: h.j.lambers.heerspink@umcg.nl
Sponsor	University Health Network ***Toronto, Ontario, Canada***
Subsidising party	Merck (investigator initiated study)

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
[Coordinating Investigator/Project leader/Principal Investigator]: Dr. David Cherney Division of Nephrology University Health Network, University of Toronto Canada Dr. Hiddo Lambers Heerspink Department of Clinical Pharmacology UMCG, Groningen, Netherlands		
[Coordinating Investigator/Project leader/Principal Investigator]: Dr. David Cherney Division of Nephrology University Health Network, University of Toronto Canada Daniel Raalte University of Amsterdam Amsterdam, Netherlands		

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
ACEi	**Angiotensin Converting Enzyme Inhibitor**
AE	**Adverse Event**
AR	**Adverse Reaction**
ARB	**Angiotensin Receptor Blocker**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
(S)AE	**(Serious) Adverse Event**
SGLT2	**Sodium Glucose Co-Transporter 2**
SGLT2i	**Sodium Glucose Co-Transporter 2 Inhibitor**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale:

Type 2 diabetes (T2D) is an epidemic that afflicts more than 350 million people world-wide. In addition to difficulties managing hyperglycemia, patients with T2D have an increased risk of heart failure (HF). Despite the use of existing medical therapies, T2D continues to cause significant morbidity and mortality, leading to large societal and financial costs to Canadians.

Newer agents called sodium glucose co-transporter-2 inhibitors (SGLT2i) have been developed to improve glycemic control and lower hemoglobin A1c by increasing glycosuria[1]. SGLT2i also reduce blood pressure[2,3] and albuminuria[4] in T2D – possibly through natriuresis [4a, 4b]. Importantly, a landmark trial "EMPA-REG OUTCOME" demonstrated that the SGLT2i "empagliflozin" is the first anti- hyperglycemic agent to reduce mortality and HF risk[5], and also to decrease the risk of progressive diabetic nephropathy. Similar benefits were also recently reported in the CANVAS Program trial with canagliflozin[6]. Despite the benefits observed in these two pivotal trials, the mechanisms responsible for beneficial effects of SGLT2i in patients with T2D with respect to the development and/or worsening of HF are not currently known.

In light of the results of EMPA-REG OUTCOME, we aim to elucidate the mechanisms whereby the SGLT2i "ertugliflozin" modifies cardiorenal interactions that regulate fluid volume and neurohormonal activation in patients with T2D and HF (T2D-HF). We will test the hypothesis that ertugliflozin increases proximal tubular natriuresis, thereby reducing plasma volume, without inducing significant renal vasoconstriction or activation of the sympathetic nervous system (SNS)[7] (see below, Figure 1). The systematic understanding of the effects of SGLT2i in the setting of HF will enable the design of rational physiology based strategies to decrease the burden of HF, which could have major clinical and research implications internationally.

Objective:

Primary:

1. Our primary goal is to determine if SGLT2i causes a persistent proximal renal tubular natriuretic effect (see below). We will capture acute (1 week) and chronic (12 weeks) responses, since physiological effects of SGLT2i agents may change over time[2,3,8]. As an extension of our primary aim, we will assess whether ertugliflozin-related effects on proximal tubule natriuresis lead to a reduction in plasma volume and extracellular body water (see below)[7].

   Secondary:

2. We will determine if volume contraction leads to a decline in hormones that are activated in HF patients such as B-type natriuretic peptides (BNP), without activating the SNS[7];
3. We will determine the impact of ertugliflozin on: renal function measured at 1 week[2,3,5] and 12 weeks as a measure of safety;
4. Blood pressure, echocardiographic measures of cardiac output (and derived systemic vascular resistance) arterial stiffness and systemic vascular resistance to better understand the blood pressure lowering effect in this patient population;
5. Heart rate and heart rate variability, to assess effects on SNS activation;
6. Urinary natriuretic modulators, such as angiotensin converting enzyme-2[9] and adenosine[10];
7. To characterize the safety of ertugliflozin vs. placebo by determining the number of hypoglycemic episodes between groups, and serious adverse events.

Study design:
This study will use a double blind, stratified randomization trial approach involving 36 T2D-HF patients taking standard HF therapies.

Study population: Male and female subjects with heart failure and T2D, as described below.

Intervention: Patients will be randomized to 15 mg tablet PO ertugliflozin daily or a matched

placebo. **Main study parameters/endpoints:** Change in natriuresis

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients visit the outpatient clinic on a more regular basis than standard patient care - i.e. at study inclusion and at each study visit for clinical assessment. Blood work for physiological assessments, renal function tests and cardiovascular assessments will be obtained as described in the protocol. 24hr urine will be collected one day prior to the hospital visit. No other invasive measurements will be executed. Patients receive restitution of all travel costs. Patients receive no priority in treatment of other diseases in the clinic during this study. There are no direct benefits for the patients to be included and participation is on a voluntary basis.

INTRODUCTION AND RATIONALE

Patients with T2D-HF have >50% 5-year mortality, leading to substantial societal and financial costs to Canadians. *The identification of new therapies is essential to improve the quality of life and survival of T2D-HF patients.* In EMPA-REG OUTCOME there was a significant decrease in the rate of hospitalization for HF after therapy for only 3 months. Despite this beneficial effect, the impact of SGLT2i in patients with overt HF remains unknown. The current gap in knowledge around SGLT2i effects in patients with existing HF highlights the urgent need for human mechanistic studies in this area. The renal and cardiovascular function effects of SGLT2i on natriuresis-related endpoints in patients with T2D and HF is not known. Our study will provide needed insights into physiological effects of SGLT2i agents on natriuresis, hemodynamic function and neurohormones in HF patients.

Current Management Strategies in Patients with HF: HF is characterized by activation of the SNS and renin angiotensin aldosterone system (RAAS) as counter-regulatory responses to maintain blood pressure and preserve renal function. Treatment of HF patients with reduced left ventricular ejection fraction (HFrEF, ejection fraction <45%) includes β-blockers and RAAS inhibitors, diuretics and digoxin to suppress neurohormones, reduce volume overload and improve cardiac contractility[11]. In HFrEF, RAAS inhibitors reduce hospitalizations and mortality[11]. In contrast, these therapies have failed to produce beneficial effects in patients with HF and *preserved* EF ("HFpEF", ejection fraction ≥45%), which is a common type of HF in T2D[11]. Accordingly, HFpEF management focuses on the treatment of co-morbidities (hypertension, coronary disease, obesity, hyperglycemia) [12]. Many of the co-morbidities associated with HFpEF can be ameliorated with SGLT2i, making them attractive therapies for HFpEF[5,13-15]. In addition to uncertain benefit, current therapies for HFpEF have clear potential to cause side effects, such as hyperkalemia, hypotension and acute kidney injury. In contrast, in EMPA-REG OUTCOME, SGLT2i reduced mortality and HF risk without increasing any of these adverse events[5]. Interestingly, SGLT2i with empagliflozin also reduced the need for loop diuretics, which could help preserve renal function and avoid side effects[16]. Empagliflozin also has a surprising neutral effect on the SNS, despite an expected contraction of plasma volume[17,18]. This has been interpreted to reflect a possible "suppressive" effect on the SNS[17-19]. Importantly, although the proportion of patients with HFrEF vs. HFpEF in EMPA-REG OUTCOME or the CANVAS Program is not known, based on the clinical characteristics of the cohort it is likely that both subtypes had large representation in the trial. Based on a strong physiological rationale, the likely proportion of HFpEF patients in EMPA-REG OUTCOME and the CANVAS Program and a large unmet clinical need in T2D-HFpEF patients, in the current study we will include patients with this physiological profile to examine the effect of SGLT2i on natriuresis, renal and systemic hemodynamic and neurohormonal function. In addition, due to similar expectations around the high prevalence of HFrEF in EMPA-REG OUTCOME and the CANVAS Program, a large unmet clinical need in these patients, and the expectation that individuals with HFrEF and volume expansion will respond to SGLT2 inhibition with a significant natriuresis, we will also include HFrEF patients with EF ≥20% in ERADICATE-HF. We anticipate that that both HFrEF and HFpEF will exhibit an expected natriuretic response and similar directional changes in renal and systemic hemodynamic parameters. Nevertheless, to maximize homogeneity in the ertugliflozin and placebo-treated groups, participants will undergo stratified randomization to assure equal numbers of HFrEF and HFpEF phenotypes in the two groups.

