DAPAMAAST: A Double-blind, Randomized, Phase IV, Mechanistic, Placebo-controlled, Cross-over, Single-center Study to Evaluate the Effects of 5 Weeks Dapagliflozin Treatment on Insulin Sensitivity in Skeletal Muscle in Type 2 Diabetes Mellitus Patients

Sponsor:	AstraZeneca AB SE-151 85 Södertälje Sweden
Principal Coordinating Investigator:	[redacted] [redacted] Maastricht The Netherlands
Sponsor Protocol No.:	D1690C00047
EudraCT No.:	2016-003991-27
Study Drug Name:	Dapagliflozin
Development Phase:	IV
Date of Protocol:	12 September 2018 Version 5.0
Date of Previous Protocol:	13 August 2018 Version 4.0

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: A double-blind, randomized, phase IV, mechanistic, placebo-controlled, cross-over, single-center study to examine the effects of 5 weeks dapagliflozin treatment on insulin sensitivity in skeletal muscle in Type 2 diabetes mellitus patients

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.

_____ _____

Anna Maria Langkilde
Global Clinical Leader
AstraZeneca R&D Gothenburg, Sweden

Date

Declaration of the Principal Coordinating Investigator

Title: A double-blind, randomized, phase IV, mechanistic, placebo-controlled, cross-over, single-center study to examine the effects of 5 weeks dapagliflozin treatment on insulin sensitivity in skeletal muscle in Type 2 diabetes mellitus patients

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation 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, as amended in 2013[1] and the guidelines on Good Clinical Practice.

Principal or Global Coordinating Investigator:

_____ _____

[redacted]
Maastricht | The Netherlands

Qualified Physician Sub Investigator:

_____ _____

[redacted]
Maastricht

PROTOCOL SYNOPSIS

Title	DAPAMAAST: A double-blind, randomized, phase IV, mechanistic, placebocontrolled-, cross-over, single-center study to examine the effects of 5 weeks dapagliflozin treatment on insulin sensitivity in skeletal muscle in Type 2 diabetes mellitus patients
Sponsor Study No.	D1690C00047
Phase	IV
Sponsor	AstraZeneca AB SE-151 85 Södertälje Sweden
Principal Coordinating Investigator	[redacted] Maastricht | The Netherlands
Study Center	It is planned to enrol subjects at one center in Maastricht: Maastricht University Medical Center + Department of Human Biology and Human Movement Sciences, 6200 MD Maastricht, The Netherlands [redacted]

Objectives

Primary:

To investigate if dapagliflozin improves skeletal muscle insulin sensitivity expressed as corrected glucose disposal rate (cGDR) in comparison with placebo after 5-week double blind treatment. Insulin sensitivity will be determined using a 2-step euglycemic hyperinsulinemic clamp (EHC) procedure.

Exploratory:

- To investigate if dapagliflozin changes Endogenous Glucose Production (EGP) in comparison with placebo after 5 weeks of double blind treatment
- To investigate if dapagliflozin improves metabolic flexibility as compared to placebo, determined by the change in respiratory exchange ratio (RER) from fasted state to insulin stimulated state during EHC after 5-week double blind treatment
- To investigate if dapagliflozin changes RER and energy expenditure as well as plasma metabolites such as beta-hydroxybutyrate and glucose as compared to placebo, before and after meals in the metabolic chamber after 5-week double blind treatment
- To investigate if dapagliflozin improves maximal capacity to form acetylcarnitine following exercise and carnitine acetyltransferase (CRAT) activity and citrate synthase activity in muscle biopsy, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin improves in vivo mitochondrial function as measured by phosphocreatinine recovery following exercise, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin improves ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin changes body composition, skeletal muscle and liver fat content, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin changes blood biomarkers such as glucagon, insulin and FGF21 in comparison with placebo after 5 weeks of double blind treatment
- To investigate if dapagliflozin changes expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue in comparison with placebo after 5-weeks of double blind treatment.
- To investigate if dapagliflozin changes body weight, BMI and systolic and diastolic blood pressure in comparison with placebo after 5-weeks of double blind treatment.

Safety:

To evaluate the safety and tolerability of dapagliflozin by assessment of DAEs/SAEs, including laboratory values and clinically significant findings after 5-week double blind treatment.

