A study to assess the *Renoprotective Effects of the SGLT2 Inhibitor Dapagliflozin in Non-Diabetic Patients with Proteinuria: a Randomized Double Blind 6-Weeks Cross-Over Trial*

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PROTOCOL TITLE: ***A STUDY TO ASSESS THE RENOPROTECTIVE EFFECTS OF THE SGLT2 INHIBITOR DAPAGLIFLOZIN IN NON-DIABETIC PATIENTS WITH PROTEINURIA: A RANDOMIZED DOUBLE BLIND 6-WEEKS CROSS-OVER TRIAL***

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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 Transport Inhibitor 2**
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:
Despite optimal treatment with renin-angiotensin-aldosterone-system (RAAS) inhibitors, many patients with non-diabetic kidney disease show progressive kidney function loss, which is associated with high residual albuminuria. Novel treatment strategies are therefore required to further decrease albuminuria and to slow kidney function decline.
Dapagliflozin is a sodium-glucose transport (SGLT2) inhibitor and inhibits the reabsorption of glucose and sodium in the proximal tubule. The increased natriuresis following dapagliflozin administration normalizes tubuloglomerular feedback resulting in a reduction in intra-glomerular hypertension, which is in turn manifested by acute reductions in glomerular filtration rate and albuminuria. Since many etiologies of non-diabetic nephropathy are characterized by intraglomerular hypertension, we hypothesize that dapagliflozin acutely decreases GFR and albuminuria in patients without diabetes at risk of progressive kidney function loss via a glucose independent hemodynamic mechanism.

Objective:
Primary:

- To assess the change baseline in 24-hr proteinuria with dapagliflozin for six weeks relative to placebo treatment in patients with non-diabetic kidney disease and proteinuria > 500 mg/day on stable ACEi or ARB treatment.

Secondary:

- To assess the effect of dapagliflozin 10 mg/d compared to placebo on Glomerular Filtration Rate (GFR) using iohexol clearance.
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on systolic/diastolic blood pressure
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on body weight
- To assess the effect of dapagliflozin 10 mg/d on selected neurohormones/biomarkers:
    - Hormones of the RAAS (plasma and urine)
    - Natriuretic peptides
    - Urinary adenosine
    - Co-peptin

    - Immunoglobulin G (plasma and urine)
- To characterize the safety of dapagliflozin vs. placebo by determining the number of hypoglycemic episodes between groups, and serious adverse events.

Study design:
Randomized placebo controlled double blind cross-over trial
Eligible participants will be randomly assigned to one of the two treatment orders: placebo-dapagliflozin *or* dapagliflozin-placebo. Each treatment period lasts 6 weeks followed by a 6 week wash-out period to avoid cross-over effects. Patients will have their study visit in the morning in fasted condition.

Study population: Male and female subjects with Chronic Kidney Disease aged between 18 and 75 years, proteinuria levels between 500 and 3500 mg/24-hour, and an eGFR ≥ 25 ml/min/1.73m^2 will be enrolled. Patients with type 1 or type 2 diabetes, polycystic kidney disease, glomerulonephritis with flares (lupus or anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis) or ongoing active renal inflammation will be excluded. Patients have to be treated with a stable dose of an ACEi or ARB for at least 4 weeks prior to enrolment.

Intervention: Dapagliflozin 10 mg/day or matched placebo

Main study parameters/endpoints: Change in 24-hr proteinuria

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients visit the outpatient clinic on a more regular base than standard patient care - i.e. at study inclusion and at start and end of each treatment period (9 hospital visits in a total study duration of 25 or up to 41 weeks) - for clinical assessment. A fasting blood sample is collected with venipuncture. Non-radioactive iohexol GFR measurements are performed at start and end of each treatment period as well as at the end of the wash-out period. 24hr urine will be collected one day prior to the hospital visit, except for the screening visit when patients collect 24hr urine after informed consent is obtained. 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.

1. INTRODUCTION AND RATIONALE

Inhibition of sodium–glucose cotransporter 2 (SGLT2i) reduces plasma glucose and HbA1c in patients with type 2 diabetes by increasing urinary glucose excretion. Beneficial effects of SGLT2i beyond glycemic control in patients with type 2 diabetes have been suggested by the EMPA-REG OUTCOME trial, in which empagliflozin treatment added to standard care significantly reduced the composite primary endpoint (i.e. death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke), compared to participants allocated to the placebo group [1]. In this study, dramatic cardiovascular protective effects were reported, even though HbA1c was only reduced by 0.36% at the end of the study. The preliminary renal outcome data were recently reported showing that individuals treated with empagliflozin had a 39% reduced risk of new onset of nephropathy; and a 46% reduced risk of the composite renal endpoint consisting of doubling of serum creatinine, end stage kidney disease (ESKD) or death due to renal disease [2]. In light of modest glucose-lowering effects in EMPA-REG Outcome, 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 [3]. 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. However, it is unclear if the renal protection of SGLT2i can be similarly applied to other proteinuric kidney diseases that are unrelated to diabetic nephropathy.

One of the general physiological principles that applies to progression of both diabetic and non-diabetic chronic kidney disease (CKD) is the concept of elevated intraglomerular pressure as a risk factor for CKD progression. More specifically, in the setting of CKD, remnant nephrons can hyperfunction – or hyperfilter - to compensate for the loss of mass due to injury from the primary disease. What started as a compensatory mechanism to maintain GFR through elevated single nephron filtration becomes a maladaptive response leading to glomerular hypertrophy and hyperfiltration over time. Intraglomerular hypertension is thought to ultimately contribute to progressive loss of renal function in diverse glomerular nephropathies, including focal segmental glomerular sclerosis, IgA nephropathy and membranous glomerulonephritis [4].

To reduce intraglomerular hypertension, current guidelines recommend the use of RAAS blockers as the gold standard therapy for individuals with non-diabetic nephropathy and proteinuria [5]. However, despite the use of these agents, renal function continues to decline. Therefore, there is a need to widen the therapeutic armamentarium for individuals with non-diabetic 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 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. 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 - including individuals without diabetes.

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 [6, 7]. The low risk of hypoglycemia offers the opportunity to test these drug classes in non-diabetic proteinuric renal disease.