SGLT2i reduces plasma glucose and HbA1c in patients with T2D by increasing urinary glucose excretion. SGLT2i are oral anti-hyperglycemic agents that decrease HbA1c through insulin-independent effects via glycosuria. They therefore have negligible hypoglycemic risk as compared to other classes of anti-hyperglycemic agents, since the degree of glucose lowering depends on the filtered load of glucose, which, in non-diabetic patients, is very low [20,21]. Beneficial effects of SGLT2i beyond glycemic control in patients with T2D have been suggested by the EMPA-REG OUTCOME and CANVAS Program trials, in which SGLT2i treatment added to standard care significantly reduced the composite primary endpoint (i.e. death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke) and also reduced the risk of being hospitalized for heart failure or developing progressive diabetic nephropathy, compared to participants allocated to the placebo group [22,23]. In these trials, dramatic cardiovascular protective effects were reported, even though HbA1c reductions were very modest at the end of the study. The renal outcome data were equally impressive and demonstrate that individuals treated with an SGLT2i had >35% reduced risk of new onset of nephropathy[23,24]. In light of modest glucose-lowering effects in the previous cardiovascular endpoint trials, renal and cardiovascular benefits have been attributed to natriuretic properties of the class, leading to hemodynamic effects in both the renal and systemic vascular circulations.

In the renal microcirculation, SGLT2i “normalizes” tubuloglomerular feedback mechanisms seen in the hyperfiltration stage of diabetic nephropathy[25]. Furthermore, effects of SGLT2i at the afferent arteriole likely account for the acute “dip” in eGFR observed in patients with and without impaired renal function, which likely reflects a reduction in intraglomerular hypertension at the single nephron level across the spectrum of renal function. It is believed that natriuresis leading to increased tubuloglomerular feedback at least partially mediates the beneficial effects of SGLT2i via this glucose-independent hemodynamic mechanism.

To reduce intraglomerular hypertension, current guidelines recommend the use of RAAS blockers as the gold standard therapy for individuals with diabetic nephropathy or in patients with heart failure [26]. However, despite the use of these agents, renal function continues to decline – a scenario that can be worsened in the presence of combined cardiac and renal disease. Therefore, there is a need to widen the therapeutic armamentarium for individuals with diabetes and cardiorenal disease nephropathy. SGLT2i lowers the threshold of proximal glucose reabsorption resulting in glycosuria, and increases distal tubular sodium delivery to macula densa cells. Macula densa cells are specialized epithelial cells located at the distal convoluted tubule that sense sodium delivery and mediate afferent arteriolar vasoconstriction in response to an increase in tubular sodium. SGLT2 inhibition increases the delivery of glucose and sodium in the distal tubule and to the juxtaglomerular apparatus, which is sensed as an increase in glomerular perfusion. This leads to a tubuloglomerular feedback signal causing afferent arteriolar vasoconstriction, and an acute fall in intraglomerular pressure. These effects are clinically manifested as acute reductions in GFR and hence albuminuria, followed by renal function preservation in the longer term – as recently reported in EMPA-REG OUTCOME and the CANVAS Program. Furthermore, natriuresis diminishes extracellular plasma volume and blood pressure. Thus, SGLT2i specifically alters renal hemodynamic function by reducing intraglomerular pressure, which could be expected to translate into improved long-term kidney outcomes.

In light of the results of EMPA-REG OUTCOME and CANVAS Program, we aim to elucidate the mechanisms whereby the SGLT2i “ertugliflozin” modifies cardiorenal interactions that regulate fluid volume and neurohormonal activation in patients with T2D and HF (T2D-HF). We will test the hypothesis that ertugliflozin increases proximal tubular natriuresis, thereby reducing plasma volume, without inducing significant renal vasoconstriction or activation of the sympathetic nervous system (SNS)[7] (see below, Figure 1). The systematic understanding of the effects of SGLT2i in the setting of HF will enable the design of rational physiology based strategies to decrease the burden of HF, which could have major clinical and research implications internationally.

1. OBJECTIVES

Primary:

1. Our primary goal is to determine if SGLT2i causes a persistent proximal renal tubular natriuretic effect (see below). We will capture acute (1 week) and chronic (12 weeks) responses, since physiological effects of SGLT2i agents may change over time[2,3,8]. As an extension of our primary aim, we will assess whether ertugliflozin-related effects on proximal tubule natriuresis lead to a reduction in plasma volume and extracellular body water (see below)[7]. FE_{Na} and FE_{Li} excretion were calculated according to FE(electrolyte) = 100 X ([(electrolyte urine) X (creatinine plasma)]/[(electrolyte plasma) X (creatinine urine)]). FE_{Li} will used as a surrogate to measure proximal tubular sodium reabsorption, whereas FE_{Na} assesses total overall (proximal and distal) tubular sodium handling. Distal sodium handling is calculated by total-proximal sodium reabsorption, as described elsewhere[8a]. As in previous work, plasma and urine lithium levels will be measured using inductively with coupled spectroscopy[8a].

Secondary:

1. We will determine if volume contraction leads to a decline in hormones that are activated in HF patients such as B-type natriuretic peptides (BNP), without activating the SNS[7];
2. We will determine the impact of ertugliflozin on: renal hemodynamic function measured at 1 week[2,3,5] and 12 weeks as a measure of safety;
3. Blood pressure, echocardiographic measures of cardiac output (and derived systemic vascular resistance) arterial stiffness and systemic vascular resistance to better understand the blood pressure lowering effect in this patient population;
4. Heart rate and heart rate variability, to assess effects on SNS activation;
5. Urinary natriuretic modulators, such as angiotensin converting enzyme-2[9] and adenosine[10];
6. To characterize the safety of ertugliflozin vs. placebo by determining the number of hypoglycemic episodes between groups, and serious adverse events.

2. STUDY DESIGN

This study will use a double blind, stratified randomization trial approach involving 36 T2D-HF patients taking standard HF therapies, who will be allocated to either ertugliflozin or placebo in a 1:1 ratio (Table 1 – inclusion and exclusion criteria). For feasibility, participants will be recruited from the >4000 HF patients followed at UHN-MSH heart failure clinics and from heart failure clinics in Groningen, the Netherlands, of whom >40% have T2D. The study will consist of a screening visit, a 4-week run-in phase for those subjects not on stable ACEi/ARB treatment, followed by a 12-week double blind treatment period.

The target population will be invited for screening. Subjects who meet all entry criteria and who are already on a stable dose (no changes in dose or type of drug) of ACEis or ARBs for at least 4 weeks proceed directly to randomization. Subjects who had their ACEi or ARB medication changed during the preceding 4 weeks of the screening visit proceed to a run-in phase during which the type and dose of these drugs are stabilized. Subjects will be randomly assigned to one of the two treatments. Subjects will have their study visit in the morning in fasted condition.