Design	This is a double-blind, randomized, mechanistic, placebocontrolled-, cross-over study, to evaluate the effect of 5 weeks dapagliflozin treatment in Type 2 Diabetes Mellitus (T2DM) patients. The maximum duration for each treatment period will be 40 days and the study will include the following: • Screening within Day -21 to Day -0 • A 5 weeks treatment period 1: Day 1 to Day 29±3 (+ 6–8 days for end of treatment assessments at site) • A 5–10 days safety follow-up after last dose of treatment (this will be a part of the 6–8 weeks wash-out period mentioned below) • A 6–8 weeks wash-out period • A 5 weeks treatment period 2: Day 1 to Day 29±3 (+ 6–8 days for end of treatment assessments at site) • A 5–10 days safety follow-up after last dose of treatment.
Treatment	There will be two treatment periods in this study: Period 1: Patients will receive either dapagliflozin 10 mg or matching placebo for a maximum of 40 days based on randomization sequence. Period 2: Patients that received 10 mg dapagliflozin in the first treatment period will receive matching placebo in the second treatment period and subjects who received placebo in the first treatment will receive 10 mg dapagliflozin in the second treatment period, for a maximum of 40 days.
Number of Patients	A total of 26 patients are planned to be randomized to have at least 22 completers in two equal sized treatment sequences. In case of more than 4 drop-outs, another 4 patients will be randomized and if this occurs a total of 30 patients will be randomized.
Population	The study population will consist of patients with diagnosed T2DM who have been on stable dose of metformin for at least last 3 months or are drug naive. The main inclusion criteria: • Women are post-menopausal (defined as at least 1 year post cessation of menses) and aged ≥ 45 and ≤ 70 years. Males are aged ≥ 40 years and ≤ 70 years. Patients should have suitable veins for cannulation or repeated venipuncture. • Patients are diagnosed with T2DM for at least the last 6 months, based on ADA 2016 standards. • Patients are on no anti-diabetic drug treatment or on stable metformin treatment for at least the last 3 months: maximum 3000 mg metformin daily dose. • Hemoglobin A1c (HbA1c) levels between 6.0% (=42 mmol/mol) and 9.0% (75 mmol/mol).

Criteria for Evaluation of Efficacy	**Primary:** Comparison of dapagliflozin versus placebo after 5 weeks treatment in skeletal muscle insulin sensitivity measured as cGDR using a 2-step EHC procedure. **Exploratory:** Comparison of dapagliflozin versus placebo after 5 weeks treatment for the following variables: • Endogenous Glucose Production (EGP) in comparison with placebo after 5 weeks of double blind treatment • Metabolic flexibility, as determined by the RER from fasted state to insulin stimulated state during EHC • RER and energy expenditure as well as plasma metabolites such as beta-hydroxybutyrate and glucose before and after meals, as measured in metabolic chambers • Maximal capacity to form acetylcarnitine following exercise as measured using ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) and by CRAT and citrate synthase activities (muscle biopsy) • In vivo mitochondrial function as measured post-exercise by phosphocreatinine recovery using ^{31}P- magnetic resonance spectroscopy • Ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry (muscle biopsy) • Body composition as measured by dual-energy X-ray absorptiometry (DEXA), and skeletal muscle and liver fat content as measured by ^{1}H-MRS. • Blood biomarkers such as glucagon, insulin and FGF21 in comparison with placebo after 5 weeks of double blind treatment • Expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue in comparison with placebo after 5 weeks of double blind treatment. • To investigate if dapagliflozin changes body weight, BMI and systolic and diastolic blood pressure in comparison with placebo after 5-weeks of double blind treatment.
Criteria for Evaluation of Safety	Safety and tolerability of dapagliflozin by assessment of the following: • Incidence of AEs (Discontinuation Adverse Events and Serious Adverse Events) • Incidence of independently adjudicated Diabetic Ketoacidosis events if any • Clinical chemistry/hematology/urine parameters • Vital signs • Physical examination