In conclusion, SGLT2i has a plausible therapeutic potential in individuals with non-diabetic nephropathy at risk of renal progression, and are likely to be well tolerated. Exploring the beneficial effects of these agents in non-diabetic proteinuric kidney disease may pave the way for a new indication for this class of drugs. In addition, trials in non-diabetic kidney disease may help to further characterize the non-glucose dependent effects, which may help to differentiate SGLT2i from other glucose lowering agents.

2. OBJECTIVES

Primary Objective:

- To assess the change from baseline in 24-hr proteinuria with dapagliflozin for six weeks relative to placebo treatment in patients with non-diabetic kidney disease and proteinuria > 500 mg/day on stable ACEi or ARB treatment.

Secondary Objectives

- To assess the effect of dapagliflozin 10 mg/d compared to placebo on Glomerular Filtration Rate (GFR) using iohexol clearance
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on systolic/diastolic blood pressure
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on body weight
- To assess the effect of dapagliflozin 10 mg/d compared to placebo on selected neurohormones/biomarkers:
    - Hormones of the RAAS (plasma and urine)
    - Natriuretic peptides
    - Urinary adenosine
    - Co-peptin
    - Immunoglobulin G (plasma and urine)
- To characterize the safety of dapagliflozin vs. placebo by determining the number of hypoglycemia episodes between groups and serious adverse events.

3. STUDY DESIGN

A double blind randomized placebo controlled cross-over study will be conducted in subjects with Chronic Kidney Disease aged between 18 and 75 years, proteinuria levels between 500 and 3500 mg/24-hour, and an eGFR ≥ 25 ml/min/1.73m^2 will be enrolled. Patients with type 1 or type 2 diabetes, polycystic kidney disease, glomerulonephritis with flares (lupus or anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis) or ongoing active renal inflammation will be excluded. Since each subject is his/her own control, this design requires fewer patients as compared with a parallel study design. A wash-out period after each treatment period has been included in the design to avoid cross-over effects.

The study will consist of a screening visit, a 4-week (up to a maximum of 16-weeks) run-in phase for those subjects not on stable ACEi/ARB treatment, 2 consecutive 6-week double blind treatment periods separated by 6-week wash-out period. Subjects will be randomly assigned to one of the treatment orders as depicted in figure 1.

Figure 1: Study design

Screening	Run-in 4 weeks	Placebo 6 weeks	Wash-out 6 weeks	Dapa 10 mg 6 weeks	Wash-out 6 weeks

Screening	Run-in 4 weeks	Dapa 10 mg 6 weeks	Wash-out 6 weeks	Placebo 6 weeks	Wash-out 6 weeks

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 treatment orders as depicted in figure 1. Each treatment period lasts 6 weeks followed by a 6 week wash-out period to avoid cross-over effects. 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 or during the patient visit.

<u>Measurements:</u>

- During the double blind treatment period, and wash-out period patients will collect a 24-hour urine collection at each visit for measurement of 24-hour protein, albumin, sodium,

potassium, creatinine, and urea excretion. Twenty-four hour urine collections will be performed at:

o Screening visit

o Run-in, day 1, week 3, and week 6 (last day on treatment) of each treatment period, and six weeks after the second treatment period (after the last wash-out period).

- Office systolic and diastolic blood pressure measurements will be performed at day 1, week 3 and 6 (last day on treatment) of each treatment period, and six weeks after the second treatment period.
- Vital signs (heart rate, blood pressure) and body weight, height
- Blood will be sampled for measurement of laboratory assessment outlined in the Table 1 and analysis will be performed by the local laboratory.
- Additional blood will be sampled at Day 1 and week 6 of each treatment period, and six weeks after the second treatment period for shipment to the UMCG for biomarker measurement including RAAS biomarkers, natriuretic peptides, co-peptin, immunoglobulin G, and urinary adenosine.
- Blood and urine samples will be stored for future exploratory biomarker analyses to study the effect of dapagliflozin in this study population
- At the screening visit Demography and significant medical history as defined below will be assessed and recorded on the Medical History/Kidney Diagnosis section of the eCRF.
    - Kidney diagnosis
    - Condition related to kidney disease (i.e. recurrent electrolyte disturbances, hypovolemia, prior events such as Acute Kidney Injury)
    - Cardiovascular diseases
    - Recurrent/severe urinary tract infections, genital infections, urosepsis
    - Recent hospital admission, and reason for admission (within the last year)
    - Reactions to radio-contrast media

- **Time and Events Table**

Parameter	Screening[a]	Run-in	Treatment Period 1				W/O	Treatment Period 2				W/O
Visits	V0	V1	V2	V3	V4	V5		V6	V7	V8	V9	V10
Week			0	1	3	6		12	13	15	18	24
Informed Consent[a]	x											
Randomization			x									
Significant Medical History & Kidney disease diagnosis	x											
Serum or Urine Pregnancy test			x[c]					X[c, g]				
24-hour urine[b]	x[d]	x	x		x	x		x		x	x	x
GFR-iohexol			x			x		x			x	x
Plasma PK sample						x					x	
Vital signs	x	x	x		x	x		x		x	x	
Blood sampling[e]	x	x	x		x	x		x		x	x	x
Additional blood sampling 'Biomarker samples'[f]			x			x		x			x	x
Physical exam	x		x		x	x		x		x	x	x
Telephone visit				x					x			
Dispense Medication			x					x				
Drug accountability						x					x	
Adverse events	x	x	x	x	x	x		x	x	x	x	x
Review medications	x	x	x		x	x		x		x	x	
DNA collection			x									

a Informed consent is obtained before any study specific procedure is done.

b Total 24-hr urinary dapagliflozin excretion will also be measured at the end of each treatment period

c WOCBP must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug.

d Subject will be asked to collect 24 hour urine after informed consent is obtained. The subject will be requested to return the urine sample to the site on the following day.

e Specify details about specimen collection will be provided in the laboratory manual. Table 1-2 will list the laboratory assessment to be performed.

f Specify details about specimen collection, storage and processing will be provided in the laboratory manual.

g Visit 6 Serum or Urine Pregnancy Test is required for Canada sites per local regulatory requirement

Study Periods and Procedures:

Screening

Chronic Kidney Disease aged between 18 and 75 years, proteinuria levels between 500 and 3500 mg/24-hour, and an eGFR ≥ 25 ml/min/1.73m^2 who are on an ACEi or ARB, or can start with and ACEi or ARB, 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.

