DAPASALT: An Open Label, Phase IV, Mechanistic, Three-Arm Study to Evaluate the Natriuretic Effect of 2-Week Dapagliflozin treatment in Type 2 Diabetes Mellitus Patients with Either Preserved or Impaired Renal Function and Non-Diabetics with Impaired Renal Function

ClinicalTrials.gov Identifier: NCT03152084

Date of Protocol: 10 January 2020

DAPASALT: An Open Label, Phase IV, Mechanistic, Three-Arm Study to Evaluate the Natriuretic Effect of 2-Week Dapagliflozin treatment in Type 2 Diabetes Mellitus Patients with Either Preserved or Impaired Renal Function and Non-Diabetics with Impaired Renal Function

Sponsor:	AstraZeneca AB SE-151 85 Södertälje Sweden
Clinical Research Organisation:	PAREXEL International (IRL) Limited, 70 Sir John Rogerson's Quay, Dublin 2, Ireland
Sponsor Protocol No.:	D1690C00049
EudraCT No.:	2016-002961-79
Study Drug Name:	Dapagliflozin
Development Phase:	IV
Date of Protocol:	10 Jan 2020, Version 6.0
Date of Previous Protocol:	23 Apr 2018, Version 5.0

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki [1], and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

Title: DAPASALT: An Open Label, Phase IV, Mechanistic, Three-Arm Study to Evaluate the Natriuretic Effect of 2-Week Dapagliflozin treatment in Type 2 Diabetes Mellitus Patients with Either Preserved or Impaired Renal Function and Non-Diabetics with Impaired Renal Function.

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, 2013 [1], and the guidelines on Good Clinical Practice.

PPD

22 JAN 2020

Date

AstraZeneca R&D Gothenburg, Sweden

Declaration of the Investigator

Title: DAPASALT: An Open Label, Phase IV, Mechanistic, Three-Arm Study to Evaluate the Natriuretic Effect of 2-Week Dapagliflozin treatment in Type 2 Diabetes Mellitus Patients with Either Preserved or Impaired Renal Function and Non-Diabetics with Impaired Renal Function.

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, electronic data capture (EDC) system, and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the patients.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local study centre

_____ _____

Signature Date

_____

Name (block letters)

_____

Title (block letters)

_____

Institution (block letters)

_____

Phone number

PROTOCOL SYNOPSIS

Title	DAPASALT: An Open Label, Phase IV, Mechanistic, Three-Arm Study to Evaluate the Natriuretic Effect of 2-Week Dapagliflozin treatment in Type 2 Diabetes Mellitus Patients with Either Preserved or Impaired Renal Function and Non-Diabetics with Impaired Renal Function.
Sponsor Study No.	D1690C00049
Phase	IV
Sponsor	AstraZeneca AB SE-151 85 Södertälje Sweden
Study Centre(s)	It is planned to enroll patients at up to 5 centres in Europe.
Objective(s)	**Primary:** To investigate change in 24-hr sodium excretion during dapagliflozin treatment between Baseline (average of Days −3 to −1) and average of Days 2 to 4 within each study group in patients with type 2 diabetes mellitus (T2DM) with preserved or impaired renal function and in non-diabetics with impaired renal function. **Secondary:** The secondary objectives to be evaluated within each study group during or following dapagliflozin treatment are: • To evaluate the change in 24-hr sodium excretion during dapagliflozin treatment from Baseline to end of treatment, and during follow-up. • To evaluate the change in 24-hr glucose excretion. • To evaluate the change in mean 24-hr systolic blood pressure. • To evaluate the change in plasma volume. • To evaluate the change in extracellular volume. • To evaluate pharmacokinetics of dapagliflozin. • To evaluate the change in 24-hr urine albumin: creatinine ratio (UACR) CCI 

	CCI [redacted]
	Safety: • To evaluate the safety and tolerability of dapagliflozin in each of the target patient populations.
Design	This is an open label, mechanistic, three-arm study to evaluate the natriuretic effect of 2 weeks dapagliflozin treatment in T2DM patients with either preserved or impaired renal function and in non-diabetic patients with impaired renal function. The study population will comprise 3 groups of patients as described in study population below. The maximum duration of the study will be 62 days including the allowed window periods for the study (±1 day for Visit 7 at Day 13). The study will allow for an up to 6-week Screening and Run-in Period. The Run-in Period should always last 6 days (Day -6 to Day -1) for patients not on insulin (Group 2 and 3); however, for patients on insulin (Group 1) the Run-in Period may be longer (Day –20 to Day −1). Patients on insulin may require a longer Run-in Period in order to be able to adjust their insulin requirements according to the caloric content of the food boxes, if needed. However, it is not mandatory for the patient on insulin to use the entire extended Run-in Period. Based on the Investigator's judgement, the Run-in Period may be shortened once each patient (on insulin) has had sufficient time to adapt to the food boxes, and it is determined that the patient's insulin requirement has stabilised sufficiently to continue in the study. The study will then include a 2-week Treatment Period (Day 1 to Day 14) and a 5-day Follow-up Period: Day 15 to Day 19. Patients will consume food from standardised food boxes (with sodium content 150 mmol) starting on Day −6 (patients not on insulin) or Day −20 at the earliest (patients on insulin) of the study until Day 18 (inclusive). Eligibility will be confirmed on Day −1 based on 24-hr urinary assessments performed on Days −3 and −2 (stable urinary sodium excretion on 2 successive 24-hr urinary sodium excretion measurements ie, <20% difference between Days −3 and −2). Eligible patients will receive dapagliflozin 10 mg tablets once daily for 14±1 days starting on Day 1. This will be followed by a Follow-up Period of 5 days.
Treatment	Patients will receive dapagliflozin 10 mg tablets once daily for 14±1 days.

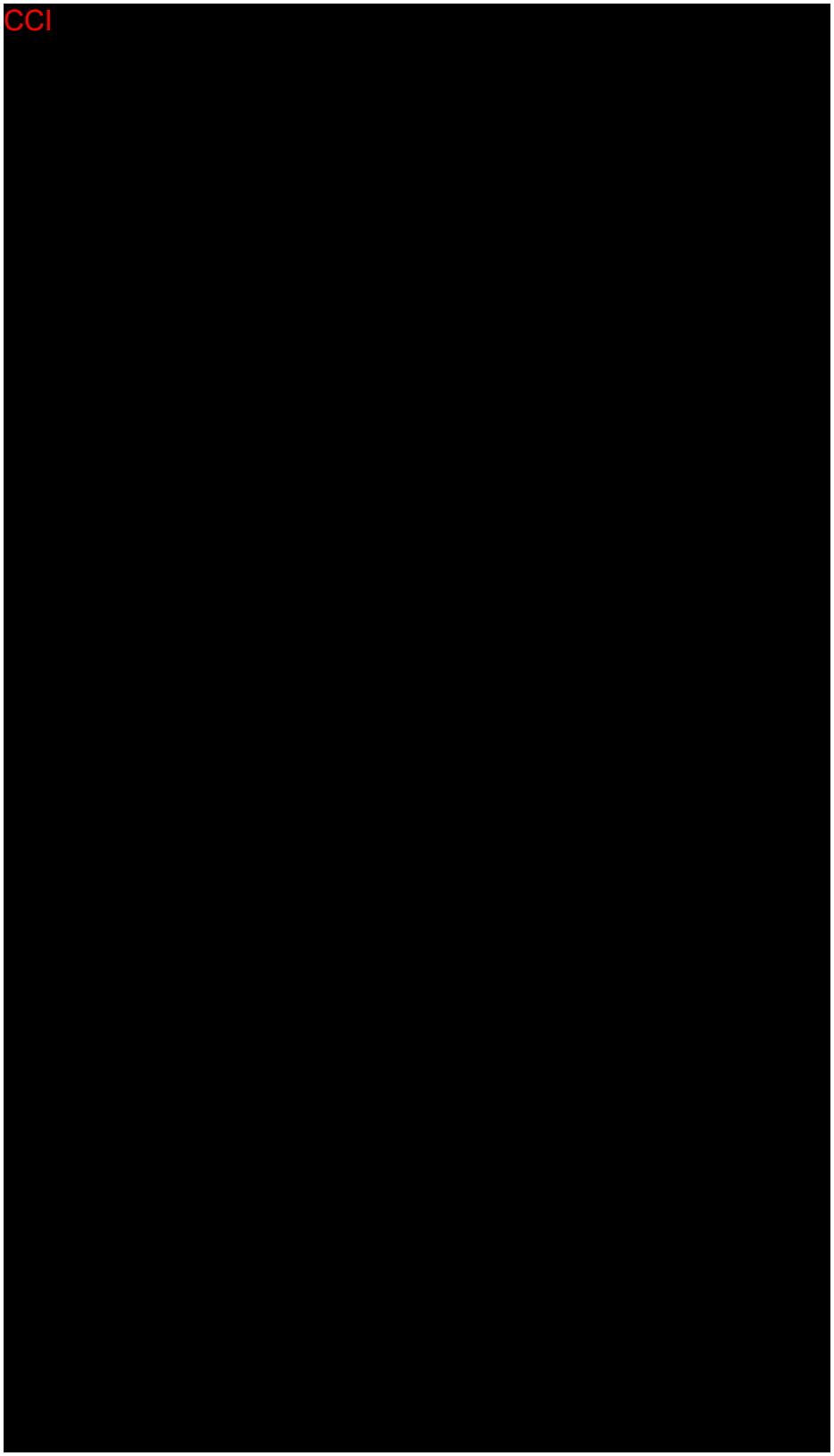
Number of Patients	We plan to enroll a total of 51 patients in the study, with 17 patients in each group.
Population	The study population will consist of Caucasian, Asian or Middle Eastern patients, but Sub-Saharan patients are not eligible. Patients are to be aged between 18 years and ≤80 years, able to provide a written informed consent, and who have one of the following: Group 1: T2DM with an estimated glomerular filtration rate (eGFR) Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI]) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit **or** Group 2: T2DM with an eGFR (CKD-EPI) between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 or older at the Screening Visit **or** Group 3: Non-diabetic with an eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit. The main inclusion criteria are: 1. In the diabetic arms - a diagnosis of T2DM with glycosylated haemoglobin (HbA1c) ≥6.5% (≥48 mmol/mol) and <10% (<86 mmol/mol); and eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 or between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 or older at the Screening Visit (Visit 1). 2. In the non-diabetic arm, HbA1c <6.5% (<48 mmol/mol) and an eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit (Visit 1). 3. Patient-specific optimal antihypertensive dose (as per Investigator's judgement) of an angiotensin receptor blocker (ARB) or Angiotensin Converting Enzyme Inhibitor (ACEi) for at least 6 weeks prior to Visit 4 (Day 1). All patients have to use an ARB or ACEi. 4. In the diabetic arm (Group 2) an appropriate stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1). 5. In the diabetic arm with impaired renal function (Group 1) a stable insulin dosing (intermediate, long-acting, premixed insulin, basal bolus insulin) for the last 12 weeks prior to Visit 4 (Day 1) as judged by the Investigator. Metformin or sulphonylurea, or metformin+sulphonylurea together with insulin would be accepted, but is not mandatory. If used, stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1) is required. 6. Stable urinary sodium excretion on 2 successive 24-hr urinary sodium excretion measurements (<20% difference between Days −3 and −2).
Criteria for Evaluation of Efficacy	**Primary:** • Average change in 24-hr sodium excretion from average Baseline to average values at Day 2 to 4 within each study group. **Secondary:**

The secondary endpoints to be evaluated during or following dapagliflozin treatment within each study group are:

- Average change in 24-hr sodium excretion from average Baseline values to average end of treatment values (Day 12 to 14); and from average end of treatment values (Day 12 to 14) to average values during follow-up (Day 15 to 17).
- Average change in 24-hr glucose excretion from average Baseline values to average values at Day 2 to 4; from average Baseline values to average end of treatment values (Day 12 to 14); and from average end of treatment values (Day 12 to 14) to average values during follow-up (Day 15 to 17).
- Change in mean 24-hr systolic blood pressure from Baseline to Day 4; from Baseline to end of treatment (Day 13); and from end of treatment (Day 13) to end of follow-up (Day 18).
- Change in plasma volume from Baseline to Day 4; from Baseline to end of treatment (Day 14); and from end of treatment (Day 14) to end of follow-up (Day 18).
- Change in extracellular volume from Baseline to Day 4; from Baseline to end of treatment (Day 14); and from end of treatment (Day 14) to end of follow-up (Day 18).
- Dapagliflozin pharmacokinetics on Day 4 and Day 14.
- Average change in mean 24-hr UACR from average Baseline to Day 4; from average Baseline values to average end of treatment values (Day 12 to 14)

CCI

[redacted]


CCI

Criteria for Evaluation of Safety	• Adverse events (AEs) starting from first dose throughout the study. • Serious adverse events (SAEs) starting from Visit 1 throughout the study. • Laboratory variables. • Vital signs. • Physical examination.
Statistical Methods	The following analysis sets will be defined: **Enrolled Set (ENS):** All patients who provided informed consent will be included in the ENS. **Run-in Set**: All patients who are provided with the standard food boxes. **Medication Dispensed Set (MDS**): All patients who dispensed at least one dose of study drug. **Evaluable Subjects Data Set**: This will be a subset of the MDS. It will exclude primary efficacy variable data which may have been affected by protocol deviations as determined by the medical monitor or agreed by the study team. All decisions to exclude data from the Run-in Set to form the Evaluable Subjects Analysis Data Set will be made prior to the database lock of the study. **Safety Set (SAF):** All patients who received at least 1 dose of study drug and who have data from at least one post-dose safety assessment available. **Efficacy:** The primary efficacy endpoint is the average change in 24-hr sodium excretion from average Baseline to average values at Days 2 to 4 within each study group. The primary analysis will be performed separately for each study group using a longitudinal repeated-measures analysis for the "change from average Baseline" as dependent variable, with a term for Baseline value. Point estimates and 95% confidence intervals will be calculated for the adjusted mean changes within each study group. **Safety:**

	All AEs will be summarised by system organ class and preferred term. All AEs will be listed for each patient. Safety laboratory, vital signs, and physical examination findings will be listed by patient and time point including changes from Baseline. Descriptive statistics will be presented by patient status for both absolute values and changes from Baseline. CCI **Partial Analysis:** As recruitment rate for Group 1 is slower than expected, a partial analysis based on all patients from Group 2 and Group 3, but none from Group 1, might be conducted. If such an analysis takes place, a formal Data Review Meeting (DRM) will be held prior to its initiation. At the DRM, all protocol deviations related to the patients in these two groups will be discussed and the impact on the analysis sets to be used for the partial analysis will be assessed. The partial analysis will include all the evaluations originally planned to be conducted, and reported, for all three groups simultaneously.
Study Plan	Refer to Section 7.1.

LIST OF STUDY PERSONNEL

Sponsor	AstraZeneca AB SE-151 85 Södertälje Sweden
Sponsor Protocol Signatories	PPD
Sponsor's Medical Expert	PPD
Scientific Coordinator	PPD
Contract Research Organisation	PAREXEL International (IRL) Limited, 70 Sir John Rogerson's Quay, Dublin 2, Ireland
Adverse Event Reporting	AstraZeneca PAREXEL Medical Services Refer to the Safety Handling Plan for details.