Statistical Methods	The following analysis sets will be defined: • The Enrolled Analysis Set will consist of all patients who sign the informed consent form. • The Randomized Analysis Set will consist of all randomized patients. • The Safety Analysis Set will consist of all patients who received at least one dose of study drug. • The Evaluable Analysis Set will be the primary analysis set for efficacy, and is a subset of the Randomized Analysis Set. This is also known as the Per-Protocol population. Relevant protocol deviations may lead to certain data points to be excluded from this analysis. All decisions to exclude data from the Evaluable Analysis Set will be made prior to the database lock of the study. **Efficacy:** The analysis of efficacy will be based on the evaluable analysis set. Comparison of cGDR between 5 weeks dapagliflozin and placebo treatment will be analyzed using a mixed effect Analysis Of Variance model. The model will include terms for treatment, sequence and period. A term representing patient nested within sequence will be included as a random effect. Least Square Mean (LSM) estimates, and 95% confidence limits will be generated from the fitted model for each treatment. For the primary comparison, the difference in LSM estimate of dapagliflozin versus placebo treatment, the corresponding 95% confidence interval and pvalue- will be generated. Statistical significance will be inferred at a (two-sided) 0.05 level. **Safety:** The analysis of safety will be based on the safety analysis set. Safety data during the treatment period will be evaluated and variables will only be summarized descriptively. DAEs and SAEs will be summarized by system organ class and preferred term. AEs will be listed for each patient. Safety laboratory, vital signs, physical examination and ECG findings will be listed by patient. Descriptive statistics will be presented by treatment for both absolute values and changes from baseline.
Schedule of Procedures	Refer to Section 7.1.

LIST OF STUDY PERSONNEL

Sponsor	AstraZeneca AB SE-151 85 Södertälje Sweden
Principal Coordinating Investigator	Dr. Patrick Schrauwen, PhD [redacted] Maastricht | The Netherlands [redacted]
Qualified Physician Sub Investigator	[redacted] Maastricht [redacted]
Adverse Event Reporting	AstraZeneca
Central Laboratory	Covance Central Laboratory Services Sàrl 7 Rue Moïse-Marcinhes, 1217 Geneva, Meyrin, Switzerland [redacted]

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AMPK	AMP-activated Protein Kinase
ANOVA	Analysis Of Variance
AST	Aspartate aminotransferase
BMI	Body Mass Index
cGDR	corrected Glucose Disposal Rate
CRAT	Carnitine Acetyltransferase
CS	Citrate Synthase
DAE	Discontinuation Adverse Events
DRL	Drug Reference List
DEXA	Dual-Energy X-ray Absorptiometry
DKA	Diabetic Ketoacidosis
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EGP	Endogenous Glucose Production
EHC	Euglycemic Hyperinsulinemic Clamp
FGF21	Fibroblast Growth Factor 21
GCP	Good Clinical Practice
GDR	Glucose Disposal Rate
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HbA1c	Hemoglobin A1c
hsCRP	high-sensitivity C-Reactive Protein
IB	Investigator's Brochure
ICF	Informed Consent Form
IEC	Independent Ethics Committee
ICH	International Conference on Harmonization
IMCL	Intramyocellular Lipids

IR	Insulin Resistance
IRB	Institutional Review Board
ITT	Intent-To-Treat
LSM	Least Square Mean
MedDRA	Medical Dictionary for Regulatory Activities
Mtfn	Mitofusin
mTOR	mechanistic Target Of Rapamycin
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NEFA	Non-Esterified Fatty Acids
NIMP	Non-Investigational Medicinal Product
NSAIDS	Nonsteroidal Anti-Inflammatory Drugs
PCr	Phospho Creatinine
RER	Respiratory Exchange Ratio
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SGLT2	Sodium Glucose co-Transporter-2
T2DM	Type 2 Diabetes Mellitus
VO_2max	Maximal Oxygen consumption
WHO	World Health Organization
W_{max}	Maximum work rate
^{1}H-MRS	^{1}H-Magnetic Resonance Spectroscopy
^{31}P-MRS	^{31}P-Magnetic Resonance Spectroscopy

1 INTRODUCTION

1.1 Background

The sodium-glucose cotransporter 2 inhibitor (SGLT2i) empagliflozin was associated with lower cardiovascular (CV) mortality in the EMPA-REG study (Zinman et al., 2015[2]). The effect was apparent within months from treatment start, suggesting that mechanisms beyond improved glucose control may be involved. Additionally, the SGLT2 inhibitor canagliflozin reduced CV death and disease in the recently presented CANVAS study (Neal et al., 2017[3]). There are a number of potential mechanisms that could help to explain the reported CV benefits. SGLT2 inhibition leads to increased urinary excretion of glucose and sodium and thus increased diuresis. As a consequence of SGLT2 inhibition, a decrease in Hemoglobin A1c (HbA1c), body weight, and blood pressure and an increase in hematocrit (Hct) is observed. However, none of these effects is believed to fully explain the reported CV benefits. A complementary hypothesis is enhanced night-time catabolism due to the increased urinary glucose excretion by dapagliflozin, which will lead to increased glycogenolysis and gluconeogenesis. The increased gluconeogenesis during night-time would inhibit mammalian Target Of Rapamycin (mTOR), which would increase autophagy/mitophagy of damaged mitochondria and biogenesis as well as fusion of mitochondria maximizing bioenergetic efficiency (Rambold, Cohen & Lippincott-Schwartz, 2015[4]). Therefore, this study will investigate the effect of dapagliflozin on energy metabolism with focus on mitochondrial function in skeletal muscle, which is more accessible for controlled and invasive investigations of mitochondrial function than the heart.