Subjects will be instructed to take the study medication once daily, in the morning, except on study days; on those days, the study drug will be taken after the patient visit.

Measurements:

- During the double blind treatment period, patients will collect a 24-hour urine collection at each visit for measurement of 24-hour protein, albumin, glucose, sodium, potassium, creatinine, and urea excretion. Twenty-four hour urine collections will be performed at: Visits 3, 4 and 8. Routine biochemistry and safety labs will be drawn at screening, during each physiological assessment and at each the specified office safety visits (see visit schedule table below).
- Office systolic and diastolic blood pressure measurements, heart rate, weight, and waist circumference will be performed at screening and at each subsequent physiological testing day and at each office visit (see visit schedule table below).
- Blood will be drawn for measurement of renal function tests, HbA1c, glucose, complete blood count, plasma albumin, RAAS biomarkers, natriuretic peptides, and neurohormones on visits 3, 4 and 8.
- Blood and urine samples will be stored for future exploratory biomarker analyses to study the effect of ertugliflozin in this study population.

- **Schedule of study procedures and events**

	Screening		Baseline	Post Visit 1/Start of Drug/Placebo	Dosing			Post Visit 2	Follow-Up	Early EOS
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
Day	**-14**	**-7**	**0**	**7**	**14- 17**	**42**	**77**	**84**	**91**	
Clinic Visit	X		X	X	X	X		X	X	X
Phone/Email Visit		X					X			
Fasting Visit			X	X				X	X	X
Demographics and Medical History	X									
Randomization			X							
ECG	X								X	X
Physical Exam	X				X	X			X	X
Vital Signs	X		X	X	X	X		X	X	X
Weight and Waist Measurement	X		X	X	X	X		X	X	X
Blood and Urine Samples	X		X	X	X	X		X	X	X
Renal Function Tests			X	X				X		
Echocardiography			X	X				X		
Arterial Stiffness			X	X				X		
Plasma Volume (Indocyanine Green Dilution)			X	X				X		
Bioimpedence spectroscopy			X	X				X		
NICOM			X	X				X		
Neurohormones/ Biomarkers			X	X				X		
Pregnancy Test	X		X	X				X		
Start Modified Diet[b]		X	Continue until V4	X			X			
Dispense Lithium	X									
Dispense Study Medication			X	X						
24 Hour Urine Collection			X	X				X		
Dispense 24 hr urine bottle	X					X				
Modified Euglycaemic clamp			X	X				X		

a Informed consent is obtained before any study specific procedure is done. [b] The diet includes a target of 140 mmol of sodium per day to avoid salt depletion and consequence activation of the RAAS 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.

Study Periods and Procedures:

Screening

Patients with a history of T2D and HF will be eligible for protocol-specific assessment during the screening period. Signature of the protocol-specific informed consent form constitutes the first procedure of the screening period, followed by the assignment of a unique subject number. Protocol-specific assessments and procedures may then be performed, as part of the screening visit, to evaluate the subject's eligibility. During the screening period, subjects will maintain their stable doses of commercially available ACEIs or ARBs, and diuretic medication.

At this visit patients will be given a 24 hour urine bottle and will be dispensed lithium pills to be taken the night before visits 3, 4 and 8.

When all inclusion and exclusion criteria have been evaluated and the requirements for entry into the study have been met, the entry into run-in period or double blind ertugliflozin or placebo period will be scheduled. If the patient does not qualify based on the laboratory data, the patient is allowed to be re-screened within 4 weeks of the original screening date. The laboratory data based on which the patient failed should be repeated.

Run-in Period

Eligible subjects who complete the screening period will enter the run-in period if they are not using a maximum tolerated dose of an ACEi or ARB. During the run-in period, ACEi or ARB will be started or up titrated towards the maximum tolerated antihypertensive dose. If a patient does not tolerate the maximum dose of an ACEi or ARB, this should be documented in the eCRF and the patient can proceed to the double blind treatment period if all other entry criteria are met.

Double-Blind Ertugliflozin/Placebo Period

Eligible subjects will enter in the randomized, double-blind, ertugliflozin/placebo period if their ACEi/ARB and diuretic medication is stable for at least 4 weeks and all entry criteria are met. A randomization code stored in each local pharmacy will be used to randomly assign subjects to an ertugliflozin/placebo arm. Subjects will continue to maintain their stable doses of commercially available ACEi or ARB, and diuretics if applicable, throughout the double blind phase. Patients will be counseled to maintain a stable habitual dietary salt intake.

Visit 2

Visit 2 will be an email visit. Research staff will remind patient to start the modified diet, to collect the 24 hour urine one day before visit 3 and to take the Lithium pill the night before visit 3.

Visit 3 (Randomization)

The randomization visit procedures should be completed on a single day under a euglycemic clamp. As part of the study procedures, blood glucose will therefore be maintained between 4-6 mmol/L during the long study days. From a patient perspective, the procedures involved in the trial involve three intravenous needles (for renal function assessments, infusion of insulin and dextrose and finally for blood-letting). The randomization visit procedures will include measures of natriuresis, renal and systemic hemodynamic function, neurohormonal assessments, echocardiography, arterial stiffness, plasma volume, bioimpedence spectroscopy, vital signs, weight and waist circumference, blood and urine samples collection. Cardiac output and systemic vascular resistance will also be measured using non-invasive cardiac monitoring (NICOM). This

non-invasive technique is a form of bioelectrical impedance ("bioreatance") to measure thoracic fluid content with electrocardiogram electrode stickers. Beat-to-beat changes in fluid content can be used to derive cardiac output. Then, using measured mean arterial pressure, systemic vascular resistance can be calculated. This entire measurement process takes approximately 15 minutes[8a]. The first dose of blinded study medication should only be administered one day after all randomization visit procedures have been completed.

Visit 4, 5 and 6
One week after initiation of ertugliflozin/placebo, subjects will return to the renal physiology laboratory for the 1-week assessment (Visit 4), with repeated measures of all physiological parameters described for Visit 3. They will then continue the same treatment allocation for the rest of the trial. A safety visit (vital signs, weight, waist circumference, and physical exam and safety laboratory tests) will be conducted approximately 7-10 days later (Visit 5), and again 4-5 weeks later (Visit 6).

Additional telephone visits will be scheduled as required to follow weight and home blood pressure, in line with usual clinical care. If the patient does not tolerate medication a visit to the out-patient clinic will be scheduled. At the end of the treatment period (at visits 3, 4, and 8), the patient will take study medication *BEFORE* physiological procedures are performed.

Visit 7 and 8
After a total of 12 weeks of dosing with ertugliflozin or placebo, patients will return for a final set of physiological assessments as described in Visit 3 (Visit 8). One email/phone visit (visit 7) will be conducted. Research staff will remind patient to start the modified diet one week prior to visit 8; collect the 24 hour urine one day before visit 8 and to take the lithium pill the night before visit 8.

In subjects who need to discontinue prematurely from the treatment period prior to the final Visit 8 at 12 weeks, every effort will be made to capture all physiological data on the last day that the investigational product is taken.

Visit 9
One week after the study medication is stopped at visit 8, the patient will return for a follow-up visit. ECG, physical exam, vital signs, weight and waist circumference will be measured and blood and urine samples will be obtained.

Biomarker analysis
Aside from planned blood and urine samples, 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. After completion of the study, samples will be shipped and stored to a central storage facility in Toronto. The results of this biomarker research will be reported either in the main publication, or separately in a scientific report or publication. The results of this biomarker research may be pooled with biomarker data from other ertugliflozin 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.