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 treatment period will be scheduled. If the patient does not qualify based on the laboratory data, the patient is allowed to be re-screened within 3 weeks of the original screening date. The laboratory data based on which the patient failed should be repeated.

Run-in Period:

Potentially eligible subjects who complete the screening visit will enter the run-in period if they are not using an ACEi or ARB, or 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 uptitrated 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.

Visit 1

Subjects who entered the run-in period will return for the run-in visit 4 weeks after starting an ACEi/ARB or after ACEi/ARB dose adjustments. The subject has to be on a stable dose of an ACEi or ARB for at least 4 weeks before randomisation. More visits may be required to make sure that the subject still meet all entry criteria at the end of the run-in period. The run-in period may take up to a maximum of 16 weeks. If the eGFR remains ≥25 ml/min/1.73m^2 and the proteinuria level remains between 500 mg/24hr and ≤3500 mg/24hr at the end of the run-in period, the patient qualifies to proceed to the double blind treatment period.

Double-Blind Treatment Period:

Eligible subjects will enter in the randomized, double-blind, treatment period if their ACEi/ARB and diuretic medication is stable for at least 4 weeks and all entry criteria are met. The

randomization codes generated by University Medical Center Groningen will be programmed into the IBM Clinical Development Merge to randomly assign subjects to a treatment arm. The investigational drug will be dispensed by the principal investigator or delegated based on the randomization number.

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

The randomization visit procedures should be completed on a single day. The randomization visit procedures will include GFR measurement by plasma clearance of nonradioactive iohexol after a single intravenous dose. The GFR measurement will last approximately 4 hours for patients. The first dose of blinded study medication should only be administered on Day 1 AFTER ALL randomization visit procedures have been completed. Patient will be treated in random order with dapagliflozin 10 mg QD or matching placebo QD.

Visit 3, 4, and 5

Subjects will be followed for a total of 6 weeks on double-blind study medication in each active treatment period. A telephone visit will be scheduled after 1 week to assess tolerability. If the patient does not tolerate medication a visit to the out-patient clinic will be scheduled. The next scheduled visit (visit 4) occurs 3 weeks after randomization. A visit window of +/- 3 days is allowed for each out-patient clinic visit. Patients are asked to collect a 24-hr urine and blood will be sampled for clinical chemistry measurement. The last scheduled visit in the first treatment period is scheduled after 6 weeks. The last visit of the treatment period will include GFR measurement by plasma clearance of nonradioactive iohexol after a single venous dose, collection of a 24-hr urine sample and clinical chemistry measurements. At the end of the treatment period, the patient will take study medication *BEFORE* the GFR measurement procedure. In addition to using blood samples for GFR measurements, the samples will also be used to measure plasma concentration dapagliflozin and its metabolite dapagliflozin-3O-glucuronide.

Visit 6,7,8, and 9

Following the last visit of the first treatment period subjects proceed to a 6 weeks wash-out period. After 6 weeks the second double blind treatment period will start. All visits and procedures in the second treatment period are similar to the first treatment period.

Visit 10

Six weeks after the second treatment period another visit will be scheduled. Procedures during this visit include GFR measurement with non-radioactive iohexol, 24-hr urine collection, blood pressure recording and blood sampling for clinical chemistry assessment.

Subjects who discontinue prematurely from the treatment period prior to visit 4, will have an abbreviated Week 12/ET visit, which will exclude measurements of GFR.

Clinical Lab Assessments

All protocol required local laboratory assessments, as defined in Table 1-2, must be conducted in accordance with the Laboratory Manual, and Time and Event Table.

Table 1

Plasma Laboratory Assessments	**Parameters**	
Hematology	Hemoglobin	Hematocrit
Clinical Chemistry	Urea	Bilirubin
	Creatinine	Phosphate
	AST	Calcium
	ALT	Glucose
	Total Protein	
Lipid Parameters	LDL	HDL
Electrolytes	Sodium	Potassium
	Chloride	Bicarbonate
Other Laboratory Test	NT-proBNP[1]	HbA1C
	Urine/Serum Pregnancy Test[2]	

[1] Sites that are unable to perform NTproBNP will arrange for samples to be sent to UMCG for central analysis.

[2] Pregnancy test: negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug.

Table 2

24-Hour Urine Laboratory Assessments	**Parameters**	
Clinical Chemistry	Sodium	Creatinine
	Potassium	Albumin
	Urea	Protein

Biomarker analysis

Serum, plasma and urine will be collected and stored for potential future analysis for exploratory biomarkers to assess correlations with disease activity and effects of study drug. These samples will be shipped and stored to a central storage facility in Groningen. 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 dapagliflozin studies to generate hypotheses to be tested in future research. Potential biomarkers to be measured include plasma albumin, RAAS biomarkers, natriuretic peptides, co-peptin, immunoglobulin G, urinary adenosine, and urinary vesicles.

DNA

Patients are asked to donate DNA as well. Participation in the pharmacogenetics study is optional and DNA will only be collected if patients consented for participation in this part of the study.

STUDY POPULATION

3.1 Population (base)

The study population will be selected from the outpatient clinical from hospitals based in the Netherlands (Groningen, Almelo, Amsterdam), Canada, (Toronto, Calgary, Vancouver) and Malaysia (Kuala Lumpur).

3.2 Inclusion criteria

- Age ≥18 and ≤75 years
- Urinary protein excretion > 500 mg/24hr and ≤ 3500 mg/24hr in a 24-hr urine collection
- eGFR ≥ 25 mL/min/1.73m^2
- On a stable dose of an ACEi or ARB for at least 4 weeks prior to randomization
- Willing to sign informed consent
- Women of Child-Bearing Potential (WOCBP):
    - WOCBP must be using an acceptable method of contraception to avoid pregnancy throughout the study and for up to 4 weeks after the last dose of study drug in such a manner that the risk of pregnancy is minimized.