TABLE OF CONTENTS

Tables in Text

Figures in Text

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ABPM	Ambulatory blood pressure monitoring
ACEi	Angiotensin converting enzyme inhibitor
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
CCI	[redacted]
ARB	Angiotensin receptor blocker
AST	Aspartate aminotransferase
CCI	[redacted]
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CSR	Clinical study report
DRM	Data Review Meeting
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
ENS	Enrolled Set
FAS	Full Analysis Set
CCI	[redacted]
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GMP	Good Manufacturing Practice
HbA1c	Glycosylated haemoglobin
IATA	International Airline Transportation Association
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MDS	Medication Dispensed Set

MedDRA	Medical Dictionary for Regulatory Activities
NHE3	Sodium-hydrogen exchanger-3
NSAIDs	Non-steroidal anti-inflammatory drugs
CCI	[redacted]
PPS	Per Protocol Analysis Set
PSSR	Project Specific Safety Requirements
CCI	[redacted]
CCI	[redacted]
SAE	Serious adverse event
SAF	Safety Set
SAP	Statistical analysis plan
SD	Standard deviation
SGLT2	Sodium glucose co-transporter-2
SOC	System organ class
SPC	Summary of Product Characteristics
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCAs	Tricyclic antidepressants
UACR	Urine albumin:creatinine ratio
CCI	[redacted]
WHO-DD	World Health Organisation - Drug Dictionary

1 INTRODUCTION

1.1 Background

Dapagliflozin is a stable, reversible, highly selective, and orally active inhibitor of human renal sodium glucose co-transporter 2 (SGLT2), the major transporter responsible for glucose reabsorption in the kidney. Dapagliflozin's mechanism of action results in the direct and insulin-independent elimination of glucose by the kidneys. Results from nonclinical and clinical studies have shown that dapagliflozin can be used to promote urinary excretion of glucose as a well-tolerated and effective method of reducing blood glucose levels in type 2 diabetes mellitus (T2DM) patients. The persistent loss of glucose with associated calories in the urine, results in a consistent and maintained reduction of the total body weight, in addition to the improved glycemic control. Moreover, dapagliflozin also has been shown to reduce blood pressure and albuminuria, 2 essential prognostic risk factors for progression of renal disease.

Notably, the total amount of glucose excreted in the urine by dapagliflozin, declines with decreasing kidney function. In a recent meta-analysis of placebo controlled clinical trials from the dapagliflozin programme it was confirmed that the glycosylated haemoglobin (HbA1c) lowering effect was smaller in patients with an estimated glomerular filtration rate (eGFR) between 45 and 60 mL/min/1.73m^2 compared to patients with an eGFR > 90 mL/min/1.73m^2 (Sjöström et al, 2016 [2]). Intriguingly however, the effects of dapagliflozin on body weight, blood pressure, albuminuria, and haematocrit were similar regardless of the eGFR level (Sjöström et al, 2016 [2]), and may therefore be independent of glycemic effects of SGLT2 inhibitors.

T2DM is associated with an elevated risk of cardiovascular morbidity and mortality as well as renal failure. The cardiovascular benefit of SGLT2 inhibition was demonstrated in the EMPA-REG study by showing a lower rate of the primary composite cardiovascular outcome and of death in the active empagliflozin treatment arm (Zinman et al, 2015 [3]). There is also a growing body of evidence indicating that SGLT2 inhibition is nephroprotective. Post-hoc analysis from the dapagliflozin phase II and phase III programme have shown in T2DM patients with moderate renal impairment on top of renin-angiotensin-aldosterone system (RAAS) blockade reduction around 40% in albuminuria and stabilization of eGFR decline for up to 1 year (Sjöström et al, 2015 [4]) and 2 years (Fioretto et al, 2015 [5]). After an initial drop in eGFR, kidney function was stable over time while a progressive decrease in eGFR was seen in the placebo group. In another trial with the SGLT2 inhibitor empagliflozin, a 38%, 44% and 55% risk reduction was found in new onset of macroalbuminuria, doubling of serum creatinine and initiation of dialysis treatment respectively. Moreover, the overall risk reduction in cardiovascular death was 22% in patients with chronic kidney disease (CKD) 3 (eGFR 30 to 59 mL/min) (Wanner et al, 2016 [6]).

The nephroprotective effect is thought to be achieved by mechanisms independent of blood glucose reduction (Rajasekeran et al, 2016 [7]), such as by reduced intra-glomerular pressure through an enhanced tubuloglomerular feedback mechanism (De Nicola, et al 2014 [8] and Thomas, 2014 [9]), reduced glucose and sodium transport over the proximal tubule cells (Pollock, et al 1991 [10] and Komala, et al 2013 [11]),

increased natriuresis (Heerspink et al, 2013 [12]) and reduced systemic blood pressure (Baker et al, 2015 [13]).

Since the sodium/volume related effects are believed to be independent of HbA1c reduction this trial will include both patients with and without T2DM.

1.2 Rationale for the Study and Justification of Study Design

1.2.1 Study Rationale

The central hypothesis of this study is that dapagliflozin drives a natriuretic effect independently of renal function level and diabetic status. Hence, the study will evaluate average 24-hr sodium excretion during dapagliflozin treatment in patients with T2DM with preserved or impaired renal function or non-diabetics with impaired renal function.

In the majority of healthy individuals blood pressure falls (dips) at night. These individuals are classified as dippers. Those who do not exhibit this nocturnal fall in blood pressure are referred to as non-dippers. In populations with CKD, non-dippers are overrepresented due to reduced natriuretic capacity (Spencer et al, 2015 and references therein [14]). Decreased natriuresis leads to sodium retention and volume expansion with clinical consequences. Patients with T2DM often have increased extracellular volume as a result of increased glucose and sodium reabsorption in the kidney (Novikov et al, 2016 [15]). We hypothesise that SGLT2 inhibition enables improved natriuresis and diuresis as both sodium and glucose excretion contribute to osmotic diuresis. The mechanisms downstream of natriuresis and diuresis have distinct impact on a number of clinically important parameters. Enhanced natriuresis enables improved systemic sodium balance, which directly impacts both volume expansion as well as systemic sodium load. The failure to maintain natriuretic and fluid balance also results in increased demand to drive natriuresis to a greater extent. Consequently, blood pressure is increased as well as maintained at raised levels during the full 24-hr period generating the non-dipping phenotype. This has clear consequence on cardiovascular fitness via demand placed on cardiac and vascular tissues. In particular sodium and fluid accumulation are associated with impaired endothelial function, vascular stiffening and consequent left ventricular hypertrophy.

As described in the background there is an apparent disconnect between HbA1c lowering and the other effects of SGLT2 inhibition by dapagliflozin (eg, blood pressure, body weight) which may be in part consequent on volume contraction or changes in tubuloglomerular feedback. Tubuloglomerular feedback is the process whereby the macular densa which sits downstream of the proximal tubule senses the delivery of sodium and chloride and provides feedback to the glomerulus to alter afferent and efferent pressure via several mechanisms including renin activity and adenosine production. CCI [redacted] At the same time diuresis leads to decrease arterial pressure and volume. Dapagliflozin is hypothesised to work through both these mechanisms to reduce renal renin activity with concomitant reductions in angiotensin II and its metabolites and alter the production of adenosine. Recent studies have also suggested a cross-talk between the SGLT2 transporter and the sodium-hydrogen exchanger-3 (NHE3) (Novikov et al 2016 [15]). NHE3 is located in the proximal tubule

and responsible for approximately 30% of the reabsorption of sodium in the kidney. Inhibition of SGLT2 results in down regulation of NHE3 sodium transport activity, which may also contribute to the natriuretic effects of dapagliflozin. As a result of the interaction between SGLT2 and NHE3, as well as potential effects (both direct and indirect, eg, via altered renal RAAS activity) on other transporters, the effect of dapagliflozin on electrolyte and fluid related parameters may persist to a significant extent in patients with lower eGFR levels. CCI [redacted] (Schey et al, 2015 [16]).

This study will further clarify the dapagliflozin mechanisms of action by investigating if and how the effect of dapagliflozin on natriuresis, blood pressure regulation, plasma volume, extracellular volume, CCI [redacted] are impacted by T2DM status and level of kidney function.

1.2.2 Study Design

This study is an open label study to evaluate the changes in average 24-hr sodium excretion during dapagliflozin treatment in patients with T2DM with preserved or impaired renal function and in non-diabetics with impaired renal function.

Optimization of Patient Population:

The study will include 3 groups of patients; T2DM patients with either impaired renal function (an eGFR by Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] between ≥25 and ≤50 mL/min/1.73m^2); or with normal renal function (an eGFR [CKD-EPI] between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 years or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69 years, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 years or older [Coresh et al, 2003 17]); and non-diabetic patients with impaired renal function as defined above. Details of eGFR estimation using CKD-EPI are provided in Section 3.1.

All patients will be required to be on patient-specific optimal antihypertensive dose (as per Investigator's judgement) of an angiotensin receptor blocker (ARB) or angiotensin converting enzyme inhibitor (ACEi) prior to being considered for the study. During the study, all patients will be provided with food in standard food boxes which contain in total 150 mmol sodium per day, starting in the Run-in Period prior to treatment initiation and to be continued throughout the duration of the study. Patients in whom 24-hr sodium excretion does not differ by more than 20% at Day −3 and −2 will be allowed to participate in the Treatment Period. This will allow a homogenous stable population to be enrolled in the study for evaluation of the study objective.

Patients with T2DM (Group 2) will be required to be on a stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for 12 weeks prior to start of treatment with dapagliflozin. In the diabetic arm with impaired renal function (Group 1), a stable insulin dosing (intermediate, long-acting, premixed insulin, basal bolus insulin) for the last 12 weeks prior to start of treatment with dapagliflozin is required. In this group metformin or sulphonylurea, or metformin+sulphonylurea together with insulin would be accepted but is not mandatory. If used, stable dose of

metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to start of treatment with dapagliflzoin is required.

Dapagliflozin Dose and Regimen:

Patients will receive one tablet dapagliflozin 10 mg per day for a total period of 14±1 days. This dose is the recommended dose for monotherapy and for add-on combination therapy with other glucose-lowering medicinal products including insulin to improve glycaemic control in T2DM.

Study Endpoints:

The primary endpoint of the study is the average change in 24-hr sodium excretion during dapagliflozin treatment from average Baseline to average values at Day 2 to 4 within each study group. This endpoint will provide information on acute changes in sodium excretion in patients based on their renal function and will enable comparison in patients with T2DM as well as non-diabetic patients. Additionally urinary sodium excretion at Days 12 to 14 will provide information on the effect of 10 mg dapagliflozin on steady state natriuresis, and Days 15 to 17 will provide data on the effect of treatment withdrawal. Additionally, changes in urinary glucose excretion, urine albumin:creatinine ratio (UACR), plasma volume, extracellular volume, and 24-hr systolic blood pressure will also be evaluated in the 3 study groups. Pharmacokinetics of dapagliflozin will also be studied in the 3 study groups.

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[redacted]

1.2.3 Safety Monitoring

Assessment of adverse events (AEs) is part of the study procedures. All serious adverse events (SAEs) will be assessed from the time of signing of informed consent and AEs will be assessed from first dose administration throughout the study including the Follow-up Period.

Patients at risk of volume depletion due to co-existing conditions or concomitant medications, will be carefully monitored for their status. Events of potential diabetic ketoacidosis will be monitored as per Section 6.2.12.

Safety laboratory assessments will be performed at Screening, immediately prior to dosing, 4 days after treatment initiation (for evaluation of serum creatinine), and at the

completion of Treatment Period (Day 14). Vital signs and physical examination will also be monitored in the study.

1.3 Risk-Benefit Assessment

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.

Potential risks

The potential risks for the treatment with dapagliflozin and other SGLT2 inhibitors are described in the Investigator's Brochure (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.

Higher proportions of patients with marked laboratory abnormalities of hyperphosphataemia 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, in patients with type I diabetes mellitus (T1DM) and T2DM, 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 by the Investigator, 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 (eg, T1DM, 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 hypoglycaemia (Defronzo et al, 2013 [18]). 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.

Patients on sulphonylurea and/or insulin at the onset of the study treatment have an increased risk of experiencing hypoglycaemic events. Blood glucose is therefore monitored at Day 4. Once patients on insulin enter the study, they will be carefully followed once starting on food boxes. Additionally, insulin dosing will be adjusted, if needed, to avoid hypoglycaema/hyperglycaemia.

In this study, indocyanine green will be used to assess changes in plasma volume. Indocyanine green has been used for decades for this purpose and has a short half-life of

only approximately 3 min (Jacob et al, 2007 [19]). The risk profile of indocyanine green is considered good (Jacob et al, 2007 [18]), but there are reports of allergic reactions including anaphylactic reactions (Speich et al, 1988 [20] and Garski et al, 1978 [21]). In patients with terminal renal insufficiency, the possibility that an anaphylactic reaction occurs seems to be increased (Summary of Product Characteristics [SPC] Verdye [22]).

Assessment of CCI [redacted] (including extracellular and CCI [redacted]) will be conducted with bioimpedance spectroscopy. This is an easy to use and non-invasive technique not considered to cause any risk to the patient if following the manufacturer's instructions.

No other study procedure is considered putting the patients at risk beyond those ordinarily encountered during the performance of routine medical examinations or routine tests.

Protection against risks

This study has been designed with appropriate measures in place to monitor and minimise any of the potential health risks to participating patients. In order to ensure the safety of all patients participating in this study, AstraZeneca will conduct a real-time review of all safety information from all ongoing clinical dapagliflozin studies as they become available.

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 judgement 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. For this study, patients with current treatment with diuretics will not be included. In hypovolaemic patients starting treatment with dapagliflozin, there is a potential risk for increased serum creatinine levels. Patients who show a greater than 50% increase in serum creatinine should therefore be discontinued on the study drug. 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 judgement

Safety signal detection will include the integration of all available sources of safety information, including clinical study data, AE reports, pre-clinical data, epidemiological studies and literature reports, to identify and characterise unrecognised 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 programme as needed. Thus real-time, active safety surveillance will be conducted during the entire duration of this study.

In addition, all dapagliflozin studies are subject to a carefully designed patient risk management plan that includes the temporary and if necessary permanent discontinuation of the investigational product in individual patients in whom a potential health risk or a laboratory abnormality of clinical concern has been identified.

Due to a potential risk of allergic reactions, the use of indocyanine green should be performed under supervision of a physician. Symptoms related to an allergic reaction

may include unrest, feeling of warmth, pruritus, urticarial, acceleration of heart rate, fall in blood pressure, shortness of breath, bronchospasm, flush, cardiac arrest, laryngospasm, facial oedema, and nausea.

Patients with a known hypersensitivity to indocyanine green, sodium iodide, or iodine, or patients who have poorly tolerated indocyanine green in the past should not use indocyanine green again (see Section 4.2 Exclusion Criteria). Additionally, patients with hyperthyroidism or with autonomic thyroid adenomas are excluded (see SPC [21]). Some medicinal products and substances can reduce or increase absorption of indocyanine green and should thus be avoided (see Section 4.2, Exclusion Criteria).

Due to the risk of allergic reactions including anaphylactic reactions, emergency equipment should be available to immediately start treatment of an allergic reaction if needed.