1.1.1 Skeletal muscle insulin resistance

Type 2 diabetes mellitus (T2DM) results from combined derangements of insulin sensitivity and beta-cell function. The main tissues contributing to reduced whole body insulin resistance (IR) are the liver, skeletal muscles and adipose tissues. One of the earliest changes in first degree relatives to T2DM patients is reduced skeletal muscle insulin sensitivity. The degree of IR in skeletal muscle is typically investigated using an euglycemic hyperinsulinemic clamp (EHC) technique and given as glucose disposal rate (GDR). This study will aim to investigate if treatment with dapagliflozin improves skeletal muscle insulin sensitivity by measuring changes in corrected glucose disposal rate (cGDR) taking into account the glucose losses in urine and determined using the EHC procedure.

The cause of the IR associated with T2DM has not yet been fully understood (Samuel & Shulman, 2016[5]).

The lipotoxicity hypothesis is based on the association between IR and increased amounts of ectopic fat in the liver (liver steatosis) and skeletal muscles, also known as intramyocellular lipids (IMCL). Increased levels of various lipids, mainly diacylglycerols, ceramides and acyl-CoA in skeletal muscles have been associated with IR. In this study, liver fat and IMCL will be assessed to determine if dapagliflozin treatment changes the amount of ectopic fat.

Another focus on this study is to investigate the mitochondrial function in skeletal muscle. A decreased mitochondrial function has been associated with skeletal muscle IR in several studies (Meex et al, 2010[6]; van de Weijer et al, 2013[7]). However, it has been unclear if reduced mitochondrial function is a cause or a consequence of IR. A possible causal relationship between mitochondrial function and skeletal muscle insulin sensitivity is the ability of the mitochondria to export excess acetyl-CoA as acetylcarnitine by the action of carnitine acetyltransferase (CRAT). Reducing acetyl-CoA levels alleviate the allosteric inhibition of the pyruvate dehydrogenase complex and allow oxidation of glucose to occur in the mitochondria (Muoio et al, 2012[8]); Noland et al, 2009[9]). A strong association between levels of acetylcarnitine in skeletal muscles and insulin sensitivity has been shown in subjects with various levels of insulin resistance, indicating that decreased mitochondrial formation of acetylcarnitine could explain skeletal muscle insulin resistance (Lindeboom et al, 2014[10]).

T2DM and IR are associated with metabolic inflexibility as first described by Kelly et al (Kelley et al, 1999[11]). Metabolic inflexibility is likely to be caused by nutrient overload resulting in substrate competition at the level of the mitochondria (Muoio, 2014[12]). Exercise intervention studies have shown improvement in metabolic flexibility, mitochondrial function and insulin sensitivity (Meex et al, 2010[6]).

Therefore, this study will also aim to investigate if treatment with dapagliflozin improves mitochondrial function and improves metabolic flexibility, including increased production of acetylcarnitine during exercise and phosphocreatinine recovery following exercise.

1.1.2 Data from previous studies investigating effects of SGLT2 inhibitors

Dapagliflozin is a sodium glucose co-transporter-2 (SGLT2) inhibitor indicated for the treatment of T2DM. The SGLT2 inhibition results in a loss of glucose and its associated energy (about 50–70 g/day) via urine. This mechanism results in a reduction in Hemoglobin A1c (HbA1c) and body weight. In a 2-year study, it was shown that dapagliflozin reduces HbA1c by 0.3%, weight by 4.5 kg and fat mass by 2.8 kg (Bolinder et al, 2014[13]). It has also been shown that the reduction in body weight is faster over the first few weeks, followed by a more gradual decline that plateaus between 24 and 50 weeks of therapy (Bolinder et al, 2012 [14]).