3. STUDY POPULATION

Population (base)

The study population will be selected from the outpatient clinics from hospitals based in the Netherlands (Groningen) and Canada (Toronto).

Inclusion criteria:

1. Male or female subjects diagnosed with T2D ≥12 months prior to informed consent;
2. eGFR ≥30 ml/min/1.73m^2;
3. Age >18 years;
4. HbA1c 6.5%-10.5%;
5. Body Mass Index (BMI) 18.5-45.0 kg/m^2;
6. Blood pressure ≤160/110 and ≥90/60 at screening,
7. Heart failure with New York Heart Association (NYHA) class 2-3 symptoms and ejection fraction ≥20%
8. Stable dose of maximally tolerated ACE inhibitor, angiotensin receptor blocker or renin inhibitor for at least 30 days
9. Stable diuretic dose for at least 30 days at the time of baseline physiological assessment
10. BNP levels at baseline ≥100 pg/ml (no atrial fibrillation), ≥200 pg/ml if in atrial fibrillation

Exclusion criteria:

1. Type 1 Diabetes;
2. Leukocyte and/or nitrite positive urinalysis that is untreated;
3. Severe hypoglycaemia within 2 months prior to screening;
4. History of brittle diabetes or hypoglycaemia unawareness based on investigator judgement;
5. Unstable coronary artery disease with acute coronary syndrome, percutaneous intervention or bypass surgery within 3 months;
6. Clinically significant valvular disease;
7. Congestive heart failure secondary to an infiltrative cardiomyopathic process (for example amyloid) or pericardial constriction;
8. Uncontrolled systemic hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >110) or systemic hypotension (systolic blood pressure < 90/60 mmHg);
9. Bariatric surgery or other surgeries that induce chronic malabsorption;
10. Anti-obesity drugs or diet regimen and unstable body weight three months prior to screening;
11. Treatment with systemic corticosteroids;
12. Blood dyscrasias or any disorders causing hemolysis or unstable red blood cells;
13. Pre-menopausal women who are nursing, pregnant, or of child-bearing potential and not practicing an acceptable method of birth control;
14. Participation in another trial with an investigational drug within 30 days of informed consent;
15. Alcohol or drug abuse within three months prior to informed consent that would interfere with trial participation or any ongoing clinical condition that would jeopardize subject safety or study compliance based on investigator judgement;
16. Liver disease, defined by serum levels of alanine transaminase, aspartate transaminase, or alkaline phosphatase >3 x upper limit of normal as determined during screening;
17. Active malignancy at the time of screening;
18. Allergy to iodine-based substances if receiving iohexol for GFR measures

Any disqualifying clinical or biochemical parameter may be repeated, at the discretion of the investigator and where there is a clinical reason to do so. The repeat value should be assessed prior to the subject recorded as having screen failed. If the repeat value falls within the ranges defined by the protocol and the patient meets all other inclusion criteria, the patient is eligible for the study.

2.1 Sample size calculation

Sample size: The sample size is based on the primary endpoint of proximal fractional sodium excretion (FE_{Na}), which will be measured using fractional excretion of lithium as a surrogate for proximal sodium handling. Based on preclinical studies, and assuming a baseline FE_{Na} of 22±7, to be able to detect a 40% increase in FE_{Na} with an 80% power and a two-tailed alpha = 0.05, 18 patients per group are required, for a total sample size of n=36. This sample size is similar to those successfully used in previous studies[2], and sufficient to measure secondary endpoints related to Na^+ handling[27,28], renal function[2], arterial stiffness, plasma volume[7], systemic vascular resistance [29] and measures of neurohormonal function[10,17,18]. Randomization will be stratified to assure equal numbers of HFrEF and HFpEF patients in the ertugliflozin and placebo-treated groups. We anticipate an approximate 50%/50% split in the proportion of participants with HFrEF vs. HFpEF, which will be assessed based on the 2D echo at the time of the baseline physiological testing.

Data Analysis: **The primary outcome** is the difference in proximal sodium reabsorption FE_{Na} (measured using FE_{Li}) with ertugliflozin vs. placebo. **Secondary outcomes** will consist of *within* and *between group* changes in hemodynamic and tubular function parameters and vasoactive mediators in response to combination vs. monotherapy regimens. *Within group* comparisons will be analyzed using repeated measures ANOVA. As in previous work, *between group* comparisons will also be made using a 2-way ANOVA with treatment allocation as the dependent variable. *Post-hoc* analyses will be used to compare means, corrected for multiple comparisons. In pre-specified, exploratory sensitivity analyses, the same endpoints will be examined only in those patients who did not have changes in RAAS inhibitors and/or diuretic agents during the treatment period.

3. TREATMENT OF SUBJECTS

3.1 Investigational product/treatment

Blinded IP will be provided to the site by Merck. Each bottle will be identified with a unique Composite ID in order to maintain the blind. By way of an Unblinding List, Merck will provide treatment allocations of each Composite ID sent to the site. 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 15 mg ertugliflozin tablet once daily or matching placebo according to randomised treatment scheme.

Doses of ertugliflozin of up to 25 mg are well tolerated (see Investigator's Brochure) in patients with T2D, with and without CKD. The proposed 15 mg dose is well tolerated and effective for the treatment of T2D and in patients with CKD. Ertugliflozin is effective at reducing blood pressure and plasma glucose. In addition, in patients with CKD, this dose was found to be well tolerated.

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.

3.2 Use of co-intervention (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), systemic corticosteroids, and immunosuppressants as these medications may interfere with the evaluation of safety, tolerability and/or efficacy.

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 physiological assessments.

4. INVESTIGATIONAL PRODUCT

4.1 Name and description of investigational product(s)

Ertugliflozin L-pyroglutamic acid: (1S,2S,3S,4R,5S)-5-(4-chloro- 3-(4-ethoxybenzyl)phenyl)-1-(hydroxymethyl)-6,8- dioxabicyclo[3.2.1]octane-2,3,4-triol, compound with (2S)-5- oxopyrrolidine-2-carboxylic acid

Product Description and Dosage Form	Dosage form and strength	Manufacturer
Ertugliflozin Tablets Total Dose 15mg	Green, film coated tablets (orally)	Merck
Placebo Matching Ertugliflozin Tablet	Green, plain, film coated tablet. Does not contain active ingredient	Merck

4.2 Summary of findings from non-clinical studies

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

4.3 Summary of findings from clinical studies

Ertugliflozin:

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Despite the presence of hyperglycaemia in T2D, reabsorption of filtered glucose continues. Ertugliflozin 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 ertugliflozin'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 are likely to be independent of GFR (see Investigator's Brochure). Ertugliflozin 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 T2D following the administration of ertugliflozin. Approximately 64 g of glucose was excreted in the urine per day (corresponding to 280 kcal/day) at an ertugliflozin dose of 15 mg/day in healthy individuals and with urine glucose excretion of 105 g/day in subjects with T2D. The increase in urine glucose excretion is sustained over time.

As of May 2016, a total of 29 Phase 1 and 2 Phase 2 studies with ertugliflozin have been completed. In addition, analyses from the primary time point in 5 Phase 3 studies were available to support this Investigator's Brochure (IB). Although these Phase 3 studies have not been completed, information from these studies is included. Table 5-1 in the Investigator's Brochure provides an overview of these studies.

4.4 Summary of known and potential risks and benefits

Potential risks

The potential risks for the treatment with ertugliflozin and other SGLT2i 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 3 studies) and genital infections has been seen.

Based on the mechanism of action of ertugliflozin 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 ertugliflozin who develop conditions that may cause hypovolaemia or electrolyte imbalance, decisions to interrupt or discontinue ertugliflozin therapy and management of patients should be based on clinical judgment.