For the bioimpedance spectroscopy measurements, patients should not be pregnant, have a pacemaker or other implanted electronic devices (see Section 4.2, Exclusion Criteria).

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 limit or no direct clinical benefit for the subjects. In studies of longer duration, in patients randomised to active drug, dapagliflozin is expected to reduce progression of renal failure and reduce cardiovascular mortality. Dapagliflozin is known to decrease body weight (or prevent weight gain) as well as lower blood pressure.

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 health care professional, 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 with respect to other study procedures, participation in this study presents a small and thus acceptable risk to patients who meet the inclusion/exclusion criteria and consent to take part in the study.

2 STUDY OBJECTIVES

2.1 Primary Objective

To investigate change in 24-hr sodium excretion during dapagliflozin treatment between Baseline (average of Days −3 to −1) and average of Days 2 to 4 within each study group in patients with T2DM with preserved or impaired renal function and in non-diabetics with impaired renal function.

2.2 Secondary Objectives

The secondary objectives to be evaluated within each study group during or following dapagliflozin treatment are:

- To evaluate the change in 24-hr sodium excretion during dapagliflozin treatment from Baseline to end of treatment, and during follow-up.
- To evaluate the change in 24-hr glucose excretion.
- To evaluate the change in mean 24-hr systolic blood pressure.
- To evaluate the change in plasma volume.
- To evaluate the change in extracellular volume.
- To evaluate the pharmacokinetics of dapagliflozin.
- To evaluate the change in 24-hr UACR.

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2.4 Safety Objectives

- To evaluate the safety and tolerability of dapagliflozin in each of the target patient populations.

3 OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

This is an open label, mechanistic, three-arm study to evaluate the natriuretic effect of 2-week dapagliflozin treatment in T2DM patients with either preserved or impaired renal function and in non-diabetics with impaired renal function.

The study population will comprise 3 groups of patients:

Group 1: T2DM patients with impaired renal function (an eGFR [CKD-EPI] between ≥25 and ≤50 mL/min/1.73m^2)

Group 2: T2DM patients with normal renal function (an eGFR [CKD-EPI] between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 years or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69 years, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 years or older)

Group 3: Non-diabetic patients with impaired renal function (an eGFR [CKD-EPI] between ≥25 and ≤50 mL/min/1.73m^2)

Estimated GFR using CKD-EPI equation is given below (Levey et al, 2009 [23]):

$$eGFR\ (mL/min/1.73m^2) = 141 \times \min(SCr/\kappa,1)^{\alpha} \times \max(SCr/\kappa,1)^{-1.209} \times 0.993^{Age} \times [1.018\ \text{if female}] \times [1.159\ \text{if black}]$$

SCr = serum creatinine (in mg/dL), κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of SCr/κ or 1, and max indicates the maximum of SCr/κ or 1.

We plan to enroll a total of 51 patients in the study, with 17 patients in each group.

The maximum duration of the study will be 62 days including the allowed window periods for the study (±1 day for Visit 7 at Day 13). The study periods and schedule of visits are presented in Table 5 for patients on insulin (Group 1) and Table 6 for patients not on insulin (Group 2 and Group 3).

Screening (Day −28 to Day −7 [patients not on insulin] and Day −42 to Day −21 [patients on insulin]); Run-in Period (Day −6 to Day −1 [patients not on insulin] and Day −20 to Day −1 [patients on insulin):

All patients will undergo study procedures as listed in Table 5 and Table 6 at the Screening Visit (Visit 1), after signing the informed consent. At this visit, the patients will also have a discussion with the study dietician regarding specific dietary requirements to be adhered to during the study from Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin) of the study. Patients on insulin may require a longer Run-in Period in order to be able to adjust their insulin requirements according to the caloric content of the food boxes, if needed. However, it is not mandatory for the patient on insulin to use the entire extended Run-in Period. During this Run-in Period, there will be regular contacts between the Investigators and patients to ensure that their blood glucose is adequately controlled (by potentially adjusting/optimising their insulin doses) and to confirm that their insulin regime remains stable while on food boxes.

Patient consultation with Investigator may occur at several times from Day -20 to Day -1. Exact dates will be decided on an as needed basis by patient and Investigator. Patients will be contacted by the study dietician by telephone on Day −7 (patients not on insulin) or Day −21 at the earliest (patients on insulin) to remind them about the dietary requirements to be followed from Day −6 (patients not on insulin) or Day −20 at the earliest (patients on insulin) of the study, respectively, and about the food boxes that will be provided to them from Day −6 (patients not on insulin) or Day −20 at the earliest (patients on insulin), respectively.

Following the Screening Visit eligible patients (eligible based on available investigations from Screening Visit) will enter the Run-in Period beginning on Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin). Based on the Investigator's judgement, the Run-in Period may be shortened once each patient (on insulin) has had sufficient time to adapt to the food boxes, and it is determined that the patient's insulin requirement has stabilised sufficiently to continue in the study. Patients will receive food boxes with a daily sodium content of approximately 150 mmol, starting on Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin), throughout the study until Day 18 (inclusive). All patients will be required to adhere to the dietary requirements throughout the period of the study until Day 18 (inclusive). Patients will be required to complete food questionnaires starting Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin) until Day 18, wherein they will record daily adherence to the dietary requirements and record liquid intake and any deviation from the recommended diet. Patients will also record study treatment intake daily in the food questionnaires.

During the Run-in Period, clinic visits are scheduled on Day −2 (Visit 2) and Day −1 (Visit 3). Patients will collect 24-hr urine samples on Day −3, and −2. Patients, in whom 24-hr sodium excretion does not differ by more than 20% from Day −3 to Day −2, will be considered eligible to proceed to the Treatment Period starting Day 1 (Dosing Initiation Visit).

The patients will commence 24-hr ambulatory blood pressure monitoring (ABPM) on Day −1 (ie, the day before the start of dosing). The patients will also collect the 24-hr urine sample on Day −1.

In case the patients are not eligible for the study based on the 24-hr sodium excretion results, the patients will be informed and the 24-hr blood pressure monitoring and 24-hr urine collection will be discontinued.

Treatment Period (Day 1 to Day 14):

On Day 1 (Dosing Initiation Visit), patients will start the administration of one dapagliflozin 10 mg tablet per day. Patients will continue with dapagliflozin until Day 14, following which they will enter a Follow-up Period from Day 15 to Day 19.

During the Treatment Period, the following clinic visits are scheduled: Visit 4 (Day 1: Dosing Initiation Visit), Visit 5 (Day 4), Visit 6 (Day 5), Visit 7 (Day 13) and Visit 8 (Day 14: end of treatment). There will be 4 consecutive 24-hr urine collections on Days 1, 2, 3, and 4 followed by 3 consecutive 24-hr urine collections on Days 12, 13, and 14. Initiation of 24-hr blood pressure monitoring will be done on Days 4 and 13.

Plasma volume assessment and bioimpedance spectroscopy for extracellular volume, CCI [redacted] will be performed on Days 1, 4 and 14. On Day 1, CCI [redacted], plasma volume and bioimpedance spectroscopy measurements will be done prior to the first dose of dapagliflozin. CCI [redacted] Blood samples for pharmacokinetics of dapagliflozin will be collected on Days 4 and 14.

A window period of ±1 day is allowed for Visit 7 at Day 13. Urine collection on Day 12 should start 1 day before Visit 7. Visit 8 will occur 1 day after Visit 7 and the follow-up period of 5 days (Day 15 to Day 19) will start 1 day after Visit 8.

Follow-up Period (Day 15 to Day 19):

On Day 15, treatment with dapagliflozin will be discontinued (last dose to be taken on Day 14) and patients will enter a 5-day Follow-up Period between Day 15 and Day 19. A clinic visit is scheduled on Day 18 to provide the last 24-hr urine samples and participate in the investigations detailed in Table 5 and Table 6. CCI [redacted]. The patients will return to the clinic for the last study visit on Day 19 and return equipment provided.

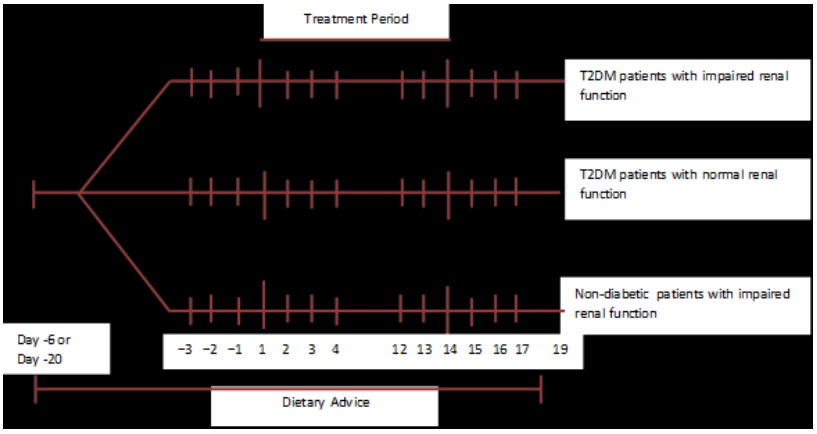
There will be 3 consecutive 24-hr urine collections on Days 15, 16, and 17. On Day 18, 24-hr blood pressure monitoring will be initiated. Plasma volume assessment and bioimpedance spectroscopy for extracellular volume, CCI [redacted] will be performed as well.

After the end of the study, patients will return to their normal clinical care plan.

Stopping rules for individual patients are described in Section 4.5. Stopping rules for the study are described in Section 9.10.

The study design is presented in Figure 1. Refer to the Study Plan (Table 5 and Table 6) for further details of the timings of planned assessments.

Figure 1: Study Design



T2DM=Type 2 diabetes mellitus
Note: lines indicate days on which 24-hr urine is collected.
Note: Day −6 indicates the start of the Run-in Period for patients not on insulin; Day −20 indicates the earliest start of the Run-in Period for patients on insulin.
Patients will be required to adhere to dietary advice and consume food only from the food boxes until Day 18 (inclusive).

3.2 Criteria for Evaluation of the Study

Refer to the study objectives in Sections 2.1, 2.2, 2.3, and 2.4.

3.2.1 Primary Endpoint

- Average change in 24-hr sodium excretion from average Baseline to average values at Day 2 to 4 within each study group.

3.2.2 Secondary Endpoints

The secondary endpoints to be evaluated during or following dapagliflozin treatment within each study group are:

- Average change in 24-hr sodium excretion from average Baseline values to average end of treatment values (Day 12 to 14); and from average end of treatment values (Day 12 to 14) to average values during follow-up (Day 15 to 17).
- Average change in 24-hr glucose excretion from average Baseline values to average values at Day 2 to 4; from average Baseline values to average end of treatment values (Day 12 to 14); and from average end of treatment values (Day 12 to 14) to average values during follow-up (Day 15 to 17).
- Change in mean 24-hr systolic blood pressure from Baseline to Day 4; from Baseline to end of treatment (Day 13); and from end of treatment (Day 13) to end of follow-up (Day 18).
- Change in plasma volume from Baseline to Day 4; from Baseline to end of treatment (Day 14); and from end of treatment (Day 14) to end of follow-up (Day 18).
- Change in extracellular volume from Baseline to Day 4; from Baseline to end of treatment (Day 14); and from end of treatment (Day 14) to end of follow-up (Day 18).
- Dapagliflozin pharmacokinetics on Days 4 and 14.
- Average change in mean 24-hr UACR from average Baseline to Day 4; and from average Baseline values to average end of treatment values (Day 12 to 14).

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[redacted]

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3.2.4 Safety Endpoints

- AEs starting from first dose throughout the study.
- SAEs starting from Visit 1 throughout the study.
- Laboratory variables.
- Vital signs.
- Physical examination.

4 STUDY POPULATION

The study population will consist of patients with one of the following:

- T2DM with an eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit or
- T2DM with an eGFR (CKD-EPI) between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 years or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69 years, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 years or older at the Screening Visit or
- Non-diabetic with an eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit.

Patients must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

4.1.1 Inclusion Criteria at Screening Visit (Visit 1)

Patients will be entered into this study only if they meet all of the following criteria:

1. Provision of signed and dated, written informed consent prior to any study-specific procedures.
2. Caucasian, Asian or Middle Eastern (sub-Saharan patients are not eligible); female and/or male aged between 18 years and ≤80 years.
3. In the diabetic arms - a diagnosis of T2DM with HbA1c ≥6.5% (≥48 mmol/mol) and <10% (<86 mmol/mol); and eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 or between >90 and ≤130 mL/min/1.73m^2 for patients aged 59 years or younger, between >85 and ≤130 mL/min/1.73m^2 for patients aged 60 to 69 years, and between >75 and ≤130 mL/min/1.73m^2 for patients aged 70 years or older at the Screening Visit (Visit 1).
4. In the non-diabetic arm, HbA1c <6.5% (<48 mmol/mol) and an eGFR (CKD-EPI) between ≥25 and ≤50 mL/min/1.73m^2 at the Screening Visit (Visit 1).
5. Patient-specific optimal antihypertensive dose (as per Investigator's judgement) of an ARB or ACEi for at least 6 weeks prior to Visit 4 (Day 1).
6. In the diabetic arm (Group 2) an appropriate stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1).
7. In the diabetic arm with impaired renal function (Group 1) a stable insulin dosing (intermediate, long-acting, premixed insulin, basal bolus insulin) for the last 12 weeks prior to Visit 4 (Day 1) as judged by the Investigator. Metformin or sulphonylurea, or metformin+sulphonylurea together with insulin would be accepted but is not mandatory. If used, stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1) is required.

8. Suitable veins for cannulation or repeated venepuncture
9. Female patients must be 1 year post-menopausal, surgically sterile, or using an acceptable method of contraception (an acceptable method of contraception is defined as a barrier method in conjunction with a spermicide) for the duration of the study (from the time they sign consent) and for 3 months after the last dose of study drug to prevent pregnancy. In addition, oral contraceptives, approved contraceptive implant, long-term injectable contraception, intrauterine device, or tubal ligation are allowed. Oral contraception alone is not acceptable; additional barrier methods in conjunction with spermicide must be used.

4.1.2 Inclusion Criteria at End of Run-in Period (Visit 3)

Patients must fulfil the following criteria in order to continue participation in the study.

10. Patient-specific optimal antihypertensive dose (as per Investigator's judgement) of an ARB or an ACEi for at least 6 weeks prior to Visit 4 (Day 1).
11. In the diabetic arm (Group 2) an appropriate stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1).
12. In the diabetic arm with impaired renal function (Group 1) a stable insulin dosing (intermediate, long-acting, premixed insulin, basal bolus insulin) for the last 12 weeks prior to Visit 4 (Day 1) as judged by the Investigator. Metformin or sulphonylurea, or metformin+sulphonylurea together with insulin would be accepted but is not mandatory. If used, stable dose of metformin or sulphonylurea, or metformin+sulphonylurea as anti-diabetic therapy for the last 12 weeks prior to Visit 4 (Day 1) is required.
13. Stable urinary sodium excretion on 2 successive 24-hr urinary sodium excretion measurements (<20% difference between Days −3 and −2).