An effect on insulin sensitivity, measured as a 17.5% increase in cGDR, has been demonstrated after 12 weeks of treatment with 5 mg dapagliflozin (Mudaliar et al, 2014[15]). This finding was repeated in a study with a treatment period of about 2 weeks where cGDR increased by 16% in the dapagliflozin group but remained unchanged in the placebo treated group ($p<0.05$ vs baseline and placebo). This study also showed that endogenous glucose production (EGP) increased as a result of dapagliflozin treatment. The increase in EGP was associated with an increase in fasting glucagon levels and decrease in fasting insulin levels (Merovci et al, 2014[16]). An increased glucagon/insulin ratio is expected to reduce hepatic de novo lipogenesis and in parallel increase hepatic fatty acid oxidation and ketone body production. In a 4-week empagliflozin study, an increase in plasma free fatty levels and fatty acid oxidation was observed. The increase in fatty acid oxidation was associated with a decrease in both glucose oxidation and nonoxidative glucose disposal. In the same study, it was shown that the increase in EGP was exactly balancing the glucose loss in urine (Ferrannini et al, 2014[17]).

Another more recent randomized placebo controlled study also investigated insulin stimulated glucose disposal in T2DM patients, in which a 36% increase in cGDR after 2 weeks in the dapagliflozin group and a 12% increase in cGDR after 2 weeks in the placebo group was observed (Daniele et al, 2016[18]). The increase in cGDR was associated with increased non-oxidative glucose uptake, but no change in glucose oxidation; carbohydrate oxidation remained depressed in the presence of a sustained increase in whole body lipid oxidation.
In summary, 3 studies have investigated the effect of 2–12 weeks of treatment with SGLT2 inhibitors on insulin sensitivity and found 16-24% net improvements in cGDR.

1.2 Rationale for the study and justification of study design

Treatment with SGLT2 inhibitors increases glucose excretion via the urine, which results in energy losses. Increased glucagon/insulin ratio explains increased hepatic glucose production, while indirect mechanisms explaining improved skeletal muscle insulin sensitivity are not known.

The aim of the study is foremost to understand the mechanisms responsible for the improved insulin sensitivity as a consequence of the increased urinary excretion of glucose during treatment with dapagliflozin. Another aim is to understand changes in energy metabolism with emphasis on mitochondrial function that could help to better understand the significant effects observed in the EMPA-REG (Zinman et al., 2015[2]) and CANVAS (Neal et al., 2017[3]) studies on cardiovascular outcome for the future development of new medicines to treat type 2 diabetes.

For this purpose, T2DM patients on metformin or on no pharmacological treatment for T2DM will be recruited and randomized to 5 weeks of treatment with 10 mg dapagliflozin or placebo in a doubleblind- cross-over study. The placebo tablets will look exactly the same as the dapagliflozin containing tablets but will not contain any active ingredient. Placebo controlled studies are the best possible way to define the effect of the active ingredient. This short-term study will be conducted in well-controlled T2DM patients and therefore comparing the effects of dapagliflozin with placebo will be associated with limited risks.

The cross-over design was chosen so that fewer patients are needed to detect a significant difference in cGDR as compared to a parallel group design.

Significant improvement in cGDR for dapagliflozin treatment over the placebo treatment will be quantified and demonstrated in primary efficacy analysis, and the expected difference between treatment groups is the basis for the size of the study. All other end-points are considered as exploratory.

A potential draw back with cross-over designs is the risk of losing the patient in case he/she does not turn up for the second period of treatment. The risk for drop-outs will be managed by recruiting only those patients who are very motivated and this risk is therefore not regarded as very high. The patient will be contacted regularly throughout the study by telephone. Main reason for the telephone contacts is to keep the patients motivated to continue the study. Another risk associated with the cross-over design is the carrying over effects of the first treatment. Studies on the effects of sedentary lifestyle, exercise and dieting on insulin sensitivity have shown that overall these interventions

result in transient effects, which are gone within a few weeks. Considering a halflife of dapagliflozin of 12.9 hours as well as 6–8 week wash-out period and 5 weeks of treatment before the next examination, the risk for carry over effects are regarded as very small.

1.3 Risk Assessment

Dapagliflozin is approved in > 80 countries and based on global cumulative sales figures up to March 2016, it is estimated that dapagliflozin has been administered during >1,000,000 patient years. Details regarding potential risks associated with administration of dapagliflozin once a day are provided in the Investigator's Brochure (IB).

Drug related risks and protection against risks

Due to its mode of action resulting in increased urinary glucose excretion, an increased risk of urinary tract infections and genital infections has been seen. The potential risks for the treatment with dapagliflozin and other SGLT2 inhibitors are described in the IB.