Ertugliflozin treatment led to small increases in serum phosphate, which were of uncertain significance. The magnitude and clinical significance of this in patients with CKD is unclear.

After the introduction of other SGLT2i 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 ertugliflozin 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. Ertugliflozin should be used with caution in these patients.

There is no reason to believe that ertugliflozin poses an undue risk of hypoglycemia in 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 (Defronzo et.al 2013).

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 ertugliflozin 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 ertugliflozin in individual patients in whom a potential health risk or a laboratory abnormality of clinical concern has been identified.

Potential benefits to patients

In this study, the dose of ertugliflozin 15 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 ertugliflozin is expected to reduce progression of renal failure and reduce CV mortality. Ertugliflozin is known to decrease body weight (or prevent weight gain) as well as lower blood pressure and albuminuria in patients with T2D.

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

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. The patient will not be disadvantaged in any way.

Conclusion

Considering the pre-clinical and clinical experience with ertugliflozin 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.

4.5 Description and justification of route of administration and dosage

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

4.6 Dosages, dosage modifications and method of administration

Ertugliflozin will be administered in a dose of 15 mg/day.

4.7 Preparation and labelling of Investigational Medicinal Product

Study medication (ertugliflozin and matching placebo) will be provided by Merck. 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 of each participating centre in accordance with relevant guidelines.

4.8 Drug accountability

All study medications will be stored at room temperature at the pharmacy department of each participating centre. Study medication will be collected from the pharmacy department by the principle investigator at each participating centre. Non-used medication will be returned to the pharmacy department where it will be destroyed.

4.9 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), as described in section 6.1.
If concerns regarding the quality or appearance of the investigational product arise, the investigational product will not be dispensed and Merck will be contacted immediately.

4.10 Drug Ordering

Initial Orders
Contact the protocol manager at the UHN for information.
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 with your pharmacy regarding existing investigational stock to assure optimal use of drug on hand.

5. NON-INVESTIGATIONAL PRODUCT

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

Inulin (Inutest) or iohexol (Omnipaque) and paraaminohippurate (PAH), lithium carbonate (300 mg one day prior to each physiological study day i.e. on three occasions).

5.2 Summary of findings from non-clinical studies

Inulin and PAH have 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. Lithium will be administered on three separate occasions to measure tubular sodium handling. Plasma volume will be measured using a non-radioactive technique (indocyanine green dilution) to assess changes in plasma volume in response to SGLT2 inhibition

5.3 Summary of findings from clinical studies

Close to 100 per cent of the intravenously injected Inulin and PAH are 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.

5.4 Summary of known and potential risks and benefits

Measurement of GFR and effective renal plasma flow by plasma clearance of Inulin and PAH has not been associated with reports of adverse events, but should be avoided in subjects with known intolerances to these agents. 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.

5.5 Description and justification of route of administration and dosage

Inulin (or iohexol) and PAH will be intravenously administered according to established UHN protocols through an injection catheter.

5.6 Drug accountability

Inulin (or iohexol) and PAH will be stored at room temperature at each site. Inulin (or iohexol) and PAH and lithium will be collected from the pharmacy department by the principle investigator at each participating center in the morning of the study visit.

6. METHODS

6.1 Study parameters/endpoints

6.1.1 Main study parameter/endpoint

- Fractional excretion of sodium, using lithium clearance as a surrogate for proximal tubular sodium handling.

6.1.2 Secondary study parameters/endpoints (if applicable)

- Glomerular Filtration Rate and Renal plasma flow measured with inulin and PAH, as well as derived renal hemodynamic parameters as described elsewhere.[25] In the event that inulin is not available to measure GFR, then iohexol will be used, provided that there is no history of allergy to iodine based substances.
- Systolic/diastolic blood pressure, heart rate
- Echocardiography for markers of systolic and diastolic function, cardiac output (29)
- Arterial stiffness
- Plasma volume will be measured using a non-radioactive technique (indocyanine green dilution) to assess changes in plasma volume in response to SGLT2i (20, 27).
- Extracellular water, will be measured non-invasively using bioimpedence spectroscopy, as described elsewhere(28).
- Cardiac output and systemic vascular resistance will also be measured using non-invasive cardiac monitoring (NICOM). This non-invasive technique is a form of bioelectrical impedance ("bioreatance") to measure thoracic fluid content with electrocardiogram electrode stickers. Beat-to-beat changes in fluid content can be used to derive cardiac output. Then, using measured mean arterial pressure, systemic vascular resistance can be calculated. This entire measurement process takes approximately 15 minutes.
- Neurohormones/biomarkers
    - Hormones of the RAAS (renin, aldosterone in plasma and urine)
    - Natriuretic Peptides
    - Sympathetic nervous system markers
    - Urinary adenosine

6.1.3 Other study parameters (if applicable)

- Number of hypoglycaemic episodes
- Serious Adverse Events
- Drug related adverse events (causality to investigational product assessed by research physician).

6.2 Randomisation, blinding and treatment allocation

Treatment ertugliflozin and placebo will be randomized. A computer generated randomized code generated by UHN will be used. The pharmacy of each participating centre will store the randomization code.

6.3 Study procedures

- Physical examination

Patients will be subjected to physical examination by the principal investigator before inclusion. This physical examination entails a routine investigation of heart, lungs and abdomen. At each visit patients will be asked for adverse events related to urinary tract infections or genital infections.

- 24hr urine collection
  Patients are asked to collect 24hr urine at start and end of each treatment period to monitor albuminuria. In total, three 24hr urines are collected during the course of this study. 24hr urine collection instructions will be provided to each patient. The volume of the urine containers is determined when the patient delivers the urine at each visit. Subsequently part of the urine is used for clinical chemistry measurement and the other part stored per instructions in the laboratory manual.
- Renal hemodynamic function
  GFR and plasma flow measurements will be performed during each of the three physiological assessment visits.
  The central laboratory at the UHN will be providing detailed instructions for the renal function tests in a separate laboratory manual. The inulin/PAH will be bought by the investigational site and should be used in accordance with the approved product label and manual instructions. In the event that inulin is not available, then iohexol will be used instead according to label and laboratory manual instructions.

Subject preparation:
After a 10-hr fast, two intravenous (IV) catheters, one in each arm, will be placed in the subject's antecubital vein. One line will be used for the injection of renal function marker solutions. The second line will be used for intermittent blood sampling. On each long study day (Visits 3, 4 and 8), study medication will be taken between after arrival in the laboratory **BEFORE** physiological measurements are obtained.

- Blood pressure measurements
  Blood pressure will be measured by office blood pressure machine during the outpatient visits. Patients will be in a semi-supine position during the blood pressure measurement. The average of three readings will be used.
- Venipuncture
  At each of the visits, approximately 20 ml will be taken for routine blood tests (approximately 20 ml/ 7 visits = 140 ml). At each of the 3 visits to assess kidney function, approximately 24 ml will be taken (total 72 ml), plus another 20 ml at each kidney function visit for measurement of hormones and other factors associated with kidney disease (approximately 20 ml for each of the 3 kidney visits = 60 ml). At the end of each of the 2 treatment periods (Visits 4 and 8), 9 blood samples of 1ml will be taken for ertugliflozin PK analysis (total 18ml). A total of approximately 290 ml of blood will be collected over 12 week period. Additional blood sampling will be take for plasma volume and lithium/sodium excretion measurements as described in the ICF.
- Laboratory measurements
  All routine laboratory measurements of this study will be assessed at the local laboratories of participating centres.
- Ertugliflozin plasma/urine concentration measurement
  At the end of the treatment period, during the GFR assessment, a 6 ml blood sample will be drawn for ertugliflozin and its metabolite ertugliflozin-3O-glucuronide measurement.