4.2 Exclusion Criteria

4.2.1 Exclusion Criteria at Screening Visit (Visit 1)

Patients will not be entered into this study if they meet any of the following criteria:

Study-related:

1. Previous enrolment in the present study or participation in another clinical study with an investigational product during the last 6 months prior to Screening Visit (Visit 1).
2. Involvement in the planning and conduct of the study (applies to both AstraZeneca staff and staff at third party vendor or at the investigational sites).
3. Hypersensitivity to dapagliflozin, indocyanine green, sodium iodide, or iodine, or patients who have poorly tolerated indocyanine green in the past.
4. Pacemaker or other implanted electronic devices.

5. Pregnancy.
6. Breastfeeding.

General health-related:

7. Known clinically significant disease or disorder; or clinically relevant abnormal findings in physical examination, clinical chemistry, haematology, and urinalysis; or unstable or rapidly progressing renal disease; or any other condition or minor medical complaint, which, in the opinion of the Investigator, may either put the patient at risk because of participation in the study, or influence the results, or the patient's ability to participate in the study and comply with study procedures, restrictions and requirements.
8. Diagnosis of T1DM.
9. Hyperthyroidism or autonomic thyroid adenomas.
10. Abnormal vital signs, after 10 minutes supine rest, defined as any of the following (Visit 1):
    - Systolic blood pressure above 180 mmHg.
    - Diastolic blood pressure above 110 mmHg.
    - Pulse <50 bpm or >100 bpm
11. Any of the following cardiovascular/vascular diseases within 3 months prior to signing the consent at Visit 1, as assessed by the Investigator: myocardial infarction, cardiac surgery or revascularization (coronary artery bypass graft [CABG]/ percutaneous transluminal coronary angioplasty [PTCA]), unstable angina, unstable heart failure, heart failure New York Heart Association Class IV, transient ischemic attack or significant cerebrovascular disease, unstable or previously undiagnosed arrhythmia.
12. Patients with severe hepatic impairment (Child-Pugh C).
13. Ongoing weight-loss diet (hypocaloric diet) or use of weight-loss agents, unless the diet or treatment has been stopped at least 3 months before Screening Visit, ensuring patients having a stable body weight with no verified body weight variability of >3 kg during the 3 months before Screening Visit.

Renal failure-related:

14. Symptoms/complaints suggestive of established neurogenic bladder and/or incomplete bladder emptying.
15. History of bladder cancer.
16. Diagnosis of polycystic kidney disease.
17. History of or current lupus nephritis.
18. UACR >1000 mg/g per day at the Screening Visit based on spot urine sample (quantitative assessment).

Concomitant Medication and/or study treatment-related:

19. Current/chronic use of the following medication: any anti-diabetic medication with the exception of metformin, sulphonylurea, insulin (insulin only allowed in Group 1), oral glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs), immune suppressants, chemotherapeutics, antipsychotics, tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors.
20. Receiving immunosuppressive or other immunotherapy for primary or secondary renal disease within 6 months prior to Screening Visit (Visit 1).
21. Current treatment or treatment within the last 2 weeks prior to Screening Visit (Visit 1) with diuretics including loop diuretics, thiazides, and mineralocorticoid antagonists.
22. A metformin dose which is outside the specified dose range for renal impairment according to local guidelines and/or Investigator's judgement.
23. Medicinal products and substances that can reduce or increase absorption of indocyanine green: anticonvulsants, bisulphite compounds, haloperidol, heroin, meperidine, metamizol, methadone, morphium, nitrofurantoin, opium alkaloids, phenobarbital, phenylbutazone (reduced absorption), and cyclopropane, probenecid, rifamycin (increased absorption).

4.3 Rescreening

Rescreening in this study will be allowed once per patient only under the following circumstances:

- If a patient consents to participate, meets the eligibility criteria but is no longer able to be enrolled on a certain date due to personal reasons (eg, family crisis, vacation, etc.).
- If the Investigator discovers during the Screening Period, that a patient requires an adjustment of their regular medication, in line with the local standard of care, for instance, any changes required with regard to their antihypertensive (ARB or ACEi) or anti-diabetic (metformin, sulphonylurea, metformin+sulphonyurea or insulin) treatment, such a patient may be rescreened once the patient's medical condition has stabilised, as judged by the Investigator. The time required before such a patient is rescreened will be assessed on a case-by-case basis as per the judgement of the Investigator.
- In case errors occurred during the 24-hr urine collection process, for instance urine spillage/loss of urine/not used the correct amount of salt sticks, etc. In such a case, the patient may be retested to accurately assess the sodium excretion. This is done by restarting the urinary sampling days with start from Day −3 again. The new urinary collections should be used for analysis.
- In case a patient previously failed screening based on older inclusion and exclusion criteria in previous versions of the protocol but may now be eligible based on amended inclusion and exclusion criteria (e.g. age-specific eGFR ranges, age etc).

- Retesting of patients will be allowed in the Screening Visit window period at the discretion of Investigator in case there is a single laboratory measurement that is out of range. In case of multiple laboratory parameters out of range and considered not clinically significant, retesting will be allowed.
- In case the eGFR performed at Screening is out of range and the Investigator is of the opinion that the patient is eligible based on local laboratory values, a single repeat of the eGFR test is allowed.

4.4 Study Restrictions

Patients entered into the study must:

1. Patients should strictly adhere to the dietary requirement including drinks in the study and consume food only from the food boxes provided to them throughout the study starting Day -6 (patients not on insulin) or Day –20 at the earliest (patients on insulin), and up to Day 18 (included). Patients on insulin may require a longer Run-in Period in order to be able to adjust their insulin requirements according to the caloric content of the food boxes, if needed. However, it is not mandatory for the patient on insulin to use the entire extended Run-in Period. Based on the Investigator's judgement, the Run-in Period may be shortened, once each patient (on insulin) has had sufficient time to adapt to the food boxes, and it is determined that the patient's insulin requirement has stabilised sufficiently to continue in the study. During this Run-in Period, there will be regular contacts between Investigators and patients to ensure that their blood glucose is adequately controlled (by potentially adjusting/optimising their insulin) and to confirm that their insulin regime remains stable while on food boxes. Patient consultation with Investigator may occur at several times from Day -20 to Day -1. Exact dates will be decided on an as needed basis by patient and Investigator.
2. Not use drugs of abuse during the entire study. One alcoholic beverage a day is only allowed between Visit 1 and Day −7 (patients not on insulin) / Day −21 at the earliest (patients on insulin). Alcohol should not be consumed on days when urine samples are collected.
3. Women of childbearing potential: use contraception as specified in inclusion criteria 9, in Section 4.1.

Restrictions for clinic visit days:

4. CCI [redacted].

Restrictions prior to measurements for plasma volume and bioimpedance spectroscopy:

5. Plasma volume assessment and bioimpedance spectroscopy measurements will be done at Day 1, Day 4, Day 14 and Day 18. Prior to the test, patients should adhere to non-caffeinated drinks.

6. During the measurement procedure, patients are allowed to move around, to eat a snack and to drink non-caffeinated drinks.

4.5 Patient Withdrawal and Discontinuation

Patients may be discontinued from study treatment and assessments at any time in the following situations.

- Patient decision. The patient is free at any time to discontinue treatment, without prejudice to further treatment.
- Severe non-compliance to protocol, as judged by the Investigator and/or AstraZeneca.
- Safety reasons, eg, AE that warrants discontinuation, as judged by the Investigator and/or AstraZeneca.
- Incorrect enrolment (ie, the patient does not meet the required inclusion/exclusion criteria) for the study.
- Patient lost to follow-up.
- Pregnancy.
- Major hypoglycaemic events.

  Subjects should not be discontinued from any treatment phase based on single episodes of hypoglycaemia or symptoms of hypoglycaemia unless clinically indicated (see the criteria below). The assessment of a single fingerstick or local laboratory glucose value should not be the sole assessment used to determine patient discontinuation due to hypoglycemia.

  Subjects should be discontinued from study drug if they experience severe hypoglycaemia as defined by the American Diabetes Association:

  - 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 return of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.
- Suspected/potential diabetic ketoacidosis based on Investigator's judgement.
- Patients who show a greater than 50% increase in serum creatinine on Day 4 should be discontinued from the study drug.

In all cases, the reason(s) for withdrawal and the primary reason must be recorded on electronic case report form (eCRF). If a patient is prematurely withdrawn from the study treatment for any reason, the Investigator must make every effort to perform the evaluations described for Visit 8.

Patients will also be withdrawn if the entire study is terminated prematurely as described in Section 9.10.

Refer to Section 4.3 for rescreening of patients for the study.

4.6 Planned Sample Size and Number of Study Centres

We plan to enroll a total of 51 patients at up to 5 study centres in 3 equal sized groups. Each group will enroll 17 patients. It is planned to enroll patients in Europe. Refer to Section 8.6 for sample size justification.

4.7 Patient Identification and Randomisation

4.7.1 Patient Identification

At Screening, each patient will receive a unique enrolment number. Patients who drop out of the study before dosing will retain their assigned enrolment number.

The enrolment number will be in the following format and will be recorded in the eCRF:

E code format (E_Site ID_Patient ID), eg, E0010001.

The enrolment number will serve as the patient identifier throughout the study and will be required in all communication between the Investigator or designee regarding a particular patient.

4.7.2 Randomisation Scheme

There is no randomisation in the study.

4.7.3 Allocation of Patients to Treatment

Start of treatment will occur at Visit 4 (Day 1) after all Screening procedures have been performed and eligibility for the study confirmed. Patients will be enrolled to specific groups based on their diagnosis. When 17 patients have been enrolled to each specific patient group enrolment into that group will stop.

5 STUDY DRUG

5.1 Identity

Dapagliflozin is a highly potent, selective and reversible inhibitor of SGLT2.

5.1.1 Investigational Products

For this study, study treatment refers to dapagliflozin 10 mg tablets.

Details of the study treatment can be found in Table 1. Dapagliflozin 10 mg tablets will be supplied by AstraZeneca in labelled bottles containing 35 tablets.

Table 1: Identity of the Study Drug

Study drug	Dosage form and strength	Manufacturer
Dapagliflozin	Plain, green, diamond-shaped, film coated tablet, 10 mg	AstraZeneca

Details of the batch numbers will be included in the Trial Master File and the final clinical study report (CSR).

5.2 Administration

The study consists of a 2-week, open label, Treatment Period. Patients will be provided with one bottle of dapagliflozin tablets on Day 1 (Visit 4) to last for the 14±1 days of the Treatment Period.

The tablet is taken orally once daily in the morning and at approximately the same time of the day.

There are no restrictions regarding timing in relation to food. Dapagliflozin tablets contain lactose, which may cause discomfort in lactose-intolerant individuals.

The first dose of dapagliflozin will be administered at Visit 4 (Day 1) at the study site after all baseline assessments (including laboratory tests, plasma volume assessments, and bioimpedance spectroscopy measurement) have been performed.

On days of study site visits where blood sampling is scheduled, ie, Visit 5 and Visit 8, the patients will be required to bring the bottle of dapagliflozin tablets to site. At Visit 5 (Day 4), the patient will consume the tablet together with breakfast after fasting blood sampling is completed at the site. At Visit 8 (Day 14), the patient will consume the tablet together with breakfast after pre-dose bioimpedance spectroscopy measurements, pharmacokinetics blood sampling, CCI [redacted].

5.3 Packaging, Labelling, and Storage

Patients will be provided with study drug according to the study plan Table 5 (Group 1) and Table 6 (Group 2 and Group 3).

The study drug will be packaged by AstraZeneca or designee according to Good Manufacturing Practice (GMP).

Labels will be prepared in accordance with GMP and local regulatory guidelines. The labels will fulfil GMP Annex 13 requirements for labelling. Label text will be translated into local language.

The study drug should be kept in a secure place under appropriate storage conditions. The study drug label on the bottle specifies the appropriate storage.

5.4 Blinding and Breaking the Blind

This is an open label study and hence blinding is not applicable.

5.5 Drug Accountability

The study drug provided for this study will be used only as directed in the study protocol.

The study personnel will account for all study drugs dispensed to and returned from the patient.

The Investigator is responsible for making sure:

- That the study drug is handled and stored safely and properly (see Section 5.3).
- That the study drug is only dispensed to study patients in accordance with this protocol.

Patients should return all unused study drug and empty containers to the Investigator.

At the termination of the clinical study or at the request of AstraZeneca, remaining drug and packaging of used study drug after final drug accountability, are to be destroyed locally in line with local regulations. On destruction of the study drug at the site, the site personnel will account for all unused study drug and for appropriate destruction documentation. Certificates of delivery, destruction and return should be signed and archived.

Each dispensing and returning of study drug will be documented in the eCRF.

5.6 Compliance

Patients will be instructed on proper use of study drug.

Compliance with study treatment will be monitored via the drug accountability assessments (tablet counts at Visit 8) and patient diary entries (ie, patients will record study drug intake daily in the food questionnaires and this will be reviewed at the clinic visits during the Treatment Period).

5.7 Previous and Concomitant Medications

Any medication the patient takes other than the study treatment, including herbal and other non-traditional remedies, is considered a concomitant medication.

All concomitant medications must be recorded in the eCRF. The following information must be recorded in the eCRF for each concomitant medication: generic name, route of administration, start date, stop date, dose, regimen, and indication.

Any changes in the dosage or regimen of a concomitant medication must be recorded in the eCRF.

At Screening, patients will be asked what medications they have taken during the last 3 months. At each subsequent study visit, patients will be asked if there have been any changes to their concomitant medications or standard of care medication for T2DM or renal failure (as applicable), and whether they have taken any other medications since the previous visit.

5.7.1 Allowed Concomitant Medications

Background therapy for T2DM and renal failure:

All patients will continue to use their ARB or ACEi at the same dose that they were on prior to start of the study.

Diabetic patients will continue on metformin or sulphonylurea, or metformin+sulphonylurea therapy at the same dose that they were on prior to start of the study. For patients on insulin (Group 1), recording of total insulin doses will be done during the study.

At each visit, the Investigator will reassess whether the concomitant medication that the patient is taking is part of the original maintenance for T2DM or renal failure or whether the patient has started taking extra concomitant medication. The status of each concomitant medication (ie, pre-existing or new) will be documented on the eCRF.

Therapies for treatment of other comorbidities are allowed as long as the dose regimen is stable in the 3 months prior to first dose and is not a prohibited medication (Section 5.7.2). All efforts should be made to keep the dose of study drug stable throughout the study.

5.7.2 Prohibited Concomitant Medications

Refer to Section 4.2 for exclusion criteria relating to prohibited medications.

The following medications are prohibited during the study:

- Immunosuppressive or other immunotherapy for primary or secondary renal disease.
- Diuretic drugs including loop diuretics, thiazides, mineralocorticoid receptor antagonists.
- Use of any anti-diabetic medications with the exception of metformin, sulphonylurea and insulin (insulin only allowed in Group 1), oral glucocorticoids, NSAIDs, immune suppressants, chemotherapeutics, antipsychotics, TCAs and monoamine oxidase inhibitors.
- Use of medicinal products and substances that can reduce or increase absorption of indocyanine green: anticonvulsants, bisulphite compounds, haloperidol, heroin, meperidine, metamizol, methadone, morphium, nitrofurantoin, opium alkaloids, phenobarbital, phenylbutazone (reduced absorption), and cyclopropane, probenecid, rifamycin (increased absorption).