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

After the introduction of dapagliflozin and other SGLT2 inhibitors, there have been post marketing reports of ketoacidosis, including diabetic ketoacidosis (DKA), in patients with Type 1 diabetes 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, will be assessed for ketoacidosis, even if blood glucose levels are < 14 mmol/L (250 mg/dL). If ketoacidosis is suspected, discontinuation or temporary interruption of dapagliflozin should be considered and the patient should be promptly evaluated.

Predisposing factors to ketoacidosis include a low beta-cell function reserve resulting from pancreatic disorders (e.g., Type 1 diabetes, 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.

Procedure related risks and protection against risks

Muscle biopsies will be performed by a skilled person under the supervision of a medical doctor. The muscle biopsy will be taken under local anaesthesia of the skin; however patients may report dull pain during the sampling of muscle material. Patients can perform normal activities after the muscle biopsy, however patients will be instructed to refrain from heavy physical labor and not remove the pressure bandage from the leg

where the biopsy was taken within the first 24 hours. In rare occasions, the patients need an oral pain killer such as paracetamol to reduce pain after the muscle biopsy. The patient will be informed that a small scar can occur where the muscle biopsy is taken.

Magnetic Resonance Spectroscopy (MRS) and Dual-Energy X-ray Absorptiometry (DEXA) (0.001 mSv) are both safe procedures, with no health risks as long as none of the exclusion criteria are met. There could be a chance that Magnetic Resonance Imaging (MRI) reveals an unexpected medical condition, of which the patient and his/ her physician will be informed.

The exercise test can cause discomfort and muscle pain. During the maximal oxygen consumption (VO_2max) cycling test, an increase in heart rate is expected which will be monitored using an ECG. This test will be performed under the supervision of trained medical staff.

EHC is a procedure performed routinely in the site laboratory without notable complications. In rare occasions, subjects could exhibit symptoms of hypoglycemia (even if their blood glucose levels are > 3 mmol/l). After successfully performing the clamp procedure, blood glucose values will be monitored for an additional 60 minutes with glucose infusion as a stand-by, in case glucose levels happen to drop. Solid food and sugar drinks will be provided after completing the clamp procedure to avoid hypoglycemia.

This study has been designed with appropriate measures in place to monitor and minimize 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.

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

Informed consent and alternatives to participation

All prospective participants will be fully informed of the possible risks 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 T2DM, and other

possible concomitant diseases will be treated according to the discretion of their health care professional; the patient will not be disadvantaged in any way.

Conclusion

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

2 STUDY OBJECTIVES

2.1 Primary Objective

To investigate if dapagliflozin improves skeletal muscle insulin sensitivity expressed as corrected glucose disposal rate (cGDR) in comparison with placebo after 5-week double blind treatment. Insulin sensitivity will be determined using a 2-step euglycemic hyperinsulinemic clamp (EHC) procedure.

2.2 Exploratory Objectives

The exploratory objectives to be evaluated are:

- To investigate if dapagliflozin changes Endogenous Glucose Production (EGP) in comparison with placebo after 5-weeks of double blind treatment.
- To investigate if dapagliflozin improves metabolic flexibility as compared to placebo, determined by the change in respiratory exchange ratio (RER) from fasted state to insulin stimulated state during EHC after 5-week double blind treatment
- To investigate if dapagliflozin changes RER and energy expenditure as well as plasma metabolites such as beta-hydroxybutyrate and glucose as compared to placebo, before and after meals in the metabolic chamber after 5-week double blind treatment
- To investigate if dapagliflozin improves maximal capacity to form acetylcarnitine following exercise and CRAT and CS activities in muscle biopsy, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin improves in vivo mitochondrial function as measured by phosphocreatinine recovery following exercise, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin improves ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry, as compared to placebo after 5-week double blind treatment
- To investigate if dapagliflozin changes body composition, skeletal muscle and liver fat content, as compared to placebo after 5-week double blind treatment.
- To investigate if dapagliflozin changes blood biomarkers such as glucagon, insulin and FGF21 in comparison with placebo after 5-weeks of double blind treatment.
- To investigate if dapagliflozin changes expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue in comparison with placebo after 5-weeks of double blind treatment.
- To investigate if dapagliflozin changes body weight, BMI and systolic and diastolic blood pressure in comparison with placebo after 5-weeks of double blind treatment.

2.3 Safety Objective

To evaluate the safety and tolerability of dapagliflozin by assessment of DAE/SAEs, including laboratory values and clinically significant findings after 5-week double blind treatment.