- WOCBP must have a negative serum or urine pregnancy test result (minimum sensitivity 25 IU/L or equivalent units of HCG) within 0 to 72 hours before the first dose of study drug.
- Women must not be breast-feeding.

WOCBP **comprises** women who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who are not post-menopausal.

3.3 Exclusion criteria

- Diagnosis of type 1 or type 2 diabetes mellitus
- Urinary protein excretion > 3500 mg/day
- Peripheral Vascular Disease
- Autosomal dominant polycystic kidney disease or autosomal recessive polycystic kidney disease, lupus nephritis, or ANCA-associated vasculitis
- Indication for immunosuppressants as per the treating physician's judgment.
- Receiving cytotoxic therapy, immunosuppressive therapy, or other immunotherapy for primary or secondary renal disease within 6 months prior to enrolment.
- Active malignancy aside from treated squamous cell or basal cell carcinoma of the skin.
- Use of the co-interventional treatments (outlined in section 4.2) within 6 weeks of screening will not be allowed.
- Any medication, surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of medications including, but not limited to any of the following:
    - History of active inflammatory bowel disease within the last six months;
    - Major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
    - Gastro-intestinal ulcers and/or gastrointestinal or rectal bleeding within last six months;
    - Pancreatic injury or pancreatitis within the last six months;
    - Evidence of hepatic disease as determined by any one of the following: ALT or AST values exceeding 3x ULN at the screening visit, a history of hepatic encephalopathy, a history of esophageal varices, or a history of portocaval shunt;
    - Evidence of urinary obstruction or difficulty in voiding at screening
- History of severe hypersensitivity or contraindications to dapagliflozin

- History of hypersensitivity or contraindications to iodinated contrast media
- Subject who, in the assessment of the investigator, may be at risk for dehydration or volume depletion that may affect the interpretation of efficacy or safety data
- Participation in any clinical investigation within 3 months prior to initial dosing.
- Donation or loss of 400 ml or more of blood within 8 weeks prior to initial dosing.
- History of drug or alcohol abuse within the 12 months prior to dosing, or evidence of such abuse as indicated by the laboratory assays conducted during the screening.
- History of noncompliance to medical regimens or unwillingness to comply with the study protocol.
- Any surgical or medical condition, which in the opinion of the investigator, may place the patient at higher risk from his/her participation in the study, or is likely to prevent the patient from complying with the requirements of the study or completing the study.
- Pregnancy or breastfeeding
- WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and up to 4 weeks after the last dose of study drug.

Any disqualifying proteinuria or eGFR level may be repeated one time, 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.

3.4 Sample size calculation

With a sample size of 50 patients completing the study and assuming a conservative standard deviation of 0.7 in log transformed proteinuria (within-subject standard deviation of 0.475), the trial will have 80% power to detect a mean reduction in albuminuria of 25% (i.e, delta=0.288 on the log-transformed proteinuria scale) or more with dapagliflozin compared to placebo (alpha 0.05). This calculation is based on individual patient data from various clinical trials on the anti-proteinuric effects of different agents in diabetes and non-diabetes trials for which we have the individual patient data including ROAD, RENAAL and IDNT trials. For calculation of the sample size, the percent change in proteinuria is log-transformed to take into account the skewed distribution of proteinuria. Under the assumption that no carry-over effects and no period effects occur, a paired-t-

test is deemed appropriate to calculate the sample size. Using these assumptions, we calculate that 50 patients completing the study will provide 80% power to detect a 25% reduction in albuminuria. Assuming that approximately 5% of patients will discontinue the study prematurely, we intend to enrol 53 subjects.

We believe a reduction of >25% with dapagliflozin versus placebo can be detected. We recently published a large analysis of patients with diabetes and demonstrated that dapagliflozin decreased proteinuria by 33%. Trials with other SGLT2 inhibitors have also documented proteinuria reductions of approximately 30%.

4. TREATMENT OF SUBJECTS

4.1 Investigational product/treatment

Dapagliflozin and matching placebo tablets will be provided by Astra Zeneca. Patients will take 10 mg dapagliflozin (once daily) or matching placebo according to randomised treatment scheme.

The marketed 10 mg dose has been demonstrated to be well tolerated and effective for the treatment of type 2 diabetes and post-hoc analyses in patients with CKD and proteinuria have shown that this dose is effective in reducing renal risk markers (i.e. blood pressure, proteinuria) independent of its glucose lowering effect[8-10]. In addition, in a dedicated CKD clinical trial this dose was found to be well tolerated[9]. From a pharmacokinetic and pharmacodynamic perspective dapagliflozin 10 mg is appropriate for use in patients with CKD.

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. Returned and unused drugs are destroyed by the local pharmacy department at the end of the study.

4.2 Use of co-intervention (if applicable)

Use of the following treatments will not be allowed from 6 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.

- Aldosterone antagonists
- Direct renin inhibitors

Systemic corticosteroids and immunosuppressants

Patients who are receiving such medication(s) should be excluded, or if ethically justified, the medication(s) may be withdrawn according to the manufacturer's/investigator's instructions prior to start of the screening period. Withdrawal of medication is done after consultation with the patient and patients' physician.

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 non-steroidal anti-inflammatory drugs (NSAIDs), ephedrine, phenylephrine, pseudoephedrine, or phenylpropanolamine, administration of such medications is recommended to be discontinued at least 48 hours before iohexol injection and should not be resumed until completion of the GFR measurement procedure. Continuous NSAID use is not permitted during the study. NSAIDs use for several days for pain management is permitted as long as it is not during the days prior to GFR / proteinuria assessments.

Drugs which lower seizure threshold, especially phenothiazide derivatives including those used for their antihistaminic properties, are not recommended for use with iohexol. Others include monoamine oxidase (MAO) inhibitors, tricyclic antidepressants, CNS stimulants, psychoactive drugs described as analeptics, major tranquilizers, or antiphychotic drugs. The dose of such medications, if applicable, are recommended to be discontinued at least 48 hours before iohexol injection and should not be resumed for at least 24 hours following iohexol injection.