5.7.3 Non-investigational Product

The product used for plasma volume measurements (indocyanine green) is considered a non-investigational product (see Section 6.1.3 for details).

6 VARIABLES AND METHODS OF ASSESSMENT

6.1 Efficacy Variables

Assessments will be performed in accordance with the Study Plan (Table 5 [Group 1] and Table 6 [Group 2 and Group 3]).

6.1.1 24-hr Urine Measurement

24-hr urine collection will be performed as per schedule provided in Table 5 and Table 6. The patients will be provided with equipment for collection and storage of urine samples. Patients receive empty 24-hr urine collection containers preceding the collection days according to instructions. At the Screening Visit, patients will be instructed to start collection of urine samples starting Day −3. The morning the patient starts with the 24-hr urine collection, the bladder is emptied. The 24-hr urine collection starts after the first morning urine is voided in the toilet. The urine is collected in the container for 24 hrs. The date and time of the start and end of the urine collection should be written on the designated labels. Patients will be advised to store the urine in the refrigerator after finishing the 24-hr urine collection until he/she delivers the urine to the clinic. The carefully sealed container will be delivered to the research nurse/physician at the day of the next clinic visit. The importance of obtaining a complete urine collection will be stressed. The patients will return the equipment on the days specified in Table 5 and Table 6.

The local laboratory will receive the urine samples and will record the volume of urine collected for every 24-hr urine sample.

For premenopausal women, the Investigator will estimate the menstrual timing and aim to avoid the 2 urine collection periods (Day −3 to 4 and Day 12 to 17) during menstruation.

6.1.2 24-hr BP Monitoring

Ambulatory blood pressure monitoring will be performed to record 24-hr systolic and diastolic blood pressure at Days −1, 4, 13, and 18.

The ABPM manual will contain more detail on the specific device and method of data capture/transfer.

6.1.3 Plasma Volume Measurements

Plasma volume will be assessed using indocyanine green distribution in the circulation. Measurements will be performed prior to dosing on Day 1 (Baseline) and following dosing on Days 4 and 14. Plasma volume will also be assessed on Day 18.

6.1.3.1 Dosage, Form Strength and Storage of Indocyanine Green

Indocyanine green (tradename Verdye, manufacturer Diagnostic Green) 5mg /mL is provided as powder for solution. Single doses of 0.25 mg/kg body weight will be administered as bolus injection. For details on storage of indocyanine green see SPC [21].

6.1.3.2 Administration of Indocyanine Green

Indocyanine green is to be reconstituted with water for injection (not solutions containing salt). See SPC [21] for more information.

After the patient is weighed, a catheter will be positioned for example in an anticubital vein for the injection of indocyanine green. A second catheter will be positioned in the contralateral arm for blood sampling.

Blood collections will start exactly 2 minutes after injection of indocyanine green and will be collected for a 3-minute period at 30 sec intervals (7 blood samples). The administration of indocyanine green is of critical importance for the quality of the plasma volume measurements. It is important that the exact time of the samples is noted. Further details can be found in the manual for plasma volume measurements.

6.1.3.3 In Case of Anaphylactic Reactions due to Indocyanine Green

The risk profile of indocyanine green is considered good, but there are reports of allergic reactions including anaphylactic reactions (see Section 1.3). Since severe anaphylactic reactions might occur, indocyanine green must only be applied under supervision of a physician. If an anaphylactic reaction should occur, immediate emergency treatment should be initiated and may include below actions as judged necessary by the physician:

- Stop further administration of indocyanine green, leave injection catheter or cannula in the vein.
- Keep airways open.
- Inject 100 to 300 mg hydrocortisone or a similar preparation by rapid intravenous injection.
- Substitute volume with isotonic electrolyte solution.
- Give oxygen, monitor circulation.
- Slowly administer antihistamines intravenously.

The following additional measures may be indicated in cases of anaphylactic shock:

- Place patient in recumbent position with legs raised.
- Rapidly substitute volume with eg, isotonic electrolyte solution (pressure infusion), plasma expanders.
- Immediately administer 0.1 to 0.5 mg adrenaline (epinephrine) diluted to 10 mL with 0.9% saline intravenously (repeat after 10 min if necessary).

6.1.4 Bioimpedance Spectroscopy Measurements (Extracellular Volume, CCI

Bioimpedance spectroscopy will be performed prior to dosing on Day 1 (Baseline) and following dosing on Days 4 and 14. Bioimpedance spectroscopy will also be performed in the Follow-up Period on Day 18. CCI [redacted] Bioimpedance spectroscopy will be assessed using the SFB7 device produced by ImpediMed.

For the bioimpedance spectroscopy, patients should void their bladder and add to the ongoing 24-hr urine collection. Patients should abstain from exercising 2 hr prior to the assessment.

An alcohol swab is used to clean the skin, allowing the alcohol to dry completely before placing the electrodes on the skin. If there is excess hair on the sites where the electrodes will be attached, not allowing proper electrode adherence, these sites will be shaved.

The patient should be put in the supine position for 5 min before the measurement. The patient should not touch any metal surfaces while in the supine position. It has to be ensured that the patient's limbs are not crossed and that the legs are completely separated. For patients who cannot effectively separate their inner thighs, it may be necessary to place insulating material (eg, a towel) between them. It has to be ensured also that the patient's arms are not touching the torso. The bioimpedance spectroscopy should be set to make 3 repeat measurements (see manual instructions for SFB7 device). The patient has to remain still and relaxed during the measurement.

When several measurements are made on the same day, the patient should not remain supine between readings. The amount of time in supine position should be the same on each assessment day and at each assessment time.

The points of contact for the electrodes should be marked on the patient‘s skin and the same positions used at all occasions. Preferably, electrodes should be connected to the right side of the body (hand and foot according to the manual instructions for SFB7).

It has to be ensured that the bioimpedance spectroscopy machine is charged and not connected to mains power during use.

6.1.5 Efficacy Laboratory Assessments

Table 2 presents the laboratory assessments that will be performed for evaluation of the endpoints. Refer to Table 5 and Table 6 study plan for schedule of assessments.

CCI [redacted].

Instructions for collection, storage, and shipment of samples will be provided in the Laboratory Manual for samples collected at central laboratories.

CCI [redacted]

CCI [redacted]

CCI [redacted]

Table 2: Efficacy Laboratory Variables

	Local Laboratory	Central Laboratories
Primary and Secondary Variable		
24-hr urinary sodium excretion	- Sodium	-
Secondary Variables		
24-hr urinary glucose excretion	- Glucose	-
Changes in plasma volume	-	- Plasma volume
Dapagliflozin pharmacokinetics	-	- Plasma concentration of dapagliflozin
Change in 24-hr UACR	- UACR	-
CCI [redacted]		
[redacted]	[redacted]	[redacted]
[redacted]		
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]		[redacted]

CCI [redacted] UACR=urine albumin: creatinine ratio

CCI [redacted]

6.2 Safety Variables and Safety Reporting

The Investigator is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

Assessments will be performed in accordance with the Study Plan (Table 5 [Group 1] and Table 6 [Group 2 and Group 3])

6.2.1 Definition of Adverse Events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including during Run-in or Wash-out Periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs.

6.2.2 Definitions of Serious Adverse Event

An SAE is an AE occurring during any study phase (ie, Run-in, Treatment, Wash-out, Follow-up), that fulfils one or more of the following criteria:

- Results in death.
- Is immediately life-threatening.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital abnormality or birth defect.
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of an SAE, see Section 11.1.

6.2.3 Recording of Adverse Events

6.2.3.1 Time Period for Collection of Adverse Events

All AEs will be collected from the time of first dose throughout the study, including the Follow-up Period.

All SAEs will be collected from the time of signing the informed consent throughout the study, including the Run-in and Follow-up Periods.

6.2.3.2 Follow-up of Unresolved Adverse Events

Any AEs that are unresolved at follow-up are followed up by the Investigator for as long as medically indicated, but without further recording in the eCRF. AstraZeneca retains the right to request additional information for any patient with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

6.2.3.3 Variables

The following variables will be collected for each AE:

- AE (verbatim).
- The date and time when the AE started and stopped.
- Intensity.
- Whether the AE is serious or not.
- Investigator causality rating against the study drug (yes or no).
- Action taken with regard to study drug.
- AE caused patient's withdrawal from study (yes or no).
- Outcome.

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for a serious AE.
- Date Investigator became aware of the SAE.
- AE is serious due to.
- Date of hospitalisation (if applicable).
- Date of discharge (if applicable).
- Probable cause of death (if applicable).
- Date of death (if applicable).
- Autopsy performed (if applicable).
- Causality assessment in relation to Study procedure(s).
- Causality assessment in relation to other medication (eg, concomitant medication, background therapy).
- Description of SAE.

The intensity of the reported AEs 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 6.2.2. 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 6.2.2. 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 6.2.2.

6.2.3.4 Causality Collection

The Investigator will assess causal relationship between the study treatment and each AE, 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?'

For SAEs the causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

A guide to the interpretation of the causality question is found in Section 11.1.

6.2.3.5 Adverse Events Based on Signs and Symptoms

All AEs spontaneously reported by the patient or reported in response to the open question from the study personnel: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, 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.

6.2.3.6 Adverse Events Based on Examinations and Tests

The results from protocol-mandated laboratory tests and vital signs will be summarised in the CSR. 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 or are the reason for discontinuation of study treatment.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (eg, 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.

6.2.4 Reporting of Serious Adverse Events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, then Investigators or other site personnel inform the appropriate AstraZeneca representatives (PAREXEL Medical services team) within one day, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative (PAREXEL Medical services team) works with the Investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site **within 1 calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives (PAREXEL Medical services team) of any follow-up

information on a previously reported SAE within one calendar day, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

Once the Investigators or other site personnel indicate an AE is serious in the eCRF, an automated email alert is sent to the designated AstraZeneca representative (PAREXEL Medical services team).

If the electronic data capture (EDC) system is not available, then the Investigator or other study site personnel reports an SAE to the appropriate AstraZeneca representative (PAREXEL Medical services team) by telephone.

The AstraZeneca representative (PAREXEL Medical services team) will advise the Investigator/study site personnel how to proceed.

The reference document for definition of expectedness/listedness is the IB for the AstraZeneca drug.

6.2.5 Laboratory Variables

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be taken in accordance with the Study Plan (Table 5 and Table 6) and sent to the local laboratory for analysis.

Additional safety samples may be collected if clinically indicated at the discretion of the Investigator. If local retesting occurs, the date, time of retesting and results (values, units and reference ranges) will be recorded in the appropriate section of the eCRF. Blood samples will be collected after at least 8 hr overnight fasting (except for the Screening Visit where fasting is not required).

Table 3 specifies the safety laboratory variables to be determined.

Standardised and validated laboratory reference ranges will be provided by the local laboratory performing the assessments.

Table 3: Laboratory Assessments

Haematology/ haemostasis (whole blood):	**Clinical Chemistry (serum [S] or plasma [P])**
B-Haemoglobin B-Haematocrit [a] HbA1c[b]	S-Creatinine [a] S-Bilirubin, total P-Glucose [a] S-ALP S-AST S-ALT
Pregnancy testing	**Urinalysis**
Women: Urine pregnancy test[b] –for women of childbearing potential (HCG minimum sensitivity of 25 IU/L), dipstick analysed at the study centre Serum βHCG (if urine pregnancy dipstick result is positive)	**Dipstick:** U-Blood U-Albumin U-Glucose **Spot urine sample:** U-Albumin/U-Creatinine[b]

ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, B=blood, HCG=human chorionic gonadotropin, S=serum, P=plasma, U=urine

a. CCI [redacted]
b. These tests will be performed for verifying inclusion/exclusion criteria and not as a safety measure.

For information on how AEs based on laboratory tests should be recorded and reported, see Section 6.2.3.6.

6.2.6 Vital Signs

The following vital signs will be assessed in accordance with the Study Plan (Table 5 and Table 6):

- Pulse (beats/minute).
- Blood pressure (systolic blood pressure and diastolic blood pressure; mmHg).

Pulse (beats/minute, during 30 seconds) will be measured before blood pressure and in a supine position after minimum 5 minutes (preferably 10 minutes) of rest. Thereafter, systolic blood pressure and diastolic blood pressure (mmHg, the cuff method on the arm opposite to the one used for blood sampling) will be measured using the same cuff, appropriate for arm circumference, and in the same position, throughout the study.

For information on how AEs based on vital signs measurements should be recorded and reported, see Section 6.2.3.6.

6.2.7 Physical Examination

Physical examinations will be performed in accordance with the Study Plan (Table 5 and Table 6).

An abbreviated physical examination will be performed at the Screening Visit (Visit 1) and at Visit 9, and include an assessment of the following: general appearance, abdomen, cardiovascular, respiratory systems, and assessment of the volume status.

Body weight will be measured as indicated in Study Plan (Table 5 and Table 6). It will be assessed in the morning after an overnight fast (except for the Screening Visit where fasting is not required) and a lavatory visit in underwear/light dressing on the same calibrated scale at each occasion.

Documentation (yes/no) that the examination was performed will be entered in the eCRF. 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 (see Section 6.2.3.6).

6.2.8 Electrocardiogram

Standard 12-lead electrocardiogram (ECG) will be performed in accordance with the Study Plan (Table 5 and Table 6).

Either a digital or a paper ECG will be performed after 10 minutes supine rest.

The Investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant and the reason for the abnormality will be recorded. Only the overall evaluation (normal/abnormal) will be recorded in the eCRF.

The Investigator may add extra 12-lead paper ECG safety assessments if there are any abnormal findings or if the Investigator considers it is required for any other safety reason. These assessments should be entered as an unscheduled assessment.

The printout of the ECG is to be signed, dated and filed in the Investigator study file along with a signed and dated copy (if the printouts are not on archive-quality paper).

6.2.9 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 type 2 diabetes. 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. 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.

If an overdose on an AstraZeneca study drug occurs in the course of the study, then the Investigator or other site personnel inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative (PAREXEL Medical services team) works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with an SAE, the standard reporting timelines apply, see Section 6.2.4. For other overdoses, reporting must occur within 30 days.

6.2.10 Pregnancy

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

6.2.10.1 Maternal Exposure

Women of childbearing potential are allowed to be included in this study as long as they agree to use the contraceptive methods specified in inclusion criterion 9 in Section 4.1.

Should a pregnancy occur, the study drug should be discontinued immediately and the pregnancy reported to AstraZeneca.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product 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, normal birth or congenital abnormality) should be followed up and documented even if the patient was discontinued from the study.

If any pregnancy occurs in the course of the study, then the Investigator or other site personnel informs the appropriate AstraZeneca representatives (PAREXEL Medical services team) within 1 day, ie, immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative (PAREXEL Medical services team) works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site within 1 or 5 calendar days for SAEs (see Section 6.2.4) and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

The pregnancy module (PREGREP) in the eCRF is used to report the pregnancy and the pregnancy outcome report (PREGOUT) is used to report the outcome of the pregnancy.

6.2.11 Volume Depletion

Patients at risk of volume depletion due to co-existing conditions or concomitant medications, such as loop diuretics (exclusion criteria in this study), should have careful monitoring of their volume status as judged by the Investigator.