3 OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

This is a double-blind, randomized, mechanistic, placebo-controlled, cross-over study, to evaluate the effect of 5 weeks dapagliflozin treatment in T2DM patients, either drugnaïve- or on metformin and/or a DPPIV inhibitor.

A total of 26 patients are planned to be randomized to have at least 22 completers in two equal sized treatment sequences. In case of more than 4 drop-outs, another 4 patients will be randomized and if this occurs a total of 30 patients will be randomized.

Before visit 1, each patient is contacted by telephone to explain the study. During this phone call, the patient will also be asked some questions related to the inclusion and exclusion criteria to verify eligibility, this is regarded a so-called pre-screening.

There will be two treatment periods in this study:

Period 1: Patients will receive either dapagliflozin 10 mg or matching placebo for a maximum of 40 days based on randomization sequence.

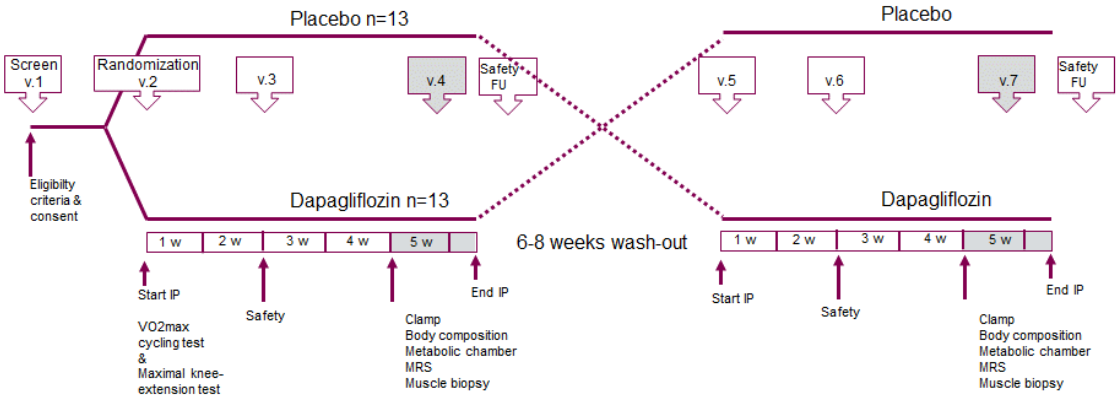
Period 2: Patients that received 10 mg dapagliflozin in the first treatment period will receive matching placebo in the second treatment period and patients who received placebo in the first treatment will receive 10 mg dapagliflozin in the second treatment period, for a maximum of 40 days.

The maximum duration for each treatment period will be 40 days and the study will include the following:

- Screening within Day -21 to Day -0
- A 5 weeks treatment period 1: Day 1 to Day 29±3 (+ 6–8 days for end of treatment assessments at site)
- A 5–10 days safety follow-up after last dose of treatment (this will be a part of the 6–8 weeks wash-out period mentioned below)
- A 6–8 week wash-out period
- A 5 weeks treatment period 2: Day 1 to Day 29±3 (+ 6–8 days for end of treatment assessments at site)
- A 5–10 days safety follow-up after last dose of treatment.

The study design is presented in Figure 1.

Figure 1: Study Design



Visit 1: Screening Visit, including C-peptide assessment, **Visit 2**: Randomization Visit, including VO_2 max cycling test, maximal knee-extension test and drug dispensing, **Visit 3**: safety, **Visit 4**: end of treatment efficacy assessments and drug dispensing, **Visit 5**: drug dispensing and safety visit, **Visit 6**: safety visit. **Visit 7**: end of treatment efficacy assessments, drug dispensing. **Safety follow up (FU) visits** taking place after end of Visit 4 and Visit 7 are telephone meetings for safety follow-up that should be made about 1 week (5-10 days) after the last dose of treatment in each period. Alternately, a visit can be made by a nurse or if needed, this visit can also take place at the site and include physical examination and laboratory assessments.
n=26 randomized

Treatment Period 1:

Visit 1:

Screening (total 1 hour)

Patients will need to sign the informed consent form and thereafter been screened to assess eligibility. During the screening, a fasted blood sample will be obtained to measure routine blood parameters including basal C-peptide, a urine sample will be obtained. Physical examination, vital signs, body weight and height will be measured and an ECG will be performed. Thereafter, medical status and history of the patients will be checked.
Only eligible patients will be included for participation in the study.