5. INVESTIGATIONAL PRODUCT

5.1 Name and description of investigational product(s)

Drug name: dapagliflozin (Forxiga, AstraZenaca, RPF-EU-12-MB102-016); Chemical structure: (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol;

Product Description and Dosage Form	Dosage form and strength	Manufacturer
Dapagliflozin 10mg Tablet	10 mg Green, plain, diamond shaped, film coated tablet (orally)	Astra Zeneca
Placebo Matching Dapagliflozin Tablet	Green, plain, diamond shaped, film coated tablet. Does not contain active ingredient	Astra Zeneca

5.2 Summary of findings from non-clinical studies

This is not applicable as dapagliflozin is already registered as antihyperglycemic agent for the treatment of type 2 diabetes in humans.

5.3 Summary of findings from clinical studies

Dapagliflozin:

SGLT2 is the predominant transporter responsible for reabsorption of glucose from the glomerular filtrate back into the circulation. Despite the presence of hyperglycaemia in type 2 diabetes, reabsorption of filtered glucose continues. Dapagliflozin improves both fasting and post-prandial plasma glucose levels by reducing renal glucose reabsorption leading to urinary glucose excretion. This glucose excretion (glycosuric effect) is observed after the first dose, is continuous over the 24-hour dosing interval and is sustained for the duration of treatment. The amount of glucose removed by the kidney through this mechanism is dependent upon the blood glucose concentration and GFR. In patients with low plasma glucose or low eGFR dapagliflozin's efficacy to lower HbA1c is low and so the risk of hypoglycemia is low as well. Effects on blood pressure, body mass index, albuminuria, hematocrit appear to be independent of GFR (Heerspink ADA 2016). Dapagliflozin does not impair normal endogenous glucose production in response to hypoglycaemia. Dapagliflozin acts independently of insulin secretion and insulin action rendering the risk of hypoglycemia low.

Increases in the amount of glucose excreted in the urine were observed in healthy subjects and in subjects with type 2 diabetes mellitus following the administration of

dapagliflozin. Approximately 70 g of glucose was excreted in the urine per day (corresponding to 280 kcal/day) at a dapagliflozin dose of 10 mg/day in subjects with type 2 diabetes for 12 weeks. Evidence of sustained glucose excretion was seen in subjects with type 2 diabetes given dapagliflozin 10 mg/day for up to 2 years.
Twelve double-blind, randomised, controlled clinical trials were conducted with 6,144 subjects with type 2 diabetes to evaluate the efficacy and safety of Forxiga; 4,164 subjects in these studies were treated with dapagliflozin.

Monotherapy:

A double-blind, placebo-controlled study of 24-week duration (with an additional extension period) was conducted to evaluate the safety and efficacy of monotherapy with dapagliflozin in subjects with inadequately controlled type 2 diabetes. Once-daily treatment with dapagliflozin resulted in statistically significant ($p<0.0001$) reductions in HbA1c compared to placebo.

Combination therapy:
In a 52-week, active-controlled non-inferiority study (with a 52-week extension period), Dapagliflozin was evaluated as add-on therapy to metformin compared with a sulphonylurea (glipizide) as add-on therapy to metformin in subjects with inadequate glycaemic control (HbA1c > 6.5% and ≤ 10%). The results showed a similar mean reduction in HbA1c from baseline to Week 52, compared to glipizide, thus demonstrating non-inferiority. At Week 104, adjusted mean change from baseline in HbA1c was -0.32% for dapagliflozin and -0.14% for glipizide. At 52 and 104 weeks, a significantly lower proportion of subjects in the group treated with dapagliflozin (3.5% and 4.3%, respectively) experienced at least one event of hypoglycaemia compared to the group treated with glipizide (40.8% and 47.0%, respectively).
In a study of 808 patients with type 2 diabetes who had inadequate glycemic control and received on average 30 units insulin, dapagliflozin 10 mg/day caused a 0.57% reduction in HbA1c relative to placebo. Dapagliflozin did not increase insulin requirements but these requirements increased progressively in the placebo group.[11]

5.4 Summary of known and potential risks and benefits

Dapagliflozin has global market approval and based on global cumulative sale figures up to March 2016 it is estimated that dapagliflozin has been administered during >1000000 patient

years. Details regarding potential risks associated with administration of dapagliflozin once daily are provided in the Investigator's Brochure (IB).

Potential risks

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

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

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

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

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

There is no reason to believe that dapagliflozin poses an undue risk of hypoglycemia in non-diabetic patients. The amount of glucose excreted in the urine depends on the total filtered glucose load (blood glucose concentration x GFR). Thus, at blood glucose levels in the low normal range, the amount of glucose excreted into the urine is not sufficient to induce hypoglycemia[12]e. In Clinical Pharmacology studies in healthy subjects single doses up to 500 mg and multiple oral doses of 2.5 to 100 mg up to 14 days have been evaluated and have shown that dapagliflozin does not induce even a single case of hypoglycemia in non-diabetic subjects. Additionally, in a recent clinical trial in 50 non-diabetes obese individuals treated with the combination of dapagliflozin and exenetide, none of the patients experienced a hypoglycemic event[13].

Protection against risks

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

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

Potential benefits to patients

In this study, the dose of dapagliflozin 10 mg was chosen based on previous clinical experience. This mechanistic study is non-therapeutic; therefore, it has limited or no direct clinical benefit for the subjects. In studies of longer duration, patients randomized to active drug, dapagliflozin is expected to reduce progression of renal failure and reduce CV mortality. Dapagliflozin is known to decrease body weight (or prevent weight gain) as well as lower blood pressure and albuminuria in patients with type 2 diabetes.

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, and the patient will not be disadvantaged in any way.

Conclusion

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

5.5 Description and justification of route of administration and dosage

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

5.6 Dosages, dosage modifications and method of administration

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

5.7 Preparation and labelling of Investigational Medicinal Product

Study medication (dapagliflozin and matching placebo) will be provided by Astra Zeneca. The pharmacy department of the University Medical Center Groningen will be responsible for labelling and distribution of study medication to the participating sites.

5.8 Drug accountability

All study medications will be stored at room temperature (<25°C) in a secure location of each participating centre. Study medication will be dispensed by the principal investigator or delegate at each participating centre. Unused and partially used study drug will be returned by the subject to the site for drug accountability and be made available for site monitor verification during onsite monitoring visit. Returned and unused study drug will be

locally destroyed per local procedure and destruction must be documented on the drug destruction record.