6.2.12 Diabetic Ketoacidosis

Patients treated with dapagliflozin who present with signs and symptoms consistent with ketoacidosis, including nausea, vomiting, abdominal pain, malaise and shortness of breath, should be assessed for ketoacidosis, even if blood glucose levels are below 14 mmol/l (250 mg/dl). If ketoacidosis is suspected by the Investigator, discontinuation of study drug and withdrawal of the patient should be considered, and the patient should be promptly evaluated.

Adjudication of potential events of diabetic ketoacidosis:

An independent Diabetic Ketoacidosis Adjudication Committee T2DM will assess available information on each potential diabetic ketoacidosis event and will classify the event in accordance with the definitions in the Diabetic Ketoacidosis Adjudication Charter T2DM, as follows:

- Definite diabetic ketoacidosis
- Probable diabetic ketoacidosis
- Possible diabetic ketoacidosis
- Not diabetic ketoacidosis

The Diabetic Ketoacidosis Adjudication Committee will be kept blinded to the study drug treatment received by each patient with a potential diabetic ketoacidosis event in the clinical study. A separate Diabetic Ketoacidosis Adjudication Manual will define and describe the procedures for the collection of diabetic ketoacidosis information, handling, adjudication criteria and reporting of these events/cases.

The decision of withdrawal of patient due to potential diabetic ketoacidosis event will be based on Investigator's judgement and will not be dependent on adjudication of these events by the Adjudication Committee.

The outcome of the classification of potential diabetic ketoacidosis cases by the Adjudication Committee will not be reported in the CSR.

6.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics are assessed at Screening. These include:

- Demographics (as appropriate to country regulations): sex, date of birth, race, ethnic group.
- Medical and surgical history:
  - Confirmation of diagnosis of T2DM.
  - Disease history (T2DM and renal impairment) including date of diagnosis.
  - Other previous and concomitant diseases within the last 6 months before Visit 1, with date of diagnosis.

    The medical history will be obtained by interviewing the patient or by inspecting his/her medical records. For coding of medical history, see Section 9.4.
- Height (in cm) and weight (in kg).
- Previous and concomitant medications at Visit 1 (see Section 5.7).
- eGFR at Screening, based on serum creatinine levels and CKI-EPD method.
- HbA1c at Screening.

6.4 Pharmacokinetics

6.4.1 Collection of Samples

Blood samples for determination of dapagliflozin concentration in plasma will be taken at the times presented in the study plan Table 5 [Group 1] and Table 6 [Group 2 and Group 3].

On Day 4, a single pre-dose sample will be obtained. On Day 14, samples will be obtained pre-dose, 1, 2 and 4 hr post-dose. It is important that the exact time of the sample is noted.

Samples will be collected, labelled stored and shipped as detailed in the Laboratory Manual.

6.4.2 Determination of Drug Concentration

Samples for determination of drug concentration in plasma will be analysed by Covance on behalf of AstraZeneca, using an appropriate bioanalytical method. Full details of the analytical method used will be described in a separate bioanalytical report.

6.4.3 Storage and Destruction of Pharmacokinetic Samples

Pharmacokinetic samples will be disposed of after the bioanalytical report finalisation or 6 months after issuance of the draft bioanalytical report (whichever is earlier), unless requested for future analyses.

Pharmacokinetic samples may be disposed of or destroyed and anonymised by pooling. Additional analyses may be conducted on the anonymised, pooled pharmacokinetic

samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the CSR.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation will not be reported in the CSR but separately in a bioanalytical report.

6.5 Volume of Blood to be Collected

CCI [redacted], as summarised in Table 4, and may change due to transfer of assay, validation, etc.

Additional blood samples for safety analyses may be taken as required in the study.

Table 4: Total Blood Volume to be Collected

Assay	Volume per sample (mL)	Total number of samples	Total blood volume (mL)
Local Laboratory			
Haematology (including haemoglobin, haematocrit, HbA1c)	**2**	**5**	**10**
haemoglobin		*3*	
haematocrit[a]		*5*	
HbA1c		*1*	
Clinical chemistry + Eyrthropoietin	**5**	**5**	**25**
AST, ALT, ALP, TBL,		*2*	
creatinine[a]		*5*	
glucose[a]		*5*	
CCI			
CCCI			
Central Laboratories			
CCI **and** CCI			
CCI			
CCI			
CCI			
Dapagliflozin pharmacokinetics	**2**	**5**	**10**
Plasma volume	**14**	**4**	**56**
Total blood volume (maximum)			CCI

ALP=alkaline phosphatase, ALT=alanine transaminase, AST=aspartate transaminase, CCI ; CCI , ECG=electrocardiogram, CCI

[redacted] HbA1c=glycosylated haemoglobin, [redacted] TBIL=total bilirubin.
[redacted]

6.6 Labelling and Shipment of Biohazard Samples

Samples will be labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix 11.2 of this Clinical Study Protocol 'International Airline Transportation Association (IATA) 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials will not be shipped and no further samples will be taken from the patient unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

6.7 Storage and Destruction of Biological Samples

Biological samples for future research will be retained at AstraZeneca or its designee for a maximum of 15 years following the finalisation of the CSR, after which they will be destroyed. [redacted]

6.8 Withdrawal of Informed Consent for Donated Biological Samples

If a patient withdraws consent to the use of donated biological samples, the samples will be disposed if not already analysed and the action documented.

As collection of donated biological samples is an integral part of the study, the patient will be withdrawn from further study participation.

AstraZeneca will ensure the laboratory holding the samples is informed about the withdrawn consent immediately and that samples are disposed of or destroyed, the action documented, and the signed document returned to the clinical site.

7 STUDY CONDUCT

7.1 Study Plan

The Study Plan displaying assessments/tasks and time points is presented in Table 5 (Group 1) and Table 6 (Group 2 and Group 3).

Table 5: Study Plan with Assessments Schedule: Group 1 (Patients on Insulin)

Study Plan	**Screening Period**		**Run-in Period**				**Treatment Period**								**Follow-up Period**				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−42 to -22**	**−21[a]**	**−20[a]**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[b]**	**15**	**16**	**17**	**18**	**19**
Informed consent	X																		
Inclusion/exclusion criteria	X					X													
Confirm final eligibility						X[c]													
Demography	X																		
Medical and surgical history	X																		
Vital signs[d]	X					X	X			X				X				X	
Physical examination	X																	X	
Height and weight[e]	X						X			X				X				X	
ECG	X																		
Briefing of patients by the study dietician regarding food boxes	X																		
Telephone call by dietician[f]		X																	
Patient consultation with the Investigator with respect to food boxes introduction[g]			←			→													

Study Plan	**Screening Period**		**Run-in Period**				**Treatment Period**								**Follow-up Period**				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−42 to -22**	**−21[a]**	**−20[a]**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[b]**	**15**	**16**	**17**	**18**	**19**
Dietary advice[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Consumption of food from food boxes			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood sampling																			
For clinical chemistry and haematology[h]	X						X			X				X				X	
CCI [redacted]							X			X				X				X	
CCI [redacted]							X			X				X				X	
Dapagliflozin PK[j]										X				X					
CCI [redacted]							X			X				X				X	
CCI [redacted]							X			X				X				X	
Urinalysis																			
Spot urine sample for urinary albumin and creatinine	X																		

Study Plan	Screening Period		Run-in Period				Treatment Period								Follow-up Period				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−42 to -22**	**−21[a]**	**−20[a]**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[b]**	**15**	**16**	**17**	**18**	**19**
CCI [redacted]							X			X				X				X	
(Dipstick): U-albumin, U-glucose, U-blood[l]	X													X					
CCI [redacted]						X				X				X			X		
CCI [redacted]						X				X				X			X		
Pregnancy test[n]	X													X					
24-hr urine[o]				X	X	X	X	X	X	X		X	X	X	X	X	X		
24-hr urine collection instructions/dispensing of containers[p]	X																		
24-hr blood pressure[q]						X				X			X					X	
Plasma volume[r]							X			X				X				X	
Bioimpedance spectroscopy[r, s]							X			X				X				X	

Study Plan	Screening Period		Run-in Period				Treatment Period								Follow-up Period				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−42 to -22**	**−21[a]**	**−20[a]**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[b]**	**15**	**16**	**17**	**18**	**19**
Dispense study drug[t]							X												
Verify drug intake and drug accountability[u]										X	X		X	X					
Review of concomitant medications	X				X	X	X			X	X		X	X				X	X
Collection of AE/SAE[v]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Food intake questionnaire[w]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

ABPM=ambulatory blood pressure monitoring, AE=adverse event, DBP=diastolic blood pressure, CCI [redacted] CCI [redacted] CCI [redacted] HbA1c=glycosylated haemoglobin, hr=hour, CCI [redacted] PK=pharmacokinetics, CCI [redacted]
SAE=serious adverse event, SBP=systolic blood pressure, UACR=urine albumin: creatinine ratio, CCI [redacted]

a. The indicated days are the earliest days for the assessments listed.
b. In case of early discontinuation, patient will attend an Early Termination Visit. This visit should take place as soon as possible after the patient stops taking study treatment. If patients can continue the study treatment up to the day of the Early Termination Visit, the observations and procedures scheduled for Visit 8 should be performed. If not, the Early Termination Visit should be handled as Visit 9.
c. Selection of a patient in the study to be done only after results of clinical chemistry, haematology, and urinalysis have been reviewed. Stable urinary sodium excretion to be confirmed on 2 successive 24-hr urinary sodium excretion measurements (<20% difference between Days −3 and −2 Inclusion criterion #13; Section 4.1.2).
d. Vital signs include heart rate and blood pressure.
e. Height to be measured only at Screening Visit. Body weight to be assessed in the morning after an overnight fast (except for Visit 1 where fasting is not required) and a visit to the lavatory in underwear/light clothing on the same calibrated scale for each individual.
f. Telephone call by dietician within 24 hours as a reminder for food boxes to be consumed from Day –20 (at the earliest) onwards and to complete food questionnaire. Dietary advice and food boxes with a daily sodium content of approximately 150 mmol sodium content will start at Day –20 at the earliest and continue throughout the study (until Visit 9).

g. During the Run-in Period, there will be regular contacts between Investigators and patients to ensure that their blood glucose is adequately controlled and to confirm that their insulin regime remains stable while on food boxes. Patient consultation with Investigator may occur at several times from Day -20 to Day −1. Exact dates will be decided on an as needed basis by patient and Investigator. Patients may have their insulin requirements adjusted according to the caloric content of the food boxes, if needed.

h. CCI
Samples should be obtained prior to dosing. At the Screening Visit and on Day 14, all safety laboratory assessments specified in Table 3 will be performed; on Day 1, only haemoglobin, creatinine, haematocrit, glucose will be assessed; and on Day 4 and Day 18, only creatinine, haematocrit, glucose will be assessed. At the Screening Visit HbA1c will be assessed for confirmation of inclusion criterion. CCI

i. CCI

j. On Day 4, a single pre-dose sample will be obtained. On Day 14, samples will be obtained pre-dose, 1, 2 and 4 hrs post-dose.

CCI

l. Urine dipstick parameters to be evaluated are blood, glucose and albumin.

CCI

n. A positive urine pregnancy test (dipstick analysed at the study centre) should be confirmed with a serum pregnancy test.

o. Patients will be given instructions on how to collect and store 24-hr urine samples. From the 24-hr urine samples, following parameters will be analysed at all timepoints: 24-hr CCI CCI glucose, sodium, CCI and UACR; and CCI will be analysed only on Days −1, 4, 14, and 17. For premenopausal women, the Investigator will estimate the menstrual timing and aim to avoid the 2 urine collection periods (Day −3 to 4 and Day 12 to 17) during menstruation. For the second collection period (Day 12 to 17), the urine collection will start 1 day before Visit 7 (Day 13±1 day) and end 4 days after Visit 7 (Day 13±1 day).

p. Patients will receive empty 24-hr urine collection containers preceding the collection days according to instructions. Dispensing of 24-hr urine containers may occur at several occasions during the study period.

q. ABPM will be used to record 24-hr SBP and DBP. Specific instructions will be provided to the patients as per the ABPM manual.

r. Assessments of plasma volume and bioimpedance spectroscopy should be done in the following order: 1) CCI. On Day 1, plasma volume assessment and bioimpedance spectroscopy will be performed pre-dose, while on Days 4 and 14, it will be assessed post dose. 2) Intake of study drug and other medications and breakfast (The time when dapagliflozin is taken should be noted as t=0). 3) One hr after dapagliflozin intake, bioimpedance spectroscopy is performed (Days 4 and 14). The timing of the assessment relative to the dapagliflozin dose should be consistent across visits for each patient. 4) Approximately 1.5 hr after dapagliflozin intake, the plasma volume assessment will be done. The timing of the assessment relative to the dapagliflozin dose should be consistent across visits for each patient. 5) On Day 14, the bioimpedance spectroscopy measurement will be conducted also at pre-dose, as well as 2 and 4 hr after dapagliflozin dose (in addition to 1 hr post-dose noted above), that is, the bioimpedance spectroscopy measurement will be done at the same time as pharmacokinetics sampling on Day 14. The bioimpedance spectroscopy measurement is always to be done

before pharmacokinetics sampling. On Days 1 and 18, bioimpedance spectroscopy is conducted 1 hr after intake of breakfast and non-study drugs (but not dapagliflozin) and plasma volume is assessed 1.5 hr after breakfast and non-study drug intake.

s. Bioimpedance spectroscopy measurements include assessment of extracellular volume, CCI [redacted]

t. Study drug administration should start in the morning at Day 1 (Visit 4) and last dose should be taken by the patient on Visit 8.

u. Intake of study treatment will be confirmed based on the completion of record of drug intake by the patient in the food questionnaire on Days 4, 5, 13, and 14. Drug accountability (tablet count) will be performed on Visit 8.

v. AEs are collected from time of first dose and SAEs are collected from provision of informed consent throughout the study.

w. Patient will record compliance with recommended food intake, liquid intake daily in the food questionnaire. Any other food and drink consumed will also be recorded. Study treatment intake will also be recorded daily during the Treatment Period.