6.4 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.

6.5 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.

6.6 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 or revered back to their general practitioner.

6.7 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.

7. SAFETY REPORTING

7.1 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.

7.2 AEs, SAEs and SUSARs

7.2.1 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 unfavourable 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)

7.2.2 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 eCRF as:

- AEs
- An SAE
- If the AE is the reason for permanent discontinuation from investigational product (DAE), if the AE leading to Investigational Product interruption or dose reduction
- An AE of interest
  - Urinary Tract Infection or Genital Infections
  - Potential DKAs
  - Volume depletion
  - Fractures
  - Major hypoglycaemic events
  - Acute kidney injury
  - Amputation or related events

7.2.3 Adverse events of interest

Urinary tract infection or genital infections

All potential events of urinary tract infection or genital infections will be recorded in the eCRF.

Diabetic ketoacidosis (DKA)

All potential events of DKA will be recorded in the eCRF.

Volume depletion

Events of volume depletion (eg, dehydration, hypovolemia, or hypotension) will be collected on the eCRF as AEs.

Fractures

All fractures will be recorded in the eCRF as AEs and on a separate eCRF page.

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. Major hypoglycaemic episodes will be recorded in the eCRF as an AE and on an additional eCRF page.

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. All acute kidney injury events will be recorded on a separate eCRF.

Amputation and related events:

Amputations and related events will be recorded in the eCRF. Information on the actual amputations will be captured on a specific eCRF page.

7.3 Recording of Adverse Events

- *Time Period for Collection of Adverse Events*

For this study, AEs, SAEs, DAEs, and AEs of special interest will be collected from the time of signing the informed consent and throughout the study, including the run-in and wash-out periods.

- *Follow-up of Unresolved Adverse Events*

Any AEs, SAEs, DAEs, and AEs of special interest that are unresolved at follow-up are followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF.

- *Variables*

The following variables will be collected for each SAE/DAE/AE of special interest:

- 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.
- AE is serious due to.
    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 AEs/SAEs/DAEs/AE of interest 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 in Section 8.2.2. 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 Section 8.2.2. 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 criteria shown in Section 8.2.2.

- *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 AEs/SAEs/DAEs/AE of special interest 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 AEs/SAEs/DAEs/AE of special interest, 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 will be reported as AEs if they fulfil any of the AE criteria. If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an 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.

7.4 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).
- The investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its status of seriousness.
- 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 investigator (sponsor) will report SAEs accompanied by a cover page mentioning the study code, patient number, country, investigator, seriousness to Merck within the same timelines.

7.4.1 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 (see chapter 8.2.2);
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

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC in the Netherlands or accredited ethics committees in other countries:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once a year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States. Concurrently, sponsor will report SUSARs to Merck within the same timelines.

Sponsor will report SAE expedited SAR to competent authorities in each member state. The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report. Concurrently, the investigator (sponsor) will report SAEs accompanied by a cover page mentioning the study code, patient number, country, investigator, seriousness to Merck within the same timelines.

There will be two sets of emergency code breaks, one set will be retained by the project leader and one set will be forwarded to the pharmacy of each participating center. The blinded emergency code break contains the details of drug treatment. In an emergency, de code can be unblinded 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 removes the scratch-off covering he/she must note the date, time and reason for removing it and record this information in the Comments section of the CRF/ on the code-break card (source data). He/she must also immediately inform the project leader and the principal investigator that the code has been broken. Even though the code is broken, 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 Investigator to ensure that there is a procedure in place to allow access to the code break cards in case of emergency. Study drug must be discontinued after unblinding but the subject will be followed until resolution of the adverse event. At the conclusion of the study, the occurrence of any emergency code breaks will be determined after return of all code break reports.

7.5 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to the sponsor and Merck.

7.5.1 Maternal exposure

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 1day of when he/she became aware of it.

7.6 Overdose

There is no specific antidote for an overdose of ertugliflozin. In the event of an overdose, employ usual supportive measures such as removing unabsorbed material from the GI tract, perform clinical monitoring, and institute supportive treatment as dictated by the subject's clinical status.

- 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.

7.7 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) 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 Merck.

7.8 Data Safety Monitoring / Safety Committee]

The oversight of safety of ertugliflozin in this trial will be performed by Drs Cherney and Heerspink.

8. STATISTICAL ANALYSIS Primary study parameter(s)

The primary outcome is the difference in FE_{Na} with ertugliflozin vs. placebo. **Secondary outcomes** will consist of *within* and *between group* changes hemodynamic and tubular function parameters and vasoactive mediators in response to combination vs. monotherapy regimens. *Within group* comparisons will be analyzed using repeated measures ANOVA. As in previous work, *between group* comparisons will also be made using a 2-way ANOVA with treatment allocation as the dependent variable. *Post-hoc* analyses will be used to compare means, corrected for multiple comparisons. In pre-specified, exploratory sensitivity analyses, the same endpoints will be examined only those patients who did not have changes in RAAS inhibitors and/or diuretic agents during the treatment period.

8.2 Other study parameters

Descriptive statistics will be used to analyse the means and distribution between all the study variables. The means of the normal distributed variables will be compared with the student T-test and non-parametric tests (Mann-Whitney U-test for continued and Chi-Square test for nominal variables) will be used for the variables that do not follow a normal distribution. Missing values will be imputed using multiple imputation techniques. Before introducing multiple imputation techniques the pattern of missing values will be examined. If the pattern of missing values indicates that missing values occur randomly a PROC MI statement in SAS will be implemented to impute missing values.

In case patients require rescue medication this will be recorded in the database. During the data-analysis sensitivity analyses will be conducted by adding an additional covariate in the mixed model to account for rescue medication required during the study.

8.3 Interim analysis

N/A

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The Medical Ethical Committee of all participating centers will approve the study.

9.2 Recruitment and consent

Patients will be enrolled to the trial from centers in the Netherlands (1 center) Canada (1 center). 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 given 2 weeks to consider their decision and will then be asked to sign their written informed consent before they take part in the study.

9.3 Objection by minors or incapacitated subjects (if applicable)

No minors or incapacitated adults will be included in this study

9.4 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.

9.5 Compensation for injury

The sponsor University Medical Center Groningen (UMCG) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. All patients will receive written information about this insurance. The University Health Network also has insurance in accordance with the legal requirements of Canada.

9.6 Incentives (if applicable)

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

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 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 15 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.

10.2 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.

10.3 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.

10.4 End of study report

The sponsor will notify the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the Competent Authority.

10.5 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.