5.9 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). If concerns regarding the quality or appearance of the investigational product arise, the investigational product will not be dispensed and Astra Zeneca will be contacted immediately.

5.10 Drug Ordering

Initial Orders

Contact the protocol manager at the University Medical Center Groningen for information.

Re-Supply

Contact your site monitor or the protocol manager at the University Medical Center Groningen (trials@apoth.umcg.nl) for information.

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

6. NON-INVESTIGATIONAL PRODUCT

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

Omnipaque 300 (iohexol)

6.2 Summary of findings from non-clinical studies

Iohexol has a very low acute intravenous toxicity in mice and rats. Animal studies have shown that iohexol has a very low protein binding and is well tolerated by the kidneys. The cardiovascular and neurotoxicity are low. The histamine release ability and the anticoagulant activity has been shown to be less than for ionic contrast media.

6.3 Summary of findings from clinical studies

For most of the hemodynamic, clinical-chemical and coagulation parameters examined following intravenous injection of iohexol in healthy volunteers, no significant deviation from preinjection values has been found. The few changes observed in the laboratory parameters were minor and considered to be of no clinical importance.

Close to 100 per cent of the intravenously injected iohexol is excreted unchanged through the kidneys within 24-hours in patients with normal renal function. The elimination half-life is approximately 2 hours in patients with normal renal function. No metabolites have been detected. The protein binding of Omnipaque is so low (<2%) that it has no clinical relevance and can therefore be neglected.

6.4 Summary of known and potential risks and benefits

Measurement of GFR by plasma clearance of iohexol has not been associated with reports of adverse events, but should be avoided in subjects with known reactions to radio-contrast media. The small amount of iohexol used for these studies is unlikely to affect GFR. Iohexol is an iodinated radio-contrast material. Adverse reactions to iohexol may include anaphylactoid reactions, which may include urticaria, angioedema, dyspnea, and shock, and typically occur within one hour of administration. These reactions are non-immunological, and are likely related to the whole molecule rather than free iodine. While allergic individuals have a somewhat increased risk of adverse reaction to radiocontrast agents, no special risk is conferred by either seafood allergy or hypersensitivity to iodine. Personnel qualified to diagnose and treat anaphylactoid reactions, as well as necessary medication and equipment, should be readily available to persons receiving iohexol.

6.5 Description and justification of route of administration and dosage

Iohexol (Omnipaque 300) will be intravenously administered.

6.6 Dosages, dosage modifications and method of administration

Omnipaque 300 mg iodine per millilitre contains 647 mg/ml iohexol. Exactly 5 mL of iohexol solution (drawn in a 5 mL syringe), containing 3.225g of iohexol will be injected intravenously through an injection catheter.

6.7 Preparation and labelling of Non Investigational Medicinal Product

The Iohexol ordered by the institution will be used. The institution may purchase Iohexol from an external vendor as appropriate. The institution must seek sponsor approval prior to use of alternative brand of Iohexel mentioned in the protocol.

6.8 Drug accountability

Iohexol will be stored at the temperature specified in the package insert in a secure location at the site.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

- 24-hour proteinuria

7.1.2 Secondary study parameters/endpoints (if applicable)

- Glomerular Filtration Rate measured with iohexol
- Systolic/diastolic blood pressure
- Neurohormones/biomarkers
    - Hormones of the RAAS (renin, aldosterone in plasma and urine)
    - Natriuretic Peptides
    - Urinary adenosine
    - Co-peptin
    - Immunoglobulin G

7.1.3 Other study parameters (if applicable)

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

7.2 Randomization, blinding and treatment allocation

Treatment sequence of dapagliflozin and placebo will be randomized. A 4-block randomization method is used. The randomization is done by hand by a UMCG pharmacist. The generated codes will be used to set-up the randomization module and subjects will be randomized by IBM Clinical Development Merge. The investigational drug will be dispensed by the principal investigator or delegate based on the randomisation number generated in Merge.The pharmacy of each participating centre will store the randomization code.

7.3 Study procedures

- Physical examination

  Patients will be subjected to physical examination by the principal investigator or delegate as specified in Time and Event table. 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, nine 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.

- GFR measurements

  GFR measurements by plasma clearance of non-radioactive iohexol will be performed at the randomization and Week 6,12,18, 24 or early termination visits. This test involves a single dose injection of 5 mL of iohexol, a non-radioactive iodinated x-ray contrast agent. GFR is estimated on the basis of plasma disappearance curves.

  The central laboratory of the UMCG will be providing detailed instructions for the GFR measurement procedure in a separate laboratory manual. The iohexol solution will be bought by the investigational site and should be used in accordance with the approved product label and manual instructions.

  The following should be considered prior to performing GFR measurements:

  - Subjects should be well hydrated prior to and following administration of iohexol.
  - If not obtained within 72 hours of the GFR measurement procedure, the subject's weight and height should be measured and recorded

  Subject preparation:

  After a 10-hr fast, two intravenous (IV) catheters, one in each arm, will then be placed in the subject's antecubital vein. One line will be used for the single dose injection of nonradioactive iohexol. The second line will be used for intermittent blood sampling. At the beginning of each treatment period (i.e. visit 2 and 6), study medication will be taken **AFTER** the iohexol GFR measurement procedure. At the end of the treatment period (i.e. visit 5 and 9) study medication will be taken **BEFORE** the iohexol GFR measurement procedure.

  Iohexol injection (time -2 to 0 minutes):

- Exactly 5 mL of iohexol solution (drawn in a 5 mL syringe), containing 3.225 g of iohexol, will be injected slowly into the injection catheter.
- The 5 mL solution should be injected over a period of 2 minutes.
- The exact time of the injection will be recorded in the eCRF and in the source document. For consistency, the same digital clock should be used throughout the procedure.
- The total, exact, volume of the solution injected must be recorded on the central laboratory requisition form and in the source document.
- Blood samples must not be taken from the arm receiving the iohexol solution.