Table 6: Study Plan with Assessments Schedule: Group 2 and Group 3 (Patients not on Insulin)

Study Plan	**Screening Period**		**Run-in Period**				**Treatment Period**								**Follow-up Period**				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−28 to -8**	**−7**	**−6**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[a]**	**15**	**16**	**17**	**18**	**19**
Informed consent	X																		
Inclusion/exclusion criteria	X					X													
Confirm final eligibility						X[b]													
Demography	X																		
Medical and surgical history	X																		
Vital signs[c]	X					X	X			X				X				X	
Physical examination	X																	X	
Height and weight[d]	X						X			X				X				X	
ECG	X																		
Briefing of patients by the study dietician regarding food boxes	X																		
Telephone call by dietician[e]		X																	
Dietary advice[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Consumption of food from food boxes			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood sampling																			
For clinical chemistry and haematology[f]	X						X			X				X				X	

Study Plan	Screening Period		Run-in Period				Treatment Period								Follow-up Period				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−28 to -8**	**−7**	**−6**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[a]**	**15**	**16**	**17**	**18**	**19**
CCI [redacted]							X			X				X				X	
CCI [redacted]							X			X				X				X	
Dapagliflozin PK[h]										X				X					
CCI [redacted]							X			X				X				X	
CCI [redacted]							X			X				X				X	
Urinalysis																			
Spot urine sample for urinary albumin and creatinine	X																		
CCI [redacted]							X			X				X				X	
(Dipstick): U-albumin, U-glucose, U-blood[j]	X													X					

Study Plan	**Screening Period**		**Run-in Period**				**Treatment Period**								**Follow-up Period**				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−28 to -8**	**−7**	**−6**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[a]**	**15**	**16**	**17**	**18**	**19**
CCI [redacted]						X				X				X			X		
CCI [redacted]						X				X				X			X		
Pregnancy test[l]	X													X					
24-hr urine[m]				X	X	X	X	X	X	X		X	X	X	X	X	X		
24-hr urine collection instructions/dispensing of containers[n]	X																		
24-hr blood pressure[o]						X				X			X					X	
Plasma volume[p]							X			X				X				X	
Bioimpedance spectroscopy[p, q]							X			X				X				X	

Study Plan	**Screening Period**		**Run-in Period**				**Treatment Period**								**Follow-up Period**				
Visits (Clinic)	**1**				**2**	**3**	**4**			**5**	**6**		**7**	**8**				**9**	**10**
Day	**−28 to -8**	**−7**	**−6**	**−3**	**−2**	**−1**	**1**	**2**	**3**	**4**	**5**	**12**	**13 ±1d**	**14[a]**	**15**	**16**	**17**	**18**	**19**
Dispense study drug[r]							X												
Verify drug intake and drug accountability[s]										X	X		X	X					
Review of concomitant medications	X				X	X	X			X	X		X	X				X	X
Collection of AE/SAE[t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Food intake questionnaire[u]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

ABPM=ambulatory blood pressure monitoring, AE=adverse event, DBP=diastolic blood pressure, [redacted], HbA1c=glycosylated haemoglobin, hr=hour, [redacted] PK=pharmacokinetics, [redacted] SAE=serious adverse event, SBP=systolic blood pressure, UACR=urine albumin: creatinine ratio, [redacted]

a. In case of early discontinuation, patient will attend an Early Termination Visit. This visit should take place as soon as possible after the patient stops taking study treatment. If patients can continue the study treatment up to the day of the Early Termination Visit, the observations and procedures scheduled for Visit 8 should be performed. If not, the Early Termination Visit should be handled as Visit 9.
b. Selection of a patient in the study to be done only after results of clinical chemistry, haematology, and urinalysis have been reviewed. Stable urinary sodium excretion to be confirmed on 2 successive 24-hr urinary sodium excretion measurements (<20% difference between Days −3 and −2 Inclusion criterion #13; Section 4.1.2).
c. Vital signs include heart rate and blood pressure.
d. Height to be measured only at Screening Visit. Body weight to be assessed in the morning after an overnight fast (except for Visit 1 where fasting is not required) and a visit to the lavatory in underwear/light clothing on the same calibrated scale for each individual.
e. Telephone call by dietician as a reminder for food boxes to be consumed from Day -6 onwards and to complete food questionnaire. Dietary advice and food boxes with a daily sodium content of approximately 150 mmol sodium content will start at Day -6 and continue throughout the study (until Visit 9).
f. Blood samples to be obtained in the morning at the same time for each individual after fasting for at least 8 hr (except at Visit 1 where fasting is not required). Samples should be obtained prior to dosing. At the Screening Visit and on Day 14, all safety laboratory assessments specified in Table 3 will be performed; on Day 1, only haemoglobin, creatinine, haematocrit, glucose will be assessed; and on Day 4 and Day 18, only creatinine, haematocrit, glucose will be

assessed. At the Screening Visit HbA1c will be assessed for confirmation of inclusion criterion. CCI [redacted].

CCI [redacted]

h. On Day 4, a single pre-dose sample will be obtained. On Day 14, samples will be obtained pre-dose, 1, 2 and 4 hrs post-dose.
i. CCI [redacted]
j. Urine dipstick parameters to be evaluated are blood, glucose and albumin.

CCI [redacted]

l. A positive urine pregnancy test (dipstick analysed at the study centre) should be confirmed with a serum pregnancy test.
m. Patients will be given instructions on how to collect and store 24-hr urine samples. From the 24-hr urine samples, following parameters will be analysed at all timepoints: 24-hr CCI [redacted], glucose, sodium, CCI [redacted], and UACR; and CCI [redacted] will be analysed only on Days −1, 4, 14, and 17. For premenopausal women, the Investigator will estimate the menstrual timing and aim to avoid the 2 urine collection periods (Day −3 to 4 and Day 12 to 17) during menstruation. For the second collection period (Day 12 to 17), the urine collection will start 1 day before Visit 7 (Day 13±1 day) and end 4 days after Visit 7 (Day 13±1 day).
n. Patients will receive empty 24-hr urine collection containers preceding the collection days according to instructions. Dispensing of 24-hr urine containers may occur at several occasions during the study period.
o. ABPM will be used to record 24-hr SBP and DBP. Specific instructions will be provided to the patients as per the ABPM manual.
p. Assessments of plasma volume and bioimpedance spectroscopy should be done in the following order: 1) CCI [redacted]. On Day 1, plasma volume assessment and bioimpedance spectroscopy will be performed pre-dose, while on Days 4 and 14, it will be assessed post dose. 2) Intake of study drug and other medications and breakfast (The time when dapagliflozin is taken should be noted as t=0). 3) One hr after dapagliflozin intake, bioimpedance spectroscopy is performed (Days 4 and 14). The timing of the assessment relative to the dapagliflozin dose should be consistent across visits for each patient. 4) Approximately 1.5 hr after dapagliflozin intake, the plasma volume assessment will be done. The timing of the assessment relative to the dapagliflozin dose should be consistent across visits for each patient. 5) On Day 14, the bioimpedance spectroscopy measurement will be conducted also at pre-dose, as well as 2 and 4 hr after dapagliflozin dose (in addition to 1 hr post-dose noted above), that is, the bioimpedance spectroscopy measurement will be done at the same time as pharmacokinetics sampling on Day 14. The bioimpedance spectroscopy measurement is always to be done before pharmacokinetics sampling. On Days 1 and 18, bioimpedance spectroscopy is conducted 1 hr after intake of breakfast and non-study drugs (but not dapagliflozin) and plasma volume is assessed 1.5 hr after breakfast and non-study drug intake.
q. Bioimpedance spectroscopy measurements include assessment of extracellular volume, CCI [redacted]
r. Study drug administration should start in the morning at Day 1 (Visit 4) and last dose should be taken by the patient on Visit 8.
s. Intake of study treatment will be confirmed based on the completion of record of drug intake by the patient in the food questionnaire on Days 4, 5, 13, and 14. Drug accountability (tablet count) will be performed on Visit 8.

t. AEs are collected from time of first dose and SAEs are collected from provision of informed consent throughout the study.
u. Patient will record compliance with recommended food intake, liquid intake daily in the food questionnaire. Any other food and drink consumed will also be recorded. Study treatment intake will also be recorded daily during the Treatment Period.

7.2 Procedures by Visit

Visit 1 should occur a maximum of 42 days before Visit 4 (ie, start of dosing). All times should be recorded using the 24-hr clock (eg, 23:20, not 11:20 pm).

7.2.1 Screening Period: Day −28 to Day −7 (Patients not on Insulin) and Day −42 to Day −21 (Patients on Insulin)

7.2.1.1 Visit 1: Screening Visit

At the Screening Visit (Visit 1), potentially suitable patients for the study will provide informed consent and will be assessed to ensure that they meet the eligibility criteria. Patients who do not meet these criteria must not be entered into the study. All assessment for Visit 1, as listed in Table 5 (patients on insulin) and Table 6 (patients not on insulin) will be performed.

At the Screening Visit, the study dietician will discuss with the patient regarding the food boxes to be provided from Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin). The dietician will emphasise the importance of adherence to the dietary requirements in the study. The patients will be explained that they should consume food only from the food boxes that will be provided on a weekly basis. The patients will be explained to record adherence to food requirements and liquid consumption throughout the day in the food questionnaires from Day -6 (patients not on insulin) or Day –20 at the earliest (patients on insulin) onwards until Visit 9 (inclusive). Patients on insulin may require a longer Run-in Period in order to be able to adjust their insulin requirements according to the caloric content of the food boxes, if needed. However, it is not mandatory for the patient on insulin to use the entire extended Run-in Period. Based on the Investigator's judgement, the Run-in Period may be shortened once each patient (on insulin) has had sufficient time to adapt to the food boxes, and it is determined that the patient's insulin requirement has stabilised sufficiently to continue in the study.

All SAEs are collected from time of signing the informed consent and throughout the study, including the Run-in Period and the Follow-up Period. Adverse events will be collected from the time of first dose throughout the study, including the Follow-up Period.

For premenopausal women, the Investigator will estimate the menstrual timing and aim to avoid the 2 urine collection periods (Day −3 to 4 and Day 12 to 17) during menstruation.

The following procedures are performed at Visit 1:

- Signing of informed consent
- Receive enrolment number
- Eligibility criteria
- Demographics
- Medical and surgical history

- Vital signs (blood pressure and pulse)
- Physical examination
- Weight and height
- ECG
- Discussion with study dietician regarding food boxes
- Blood sampling
  - Safety laboratory assessments (haematology and clinical chemistry)
  - HbA1c
- Urine samples
  - Spot urine sampling for estimation of urinary albumin excretion (UACR)
  - Urine dipstick for safety laboratory assessments
  - Urine pregnancy test for women of childbearing potential
- Concomitant medication
- SAEs
- Dispense equipment for 24-hr urine collection

7.2.2 Run-in Period: Day −6 to Day −1 (Patients not on Insulin) and Day −20 (at the earliest) to Day −1 (Patients on Insulin)

Patients meeting all eligibility criteria (based on available investigations) will be scheduled for the Run-in Period starting on Day −6 (patients not on insulin) or Day −20 at the earliest (patients on insulin).

- Patients will receive a phone call from the dietician on Day −7 (patients not on insulin) or Day –21 at the earliest (patients on insulin) as a reminder for the food boxes that they will have to start using from Day −6 or Day −20 (at the earliest) onwards, respectively, until the end of study; and to remind them to complete the food questionnaire starting Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin)
- There will be regular contacts between Investigators and patients to ensure that their blood glucose is adequately controlled (by potentially adjusting/optimising their insulin doses) and to confirm that their insulin regime remains stable while on food boxes. Patient consultation with Investigator may occur at several times from Day -20 to Day -1. Exact dates will be decided on an as needed basis by patient and Investigator.
- Dietary advice and food boxes with a daily sodium content of approximately 150 mmol will start at Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin)
- Patients will also start completing food questionnaires starting at Day −6 (patients not on insulin) or Day –20 at the earliest (patients on insulin)
- Patients will start collection of first 24-hr urine sample on Day −3

7.2.2.1 Visit 2: Day −2

- Patient to start collection of 24-hr urine sample
- Obtain 24-hr urine sample from previous day
- Dietary advice
- Concomitant medications
- SAEs
- Review of food questionnaires completed by the patient

7.2.2.2 Visit 3: Day −1

Following procedures will be performed at this visit:

- Eligibility criteria check:

  At this visit the inclusion criterion pertaining to stable urinary sodium excretion on 2 successive 24-hr urinary sodium excretion measurements will be confirmed, based on <20% difference between samples obtained on Day −3 and Day −2 (inclusion criterion 13, Section 4.1.2).
- Vital signs (blood pressure and pulse)
- Obtain 24-hr urine sample from previous day
- Patient to start collection of 24-hr urine sample
- Start 24-hr ABPM
- Dietary advice
- Concomitant medication
- SAEs
- Review of food questionnaires completed by the patient

Patients will be instructed the following:

- CCI [redacted]

7.2.3 Treatment Period (Day 1 to Day 14)

7.2.3.1 Visit 4: Day 1 (Dosing Initiation Visit)

Treatment with dapagliflozin 10 mg/day will be initiated on Day 1 for eligible patients after blood sampling.

- Obtain 24-hr urine sample from previous day (ie, Day −1)
- Patient to start collection of 24-hr urine sample
- Discontinue 24-hr ABPM
- Vital signs (blood pressure and pulse)
- Body weight
- Pre-dose blood and urine samples
  - Safety laboratory assessments (haemoglobin, creatinine, haematocrit, and glucose all of which [except haemoglobin] are also CCI [redacted])

- CCI [redacted]
- CCI [redacted]

- Patient will have breakfast at the study site along with non-study drugs
- Bioimpedance spectroscopy measurement (pre-dose; 1 hr after food intake)
- Measurements for plasma volume (pre-dose; approximately 1.5 hr after food intake)
- Administration of first dose of study drug at the study site
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient
- Dispensation of study drug sufficient for up to 15 days of treatment duration

Patients will be asked to follow the procedures below until the next clinic visit:

- collect 24-hr urine samples on Day 2 and Day 3
- follow dietary advice daily
- complete food questionnaires daily
- CCI [redacted] for the next clinic visit on Day 4 and to bring the study drug to the clinic for consumption after the blood sampling

7.2.3.2 Visit 5: Day 4

Following procedure will be performed at this visit:

- Obtain 24-hr urine sample from previous days (ie, Days, 1, 2 and 3)
- Patient to start collection of 24-hr urine sample
- Vital signs (blood pressure and pulse)
- Body weight
- Pre-dose blood and urine samples
  - Safety laboratory assessments (creatinine, haematocrit, and glucose all of which are CCI [redacted])
  - CCI [redacted]
  - Pre-dose blood sample for dapagliflozin pharmacokinetics
  - CCI [redacted]
- Patient will have breakfast at the study site along with study drug (t=0) and non-study drugs
- Bioimpedance spectroscopy measurement (1 hr after study drug intake)
- Measurements for plasma volume (approximately 1.5 hr after study drug intake)
- Start 24-hr ABPM

- Verify study treatment intake from diaries
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient

7.2.3.3 Visit 6: Day 5

Following procedure will be performed at this visit

- Obtain 24-hr urine sample from previous day (ie, Day 4)
- Discontinue 24-hr ABPM
- Verify study treatment intake from diaries
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient

Patients will be asked to follow the procedures below until the next clinic visit (Visit 7):

- collect 24-hr urine samples starting 1 day before Visit 7.
- follow dietary advice daily
- complete food questionnaires daily

7.2.3.4 Visit 7: Day 13

A window of ± 1 day is allowed for this visit; however, 24-hr urine sample collection should be started a day prior to the visit.

The following procedure will be performed at this visit:

- Obtain 24-hr urine sample from previous day
- Patient to start collection of 24-hr urine sample
- Start 24-hr ABPM
- Verify study treatment intake from diaries
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient

Patients will be instructed the following:

- CCI) for the next clinic visit on the next day and to bring the study drug to the clinic for consumption after the blood sampling

7.2.3.5 Visit 8: Day 14

Visit 8 is the final Treatment Period Visit and will occur 1 day after Visit 7 (Day 13 ±1 day). Patients will take their last dose of dapagliflozin on Visit 8.