11. STRUCTURED RISK ANALYSIS

11.1 Potential issues of concern

a. Level of knowledge about mechanism of action

- The Sodium glucose co-transporter 2 (SGLT2), located in the proximal tubule of the

kidney, is an effective transporter system which is responsible for the nearly complete reabsorption of glucose in order to maintain appropriate glucose levels. Each glucose molecule that is reabsorbed is accompanied by reabsorption of a sodium molecule in a 1:2 ratio. SGLT2i reversely inhibit the SGLT-2 transporter, which leads to enhanced glucose and sodium excretion and a reduction in plasma glucose and HbA1c. The effects of ertugliflozin on the SGLT2 transporter are well characterized and sufficient knowledge is available about the mechanisms of action.[30,31]

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

- Twelve phase 3 randomized controlled clinical trials were conducted involving more than 6000 patients with T2D of whom ~4000 were treated with ertugliflozin. Eleven studies were 24-weeks in duration with extension in 6 studies up to 78 weeks. One study was 52 weeks in duration with extension of another 52 weeks.
- As of the data cut for the most recent edition of the Investigator's Brochure, a total of 8446 human subjects, including 7852 (93.0%) with T2DM, have been exposed to ertugliflozin. Oral doses as high as 300 mg (single dose), 100 mg q.d. for up to 14 days, and 25 mg q.d. for up to 12 weeks have been found to be safe and well tolerated. The maximum tolerated dose in the clinical setting has not been identified.
- Other SGLT2 inhibitors canagliflozin and empagliflozin are registered in the US and Europe. In addition, a number of other SGLT-2 inhibitors are in various stages of clinical development.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

- Various animal models and cell lines are available to study the effect of SGLT2 inhibitors in more detail at a tissue/cell level.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

SGLT2 is highly localized to the kidney and major effects are expected to be related to glycosuria and natriuresis.

e. Analysis of potential effect

In placebo controlled clinical trials the following adverse reactions have been identified:

- *Infections and infestations*: vulvovaginitis, balanitis and genital infections, urinary tract infections (Common)
- *Metabolism and nutrition disorders:* Hypoglycemia; volume depletion (uncommon)
- *Gastrointestinal disorders:* Constipation (uncommon)
- *Musculoskeletal*: Back pain (Common)
- *Renal and urinary disorders*: Dysuria; Polyuria (Common); Nocturia (uncommon)

Common (>1/100 to <1/10) and uncommon (>1/1000 to <1/100)

Few adverse events led to discontinuation of treatment and adverse events were balanced across study groups. The most commonly reported events leading to discontinuation in patients treated

with ertugliflozin 10 mg/day were increased blood creatinine (0.4%), urinary tract infections

(0.3%), nausea (0.2%), and rash (0.2%). It should be noted that the transient rise in serum creatinine may reflect a reduction in intra-glomerular pressure which may be associated with long-term structural renal protection.

f. Pharmacokinetic considerations

The potential for clinically significant drug-drug interactions (DDIs) resulting from ertugliflozin-mediated inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A activity, or induction of CYP1A2 or CYP3A4, is low. Hence, clinically meaningful DDIs between ertugliflozin and other oral antidiabetic drugs that are oxidatively metabolized by CYP2C9 and CYP2C8 are not anticipated. Furthermore, clinically meaningful drug interactions with concomitant medications that are substrates of breast cancer resistance protein (BCRP), P-glycoprotein (P-gp), human organic cation transporter (hOCT)2 (e.g. metformin), human organic anion transporter (hOAT)1, hOAT3 (e.g. sitagliptin), hOATP1B1 (statins), or hOATP1B3 are not anticipated.

In vitro studies with Caco-2, MDR1-MDCK, and BCRP-MDCK cells suggest that ertugliflozin may be a substrate for P-gp and BCRP-mediated efflux. However, ertugliflozin has high aqueous solubility and moderate permeability, and, following a 25-mg oral dose of [14C]ertugliflozin, more than 50% of the dose was absorbed in humans. These data, along with the dose-proportional increases in exposure observed in humans over the dose range of 0.5 to 300 mg, indicate that neither P-gp nor BCRP is likely to be a limiting factor for oral absorption of ertugliflozin.

Co-administration of multiple doses of rifampin resulted in a 38.8% reduction in AUC for ertugliflozin. There were no clinically meaningful DDIs between ertugliflozin and sitagliptin, metformin, glimepiride, or simvastatin. Dose adjustment for ertugliflozin is not required in patients with renal impairment, or when ertugliflozin is coadministered with rifampin, sitagliptin, metformin, glimepiride, or simvastatin.

In healthy subjects, a supratherapeutic dose of ertugliflozin was not associated with QTc interval prolongation at Cmax values >6× the mean Cmax,ss for q.d. administration of 15 mg.

g. Study population

The enrolled population is in a stable condition and no unexpected serious adverse events are foreseen.

h. Interaction with other products

See section f above.

i. Predictability of effect

Efficacy is monitored by measuring albuminuria / blood pressure / Hba1c which are accepted and accurate surrogates to evaluate efficacy.

j. Can effects be managed?

Patients are regularly monitored and asked about adverse effects of urinary tract infections or infestations. As reported above, ertugliflozin administration is associated with an increased risk of infections which can be managed with standard antimicrobial treatment. [32]

11.2 Synthesis

The available data show that ertugliflozin decreases HbA1c, blood pressure and body weight in patients with T2D and increases incidence of urinary tract infections. This adverse effect led in rare instances to treatment discontinuation and is manageable with standard antimicrobial treatment.

12. REFERENCES

1. Musso G, Gambino R, Cassader M, Pagano G. A novel approach to control hyperglycemia in type 2 diabetes: sodium glucose co-transport (SGLT) inhibitors: systematic review and meta-analysis of randomized trials. Ann Med 2012;44:375-93.
2. Cherney DZI, Perkins BA, Soleymanlou N, et al. The Renal Hemodynamic Effect of SGLT2 Inhibition in Patients with Type 1 Diabetes. Circulation 2014;129:587-97.
3. Ferrannini E, Solini A. SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol 2012;8:495-502.
4. Cherney DZI, Lund SS, Perkins BA, et al. Empagliflozin Reduces Microalbuminuria and Macroalbuminuria in Patients with Type 2 Diabetes ASN poster, Philadelphia 2015;SA-PO-1109.

4a. David Cherney, Bernard Zinman, Silvio E. Inzucchi, Audrey Koitka-Weber, Maximilian von Eynatten, Christoph Wanner. Effect of Empagliflozin on Albuminuria in Patients with Type 2 Diabetes and High Cardiovascular Risk. Lancet Diabetes Endocrinology 2017;5:610-621

4b. David Cherney, Maximilian von Eynatten, Soeren S. Lund, Stefan Kaspers, Susanne Crowe, Hans J. Woerle, Maximilian von Eynatten. The Effect of Sodium Glucose Cotransport 2 (SGLT2) Inhibition with Empagliflozin on Microalbuminuria and Macroalbuminuria in Patients with Type 2 Diabetes. Diabetologia. 2016;59:1860-70.

5. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-28.
6. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 2017.
7. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15:853-62.
8. Skrtic M, Cherney DZ. Sodium-glucose cotransporter-2 inhibition and the potential for renal protection in diabetic nephropathy. Curr Opin Nephrol Hypertens 2015;24:96-103.

8a. Lovshin, JA, Rajasekeran, H, Lytvyn, Y, Lovblom, E, Khan, S, Alemu, R, Perkins, B, Wang, W, Drucker, DJ, **DZ Cherney**. Dipeptidyl Peptidase-4 Inhibition in Patients with Type 2 Diabetes Stimulates Distal Tubular Natriuresis and Increases in SDF-1α^{1-67}. Diabetes Care 2017:40;1073-1081

9. Cherney DZ, Perkins BA, Soleymanlou N, et al. Sodium glucose cotransport-2 inhibition and intrarenal RAS activity in people with type 1 diabetes. Kidney Int 2014;86:1057-8.
10. Rajasekeran H, Lytvyn Y, Bozovic A, et al. The Effect of SGLT2 Inhibition on Adenosine Excretion in Patients with Type 1 Diabetes. Poster No: X Presented at the American Diabetes Association (ADA) 76rd Scientific Sessions 2016.
11. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 2013;62:e147-239.
12. Mentz RJ, Broderick S, Shaw LK, Fiuzat M, O'Connor CM. Heart failure with preserved ejection fraction: comparison of patients with and without angina pectoris (from the Duke Databank for Cardiovascular Disease). J Am Coll Cardiol 2014;63:251-8.
13. Wanner C. Empagliflozin and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Chronic Kidney Disease American Society of Nephrology 2015;High Impact Late Breaking Clinical Trials session.
14. Tikkanen I, Narko K, Zeller C, et al. Empagliflozin Reduces Blood Pressure in Patients With Type 2 Diabetes and Hypertension. Diabetes Care 2014;38:420-8.
15. Fitchett D, Zinman B, Wanner C, et al. Heart failure outcomes with empagliflozin in patients with

type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME(R) trial. Eur Heart J.
16. Fitchett D, Zinman B, Wanner C, et al. Heart failure outcomes with empagliflozin in patients with type 2 diabetes at high cardiovascular risk: results of the EMPA-REG OUTCOME(R) trial. Eur Heart J 2016;37:1526-34.
17. Jordan J, Tank J, Heusser K, et al. Empagliflozin has no discernible effect on muscle sympathetic nerve activity in patients with type 2 diabetes despite reductions in blood pressure and weight. Diabetes 2014;63:A265 [1030-P].