Blood Collection:

Blood samples for the determination of GFR by plasma clearance of iohexol will collected prior the injection of the iohexol solution as well as during the last 2 hours of the procedure. **Initiation of the GFR procedure will occur at time - 2 minutes and is defined as the time at which the iohexol solution is injected. Time 0 represents the end of the iohexol injection. The timing for the blood samples are relative to the end of the iohexol injection (time 0).**

The table below summarizes the timepoints

Timepoint	Suggested Time	Procedure
Prior to Time -2 min	~7:57 AM	Baseline blood and PK sample
Time -2 min	~7:58 AM	Start iohexol injection
Time 0 min	~8:00 AM	End of iohexol injection
Time 30 min[a]	~8:30AM	Blood sample PK
Time 60 min[a]	~9:00 AM	Blood sample PK
Time 90 min[a]	~9:30 AM	Blood sample PK
Time 120 min	~10:00 AM	Blood sample GFR / PK
Time 150 min	~10:30 AM	Blood sample GFR / PK
Time 180 min	~11:00 AM	Blood sample GFR / PK
Time 210 min	~11:30 AM	Blood sample GFR / PK
Time 240 min	~12:00 AM	Blood sample GFR / PK

[a] PK samples are only collected at the end of the active treatment period

- Blood pressure measurements

  Blood pressure will be measured by office blood pressure during the outpatient visits. Patients will be in a semi-supine position during the blood pressure measurement.

Average of three readings will be used. The blood pressure measurement should be performed per local practise and the same arm should be used to take the reading for each visit.

- Venipuncture

  At each of the visits, approximately 20 ml will be taken for routine blood tests (approximately 20 ml/ 9 visits = 180 ml). At each of the 5 visits to assess kidney function, approximately 24 ml will be taken (total 120 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 5 kidney visits = 100 ml). At the end of each of the 2 treatment periods, 9 blood samples of 1ml will be taken for dapagliflozin PK analysis (total 18ml). A total of approximately 418 ml of blood will be collected over a 6 month (24 week) period

- Laboratory measurements

  All routine laboratory measurements of this study will be assessed at the local laboratories of participating centres.

- Dapagliflozin plasma concentration measurement

  At the end of the treatment period, during the GFR assessment, 9 blood samples of 1 ml will be drawn for dapagliflozin and its metabolite dapagliflozin-3O-glucuronide measurement.

7.4 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.

7.5 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. The patient proceeds to the randomization visit if the proteinuria values are within the predefined window (i.e. >500 mg/24hr and ≤3500 mg/24hr). When patients decide to withdraw after start of the treatment period, no new patients can be admitted to the study due to a limited availability of study medication. Although, in our sample size calculation we have taken into account a certain amount of withdrawal and according to our experience, we don't expect withdrawal to exceed this number.

7.6 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.

7.7 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.

8. SAFETY REPORTING

8.1 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 jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

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

8.2.2 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.)

Per Health Canada requirement, sites in Canada will collect and record all Adverse Events in the eCRF. For Netherlands and Malaysia sites AEs should be recorded in the eCRF only if it qualifies as:

- 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

8.2.3 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.

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.

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 as an AE.

Amputation and related events:

Amputations and related events will be recorded in the eCRF. Information on the actual amputations will be captured as an AE.

8.3 Recording of Adverse Events

- *Time Period for Collection of Adverse Events*

For this study, SAEs, discontinuations due to AEs (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. Information about all adverse events (serious or not) will be recorded in source documents (eg, progress notes) according to good clinical practice, and retained at the investigative sites.

- *Follow-up of Unresolved Adverse Events*

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

- *Variables*

The following variables will be collected for each SAE/DAE/AE 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.

a. Date of hospitalisation (if applicable).
b. Date of discharge (if applicable).
c. Probable cause of death (if applicable).

d. Date of death (if applicable).
e. Autopsy performed (if applicable).
f. Causality assessment in relation to Study procedure(s).
g. Causality assessment in relation to other medication (e.g., concomitant medication, background therapy).

Description of intensity.
The intensity of the reported 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 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 SAEs/DAEs/AEs 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 should therefore only be reported as AEs if they fulfil any of the SAE criteria, are the reason for discontinuation of study treatment, or fulfil criteria of AEs of special interest.
If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an SAE/DAE or AE of special interest 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.

8.4 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 sponsor's principal investigator and project leader will report SAEs accompanied by a cover page mentioning the study code, patient number, country, investigator, seriousness to AstraZeneca via the e-mail box AEMailboxClinicalTrialTCS@AstraZeneca.com within the same timelines.

8.4.1 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 AstraZeneca via the e-mail box AEMailboxClinicalTrialTCS@AstraZeneca.com 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 AstraZeneca via the e-mail box AEMailboxClinicalTrialTCS@AstraZeneca.com within the same timelines.

In an emergency, unblinding can be performed in the electronic data capture (EDC) system IBM Clinical Development (eClinicalOS) to identify the treatment given to that subject. Unblinding is not to be performed for any reason, other than an emergency where unblinding is required. When the Investigator unblinds the subject he/she must note the date, time and reason for removing it and record this information in the Comments section of the CRF and in the source data. He/she must also immediately inform the project leader/principal investigator at the UMCG (sponsor) and the site monitor that the on the unblinding of the subject treatment. Even though the subject has been unblinded, any blood samples for safety or pharmacodynamic assessments will continue to be drawn, for at least 24hr following the last dose as long as doing so will not compromise subject welfare.
It is the responsibility of the Principal Investigator to ensure the investigator site staff are appropriately trained on the unblinding procedure in case of emergency. Study drug must be discontinued after unblinding but the subject will be followed until resolution of the adverse event. It is the intent that subjects who discontinue treatment with the study drug will continue in the study according to the visit schedule described in the Time and Events Table. At the conclusion of the study, the occurrence of any unblinding can be reported.

8.5 Pregnancy

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

8.5.1 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.

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.

8.6 Overdose

Dapagliflozin has been well tolerated at doses of up to 500 mg/day in single dose testing in healthy volunteers and up to 100 mg/day in repeat dose testing for 14 days in healthy volunteers and patients with T2D. Suspected single intake of less than 50 tablets of 10 mg dapagliflozin tablets or repeated intake of less than 10 tablets of 10 mg dapagliflozin tablets should not be reported on the eCRF overdose module unless the patient is experiencing a symptomatic overdose. Overdose on IMP will be recorded in the eCRF dosing record module. If an overdose is suspected, monitoring of vital functions as well as treatment should be performed as appropriate.