The following procedure will be performed at this visit:

- Obtain 24-hr urine sample from previous day
- Patient to start collection of 24-hr urine sample
- Discontinue 24-hr ABPM
- Vital signs (blood pressure and pulse)
- Body weight
- Pre-dose blood and urine samples
  - Safety laboratory assessments (haematology and clinical chemistry)
  - CCI [redacted]
  - CCI [redacted]
  - Urine dipstick
  - Urine pregnancy test for women of childbearing potential
  - Pre-dose blood sample for dapagliflozin pharmacokinetics
- Pre-dose bioimpedance spectroscopy measurement
- Patient will have breakfast at the study site along with study drug (t=0) and non-study drugs
- Bioimpedance spectroscopy measurement (1 hr post-dose)
- Blood sample for dapagliflozin pharmacokinetics (1 hr post-dose)
- Measurements for plasma volume (approximately 1.5 hr post-dose)
- Bioimpedance spectroscopy measurement (2 hr post-dose)
- Blood sample for dapagliflozin pharmacokinetics (2 hr post-dose)
- Bioimpedance spectroscopy measurement (4 hr post-dose)
- Blood sample for dapagliflozin pharmacokinetics (4 hr post-dose)
- Verify study treatment intake from diaries
- Drug accountability (based on tablet counts)
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient

Patients will be asked to follow the procedures below until the next clinic visit:

- collect 24-hr urine samples on Day 15, Day 16, and Day 17
- follow dietary advice daily
- complete food questionnaires daily

- CCI [redacted] for the next visit on Day 18

7.2.4 Follow-up Period (Day 15 to Day 19)

The patients will have a Follow-up Period for 5 days starting 1 day after Visit 8.

7.2.4.1 Visit 9: Day 18

Visit 9 will occur on the 4th day of the Follow-up Period.

Following procedures will be performed at this visit:

- Obtain 24-hr urine sample from previous days (ie, Days 14, 15, 16, and 17)
- Vital signs (blood pressure and pulse)
- Physical examination
- Body weight
- CCI [redacted] and urine samples

CCI [redacted]

- CCI [redacted]

- Patient will have breakfast at the study site along with non-study drugs
- Bioimpedance spectroscopy measurement (1 hr after food intake)
- Measurements for plasma volume (approximately 1.5 hr after food intake)
- Start 24-hr ABPM
- Dietary advice
- Concomitant medication
- AEs/SAEs
- Review of food questionnaires completed by the patient

7.2.4.2 Visit 10: (End of Study) Day 19

Visit 10 is the final visit in the study and will occur on the 5th day of the Follow-up Period. Following procedures will be performed at this visit:

- Discontinue 24-hr ABPM
- Return equipment
- Concomitant medication
- AEs/SAEs

7.2.5 Unscheduled Visits

An unscheduled visit can be performed as required for evaluation of a safety concern or any other reason.

The following will be performed at unscheduled visit. Additional investigations/procedures can be performed as per the Investigator's clinical judgement.

- AEs

- Concomitant medication
- Vital signs (blood pressure and pulse)

7.2.6 Early Termination Visit

Patients who discontinue early from the study should, if possible, have an Early Termination Visit. This visit should take place as soon as possible after the patient stops taking study treatment. If patients can continue the study treatment up to the day of the Early Termination Visit, the observations and procedures scheduled for Visit 8 including repeated PK and BIS (Section 7.2.3.5) should be performed. If not, the Early Termination Visit should be handled as Visit 9 (Section 7.2.4.1).

8 STATISTICAL METHODS

The statistical considerations summarised in this section outline the plan for data analysis of this study.

A separate statistical analysis plan (SAP) will be finalised prior to database lock.

Any deviations from the planned analyses will be described in a SAP amendment. Any deviations from the planned analyses will be described and justified in the final integrated CSR.

CCI [redacted]

8.1 Study Patients

8.1.1 Analysis Sets

The following analysis sets will be defined for this study. Further details regarding the inclusion and exclusion of patients from each of these analysis sets will be described in the SAP.

Analysis Set	Definition
Enrolled Set (ENS):	All patients who provided informed consent will be included in the ENS.
Run-in Set:	All patients who are provided with the standard food boxes.
Medication Dispensed Set (MDS):	All patients who dispensed at least one dose of study drug.
Evaluable Subjects Data Set:	This will be a subset of the MDS. It will exclude primary efficacy variable data which may have been affected by protocol deviations as determined by the medical monitor or agreed by the study team. All decisions to exclude data from the Run-in Set to form the Evaluable Subjects Analysis Data Set will be made prior to the database lock of the study.
Safety Set (SAF):	All patients who received at least 1 dose of study drug and who have data from at least one post-dose safety assessment available.

All safety analyses will be based on the SAF.

Demographic and baseline characteristics will be summarised for the SAF.

8.2 General Considerations

Statistical analyses will be performed using SAS® (SAS Institute Inc., Cary, NC, US) Version 9.3 or higher unless otherwise specified.

All statistical tests will be 2-sided and will be performed at the 5% level of significance, unless otherwise stated.

8.3 Efficacy Analyses

The Baseline for 24-hr urinary related endpoints is the average of the daily urinary sodium excretion from Days −3, −2 and −1 assessments prior to the date of the first dose of the study treatment (with the exception of CCI [redacted] for which the Baseline is the urinary assessment from Day −1).

8.3.1 Primary Efficacy Analysis

The primary efficacy endpoint is the average change in 24-hr sodium excretion from average Baseline to average values at Day 2 to 4 within each study group. This will be analysed as follows "average change in 24-hr sodium excretion during dapagliflozin treatment between Baseline (average of Days −3 to −1) and average of Days 2 to 4 within each study group".

The primary analysis will be performed separately for each study group using a longitudinal repeated-measures analysis for the "change from average Baseline" as dependent variable, with a term for Baseline value. Point estimates and 95% confidence intervals will be calculated for the adjusted mean changes within each study group.

The primary analysis will be based on the Evaluable Subjects Analysis Set.

8.3.2 Secondary Efficacy Analyses

The secondary analyses will be based on the Evaluable Subjects Analysis Set.

The endpoints listed in Section 3.2.2 will be analysed using the same model and presentation as for the primary efficacy analysis.

8.3.2.1 Secondary Pharmacokinetic Analyses

Plasma concentrations of dapagliflozin will be listed, summarised and plotted vs time relative to intake of dapagliflozin. A population pharmacokinetic analysis of data may be performed as outlined in a separate population pharmacokinetic analysis plan.

CCI [redacted]

8.3.4 Food Intake Questionnaires

Results from patients' food intake questionnaire will be listed.

8.4 Safety Analyses

8.4.1 Adverse Events

The analysis of safety will be based on the SAF. Safety data gained during the study period will be evaluated and variables will only be summarised descriptively.

Adverse events will be presented by preferred term and system organ class.

Continuous data will be summarised with number of observations, mean, standard deviation, minimum and maximum value, and median.

Categorical data will be summarised with absolute and relative frequency.

CCI [redacted]

8.5 Partial Analyses

As recruitment rate for Group 1 is slower than expected, a partial analysis based on all patients from Group 2 and Group 3, but none from Group 1, might be conducted. If such an analysis takes place, a formal Data Review Meeting (DRM) will be held prior to its initiation. At the DRM, all protocol deviations related to the patients in these two groups will be discussed and the impact on the analysis sets to be used for the partial analysis will be assessed. The partial analysis will include all the evaluations originally planned to be conducted, and reported, for all three groups simultaneously.

Further details on the planned analyses will be given in the study SAP.

8.6 Determination of Sample Size

With a sample size of 15 patients per group and assuming a conservative standard deviation (SD) of 25 mEq/24 hrs in change from average Baseline in 24-hr sodium excretion, at an assumed increase of 20 mEq/24 hrs (delta), the study will have 80% power to detect an increase of at least 20 mEq/24 hrs in average change from average Baseline 24-hr sodium excretion with dapagliflozin within each group at a two-sided alpha level of 0.05. Under these conventions the minimum detectable difference in change from average Baseline in 24-hr sodium excretion is approximately 13.6 mEq/24 hrs. Similarly, under sample size conventions when fixing the change from average Baseline in 24-hr sodium excretion (delta) of 20 mEq/24 hrs, the maximum SD at which statistical significance can be demonstrated is approximately 37 mEq/24 hrs.

It is assumed that approximately 10% of the enrolled patients will not provide evaluable data with regards to the primary endpoint, leading to 17 patients to be enrolled in each of the groups. If the non-evaluable rate is higher than expected, additional patients might be enrolled in order to ensure 15 evaluable patients.

9 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 Data Quality Assurance

AstraZeneca or designee will conduct a site visit to verify the qualifications of each Investigator, inspect the site facilities, and inform the Investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded on the eCRF for this study must be consistent with the patients' source documentation (ie, medical records).

9.1.1 Database Management and Quality Control

All data generated by the site personnel will be captured electronically at each study centre using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data centre, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.

If additional corrections are needed, the responsible monitor or data manager will raise a query in the EDC application. The appropriate staff at the study site will answer queries sent to the Investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.

The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

9.2 Case Report Forms and Source Documentation

All data obtained during this study should be entered in the eCRFs promptly. All source documents from which eCRF entries are derived should be placed in the patient's medical records. Measurements for which source documents are usually available include laboratory assessments.

Data that will be entered directly into the eCRF (ie, for which there is no prior written or electronic record of data) are considered to be source data.

The original eCRF entries for each patient may be checked against source documents at the study site by the PAREXEL site monitor. After review by the site monitor, completed eCRF entries will be uploaded and forwarded to PAREXEL. Instances of missing or uninterpretable data will be discussed with the Investigator for resolution.

The specific procedures to be used for data entry and query resolution using the eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the eCRF.

9.2.1 Data Collection

The Investigators (and appropriately authorised staff) will be given access to an online EDC system which is 21 CFR Part 11 compliant. This system is specifically designed for the collection of the clinical data in electronic format. Access and right to the EDC system will be carefully controlled and configured according to each individual's role throughout the study. In general, only the Investigator and authorised staff will be able to enter data and make corrections in the eCRFs.

The eCRF should be completed for each patient included in the study and should reflect the latest observations on the patients participating in the study. Therefore, the eCRFs are to be completed as soon as possible during or immediately after the patient's visit or assessment. The Investigator must verify that all data entries in the eCRF are accurate and correct. If some assessments cannot be done, or if certain information is unavailable, not applicable or unknown, the Investigator should indicate this in the eCRF.

Computerised data-check programs and manual checks will identify any clinical data discrepancies for resolution. Corresponding queries will be loaded into the system and the site will be informed about new issues to be resolved online. All discrepancies will be solved online directly by the Investigator or by authorised staff. Off-line edit checks will be done to examine relationships over time and across panels to facilitate quality data.

After completion, the Investigator will be required to electronically sign off the clinical data.

Data about all study treatment dispensed and returned from the patient will be tracked on the eCRF.

Data from external sources (such as central laboratory data and data for blood pressure measurements from 24-hr ABPM) will be imported into the database using SAS.

9.3 Access to Source Data

During the study, a monitor will make site visits to review protocol compliance, compare eCRF entries and individual patient's medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

Checking of the eCRF entries for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the study. Moreover, Regulatory Authorities of certain countries, Institutional Review Boards (IRBs), Independent Ethics Committees (IECs), and/or AstraZeneca's Clinical Quality Assurance Group may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The Investigator assures PAREXEL and AstraZeneca of the necessary support at all times.

9.4 Data Processing

All data will be entered by site personnel into the eCRF (as detailed in Section 9.2.1).

The data-review and data handling document, to be developed during the initiation phase of the study, will include specifications for consistency and plausibility checks on data and will also include data handling rules for obvious data errors. Query/correction sheets for unresolved queries will be sent to the study monitors for resolution with the Investigator. The database will be updated on the basis of signed corrections.

Previous and concomitant medications will be coded using the latest available version of World Health Organisation - Drug Dictionary (WHO-DD). Medical history/current medical conditions and AEs will be coded using the latest available version of Medical Dictionary for Regulatory Activities (MedDRA) terminology.

Previous and concomitant diseases as well as AEs will be coded with latest available version of MedDRA.

The versions of the coding dictionaries will be provided in the CSR.

9.5 Archiving Study Records

According to International Council for Harmonisation (ICH) guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. However, these documents should be retained for a longer period if required by the applicable legal requirements.

9.6 Good Clinical Practice

The procedures set out in this study protocol are designed to ensure that AstraZeneca and Investigator abide by the principles of the Good Clinical Practice (GCP) guidelines of the ICH, and of the Declaration of Helsinki (2013) [1]. The study also will be carried out in keeping with local legal requirements.

9.7 Informed Consent

Before each patient is admitted to the study, informed consent will be obtained from the patient according to the regulatory and legal requirements of the participating country. This Informed consent form (ICF) must be dated and retained by the Investigator as part of the study records. The Investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all patients subsequently enrolled in the study as well as those currently enrolled in the study.

9.8 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. AstraZeneca must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written and approval obtained from the appropriate personnel and IRB/IEC/Competent Authority prior to implementation (if appropriate).

Administrative changes (not affecting the patient benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.

9.9 Duration of the Study

For each individual patient, the maximum duration of the study will be up to 62 days (including up to 6 weeks for Screening and Run-in Period, 2-week Treatment Period, and 5-day Follow-up Period, and 1 additional day for allowed window periods).

The study will close when all patients have completed the last visit (Visit 10).

9.10 Premature Termination of the Study

9.10.1 Stopping Rules for an Individual Patient

Withdrawal criterial for an individual patient are specified in Section 4.5.

9.10.2 Stopping Rules for the Study

If the Investigator, AstraZeneca, or the medical monitor becomes aware of conditions or events that suggest a possible hazard to patients if the study continues, the study may be terminated after appropriate consultation between the relevant parties. The study may also be terminated early at AstraZeneca's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study.
- Failure to enroll patients at an acceptable rate.
- A decision on the part of AstraZeneca to suspend or discontinue development of the study drug.

9.11 Confidentiality

All study findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from AstraZeneca.

The anonymity of participating patients must be maintained. Patients will be identified on eCRFs and other documents submitted to PAREXEL by their patient number, not by

name. Documents not to be submitted to PAREXEL that identify the patient (eg, the signed informed consent) must be maintained in confidence by the Investigator.

10 REFERENCE LIST

1 World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996, the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000, Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002, Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004, 59th WMA General Assembly, Seoul, South Korea, October 2008, and 64th WMA General Assembly, Fortaleza, Brazil, October 2013.

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11 APPENDICES

11.1 Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life-threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important Medical Event or Medical Intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity but may jeopardise the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

- Angioedema not severe enough to require intubation but requiring intravenous hydrocortisone treatment.
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine.
- Intensive treatment in an emergency room or at home for allergic bronchospasm.
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalisation.
- Development of drug dependency or drug abuse.

A Guide to Interpreting the Causality Question

When making an assessment of causality, consider the following factors when deciding if there is a 'reasonable possibility' that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? Or could the AE be anticipated from its pharmacological properties?
- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.
- Re-challenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a re-challenge.
- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Causality of ‘related’ is made if following a review of the relevant data, there is evidence for a ‘reasonable possibility’ of a causal relationship for the individual case. The expression ‘reasonable possibility’ of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough information to make an informed judgement. With limited or insufficient information in the case, it is likely that the event(s) will be assessed as ‘not related’.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

11.2 International Airline Transportation Association (IATA) 6.2 Guidance Document

Labelling and shipment of biohazard samples

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between risk groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are e.g., hepatitis A, B, C, D, and E viruses, human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable
- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.