18. Cherney DZ, Perkins BA, Soleymanlou N, et al. The effect of empagliflozin on arterial stiffness and heart rate variability in subjects with uncomplicated type 1 diabetes mellitus. Cardiovasc Diabetol 2014;13:28.
19. Heerspink HJ, Perkins BA, Fitchett DH, Husain M, Cherney DZ. Sodium Glucose Cotransporter 2 Inhibitors in the Treatment of Diabetes: Cardiovascular and Kidney Effects, Potential Mechanisms and Clinical Applications. Circulation 2016;134:752-72.
20. Sha S, Devineni D, Ghosh A, et al. Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, dose dependently reduces calculated renal threshold for glucose excretion and increases urinary glucose excretion in healthy subjects. Diabetes Obes Metab 2011;13:669-72.
21. Sarashina A, Koiwai K, Seman LJ, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of single doses of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, in healthy Japanese subjects. Drug Metab Pharmacokinet 2013;28:213-9.
22. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-28.
23. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med.
24. Christoph Wanner JML, David H. Fitchett, Silvio E. Inzucchi, Maximilian von Eynatten, Michaela Mattheus, Odd Erik Johansen, Hans-Jürgen Woerle, Uli Christian Brödl, Bernard Zinman. Würzburg, Germany. Empagliflozin and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Chronic Kidney Disease. ASN renal week High Impact Clinical trial 2015.
25. Cherney DZ, Perkins BA, Soleymanlou N, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. Circulation 2014;129:587-97.
26. group Kcpgw. KDIGO clinical practice guideline for glomerulonephritis. Kidney Int 2012;2.
27. Montanari A, Pela G, Musiari L, et al. Nitric oxide-angiotensin II interactions and renal hemodynamic function in patients with uncomplicated type 1 diabetes. Am J Physiol Renal Physiol 2013;305:F42-51.
28. Montanari A, Biggi A, Cabassi A, et al. Renal hemodynamic response to L-arginine in uncomplicated, type 1 diabetes mellitus: the role of buffering anions and tubuloglomerular feedback. Am J Physiol Renal Physiol;303:F648-58.
29. Valvo E, Bedogna V, Casagrande P, et al. Captopril in patients with type II diabetes and renal insufficiency: systemic and renal hemodynamic alterations. Am J Med 1988;85:344-8.
30. Obermeier M, Yao M, Khanna A, et al. In vitro characterization and pharmacokinetics of dapagliflozin (BMS-512148), a potent sodium-glucose cotransporter type II inhibitor, in animals and humans. Drug Metab Dispos 2010;38:405-14.
31. Defronzo RA, Hompesch M, Kasichayanula S, et al. Characterization of Renal Glucose Reabsorption in Response to Dapagliflozin in Healthy Subjects and Subjects With Type 2 Diabetes. Diabetes Care 2013.
32. Johnsson KM, Ptaszynska A, Schmitz B, Sugg J, Parikh SJ, List JF. Urinary tract infections in patients with diabetes treated with dapagliflozin. J Diabetes Complications 2013;27:473-8.

APPENDIX A – Lab samples/visit – ERADICATE-HF

Sample Analysis performed at UHN

BLOOD:

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
-14	-7	0	7	14-17	42	77	84	91
Screening		Baseline	Start of Drug/placebo	Dosing			Post Visit 2	Follow Up
Sodium		Sodium	Sodium	Sodium	Sodium		Sodium	Sodium
Potassium		Potassium	Potassium	Potassium	Potassium		Potassium	Potassium
Urea		Urea	Urea	Urea	Urea		Urea	Urea
Creatinine		Creatinine	Creatinine	Creatinine	Creatinine		Creatinine	Creatinine
CBC & Diff		CBC & Diff	CBC & Diff	CBC & Diff	CBC & Diff		CBC & Diff	CBC & Diff
AST		AST		AST	AST			AST
ALT		ALT		ALT	ALT			ALT
Total Bilirubin		Total Bilirubin	Total Bilirubin	Total Bilirubin	Total Bilirubin		Total Bilirubin	Total Bilirubin
Phosphate		Phosphate	Phosphate	Phosphate	Phosphate		Phosphate	Phosphate
Calcium		Calcium	Calcium	Calcium	Calcium		Calcium	Calcium
BNP		BNP	BNP	BNP	BNP		BNP	BNP
HbA1c		HbA1c		HbA1c	HbA1c			HbA1c
Glucose		Glucose	Glucose	Glucose	Glucose		Glucose	Glucose
Total Protein		Total Protein	Total protein	Total Protein	Total Protein		Total protein	Total Protein
Lipid Profile		Lipid Profile	Lipid Profile	Lipid Profile	Lipid Profile		Lipid Profile	Lipid Profile
Bicarbonate		Bicarbonate		Bicarbonate	Bicarbonate			Bicarbonate
Chloride		Chloride		Chloride	Chloride			Chloride
beta hCG								

URINE Analysis at UHN: (24 hour urine)

Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**
-14	**-7**	**0**	**7**	**14-17**	**42**	**77**	**84**	**91**
Screening		**Baseline**	**Start of Drug/placebo**	**Dosing**			**Post Visit 2**	**Follow Up**
		Sodium	Sodium				Sodium	
		Potassium	Potassium				Potassium	
		Urea	Urea				Urea	
		Creatinine	Creatinine				Creatinine	
		Albumin	Albumin				Albumin	
		Protein	Protein				Protein	

Spot Urine (done at each visit)

Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
-14	-7	0	7	14-17	42	77	84	91
Screening		Baseline	Start of Drug/placebo	Dosing			Post Visit 2	Follow Up
Sodium		Sodium	Sodium	Sodium	Sodium		Sodium	Sodium
Potassium		Potassium	Potassium	Potassium	Potassium		Potassium	Potassium
Urea		Urea	Urea	Urea	Urea		Urea	Urea
Creatinine		Creatinine	Creatinine	Creatinine	Creatinine		Creatinine	Creatinine
Albumin		Albumin	Albumin	Albumin	Albumin		Albumin	Albumin
Protein		Protein	Protein	Protein	Protein		Protein	Protein

1. Lovshin JA, Rajasekeran H, Lytvyn Y, Lovblom LE, Khan S, Alemu R, Locke A, Lai V, He H, Hittle L, Wang W, Drucker DJ, Cherney DZI. Dipeptidyl Peptidase-4 Inhibition Stimulates Distal Tubular Natriuresis and Increases in Circulating SDF-1alpha1-67 in Patients With Type 2 Diabetes. *Diabetes Care.* 2017.