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

8.7 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) adverse reactions and serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
– a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

All safety reports should be simultaneously communicated to Astra Zeneca.

8.8 Data Safety Monitoring Board (DSMB) / Safety Committee]

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

9. STATISTICAL ANALYSIS

9.1 Primary study parameter(s)

The log-transformed 24-hour proteinuria values will be analyzed by a Proc Mixed statement in SAS with sequence, treatment, and period as fixed factors and patients (nested in sequence) as a random factor. A contrast statement will be used to determine difference among treatment groups. Statistical analyses will be conducted with SAS version 9.4.

9.2 Secondary study parameter(s)

The change in secondary outcomes, GFR, systolic blood pressure, and neurohormones will be analyzed by a Proc Mixed statement in SAS with sequence, treatment, and period as fixed factors and patients (nested in sequence) as a random factor. Statistical analyses will be conducted with SAS version 9.4.

9.3 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 indicate 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.

9.4 Interim analysis

N/A

10. ETHICAL CONSIDERATIONS

10.1 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.

10.2 Recruitment and consent

Patients will be enrolled to the trial from centers in the Netherlands, Canada, and Malaysia. 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.

10.3 Objection by minors or incapacitated subjects (if applicable)

No minors or incapacitated adults will be included in this study

10.4 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. The risks associated with the use of dapagliflozin in patients with non-diabetic kidney disease are considered low. First, dapagliflozin has been used in non-diabetic individuals in doses up to 500 mg/day without reports of adverse events. Secondly, safety data in patients with diabetic kidney disease has been generated in phase 3 trials and demonstrated no additional side effects compared to type 2 diabetes patients without kidney disease. Given the SGLT2i mode of action no further safety issues are anticipated in non-diabetic CKD patients as compared to generally more vulnerable diabetic kidney disease patients included in the past phase 3 studies.

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.

10.5 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7, subsection 6 of legal requirements in the Netherlands (Article 7 WMO). This insurance applies to all participating sites in the Netherlands. An additional liability insurance has been arranged for the participating sites in Canada and Malaysia.

The sponsor University Medical Center Groningen (UMCG) has an insurance for all participating sites in the Netherlands, 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.
For the participating sites in Canada and Malaysia the sponsor has another insurance to cover damage to research subjects in these countries. This insurance policy is in accordance with the legal requirements in Canada and Malaysia.

10.6 Incentives (if applicable)

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

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 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.

11.2 Monitoring and Quality Assurance

Independent monitors will monitor the study in each country according to a pre-specified monitoring plan. The monitors are trained in GCP and will be trained on study specific tasks and processes. As part of George Clinical' s Quality Management Strategy monitor oversight will be implemented through regular documentation reviews and co-monitoring activities.

11.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the ethics committee (METC) application, or 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 METC and to the competent authority.

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

11.4 Annual progress report

The sponsor's principal investigator and project leader will submit a summary of the progress of the trial to the accredited METC once a year. 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.

11.5 End of study report

The sponsor will notify the accredited METC and 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 principal investigator and project leader from the UMCG will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 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 sponsor, nor the principal investigator has a right of veto regarding the way of publishing the results.

12. STRUCTURED RISK ANALYSIS

12.1 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. SGLT2 inhibitors 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 dapagliflozin on the SGLT2 transporter are well characterized and sufficient knowledge is available about the mechanisms of action.[14, 15]

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 type 2 diabetes of whom ~4000 were treated with dapagliflozin. 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.

Another SGLT2 inhibitor is registered in the US and filed for registration in Europe (canagliflozin). A third SGLT2 inhibitor (empagliflozin) is filed for registration 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 dapagliflozin 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.

A pooled analysis of 3152 patients who received dapagliflozin in doses between 2.5 and 10 mg/day showed that the incidence of urinary tract infection was increased with dapagliflozin dosage. Most diagnosed infections were mild to moderate and responded to standard antimicrobial treatment.[16]

Safety data in patients with diabetic kidney disease has been generated in phase 3 trials and demonstrated no additional side effects compared to type 2 diabetes patients without kidney disease. Given the SGLT2i mode of action no further safety issues are anticipated in non-diabetic CKD patients as compared to generally more vulnerable diabetic kidney disease patients included in the past phase 3 studies.

Overdose:
Dapagliflozin did not show any toxicity in healthy subjects at single oral doses up to 500 mg (50 times the dose aimed to be used in the present study). These subjects had detectable glucose in the urine for a dose-related period of time (at least 5 days for the 500 mg dose) with no reports of dehydration, hypotension, or electrolyte imbalance and with no clinically meaningful effect on QT_c interval. The incidence of hypoglycaemia was similar to placebo. In clinical studies where once-daily dosing of up to 100 mg dapagliflozin were administered for 2 weeks in healthy subjects and type 2 diabetes subjects the incidence of hypoglycaemia was slightly higher than placebo and was not dose-related.

f. Pharmacokinetic considerations

In *in-vitro*- studies, dapagliflozin neither inhibited cytochrome P450, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 nor induces CYP1A2, CYP2B6, CYP3A4. Therefore, dapagliflozin is not expected to alter the metabolic clearance of coadminstered medicinal products that are metabolized by these enzymes.

Interaction studies conducted in healthy subjects, using mainly a single dose design, suggest that the PK of dapagliflozin is not altered by metformin, pioglitazone, sitagliptin, glimepiride, hydrochlorothiazide, bumetanide, valsartan, or simvastatin.

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 f

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, dapagliflozin administration is associated with an increased risk of infections which can be managed with standard antimicrobial treatment. [16]

12.2 Synthesis

The available data show that dapagliflozin decreases HbA1c, blood pressure and body weight in patients with type 2 diabetes. The drug received marketing approval from the EMA and is registered in various EU-countries. Dapagliflozin increases incidence of urinary tract infections. This adverse effect led in rare instances to treatment discontinuation and is manageable with standard antimicrobial treatment.

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