Visit 2 (Day 1):

Randomization + VO_2-max cycling test (total 1 hour) and maximal knee-extension test (total 1.5 hours)

Eligibility criteria will be checked. The blood pressure, pulse rate and weight of randomized patients will be recorded. A maximal aerobic cycling test (VO_2 max) will be performed to characterize the patient population and to ensure standardized cycling intensity during the acetylcarnitine MRS measurement scheduled for Visits 4 and 7; for the latter 70% of maximal performance (Wmax) will be used . Also maximal knee extension test will be performed to ensure standardized knee extension intensity during the phosphocreatine recovery ^{31}P-MRS measurements scheduled for Visits 4 and 7.

About 60% of the individual maximal weight obtained will be used during the ^{31}P-MRS measurements during Visits 4 and 7.

Patients will start the treatment and will receive dapagliflozin 10 mg per day or placebo depending on randomized allocation. Patients will continue dapagliflozin or placebo until end of study period; Day 29±3 (+ 6–8 days for final assessments).

The patient will be given a lifestyle card. This is a card with a set of lifestyle advices for the patient to adhere to during the course of the study, including keeping their eating pattern, alcohol consumption and physical exercise behaviour during the study.

Visit 3 (Day 15±3):

Safety

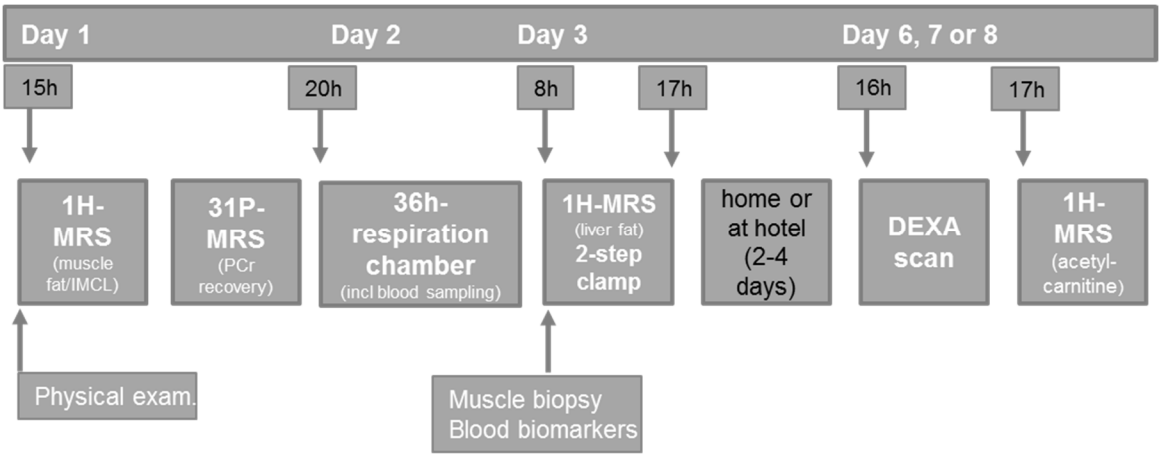
The blood pressure, pulse rate and weight of patients will be recorded and occurrence of any DAEs and SAEs will be reported.

Visit 4 and Visit 7 [Day 29±3 (+6–8 days for final assessments)]:

End of treatment period

An overview of the examinations taking place at Visit 4 and Visit 7 is presented in Figure 2.

Figure 2: Overview of the examinations taking place at Visit 4 and Visit 7



Visit 4 and Visit 7 will take place over 6–8 days. Four days (Day 1-3 and Day 6, 7 or 8) will be at the site. Two to four days after Day 3 will be spent at home or at the hotel. Fasting blood samples for biomarker analyses and muscle biopsy will take place in the morning of Day 3.
15:00h, 20:00h, 8:00h, 17:00h, 16:00h, and 17:00h denote the time of the day when the examination are taking place.

The end of treatment visit will take place over 6–8 days: four days (Days 1–3 and Day 6, 7 or 8) will be spent at the site and 2–4 days after Day 3 will be spent either at home or at the hotel. The last dose will be given on the last day of the end of treatment visit (at Visit 4 or Visit 7).

On Day 1 of the end of treatment visit, physical examination, the blood pressure, pulse rate and weight of patients will be recorded and occurrence of any DAEs and SAEs will

be reported. Blood samples will be collected to determine safety parameters. After this, the intramyocellular lipid content will be measured using ^{1}H-MRS and PCr recovery measurement will be done using ^{31}P-MRS methodology.

From the end of Day 1 until the morning of Day 3 of the end of treatment visit (36 hours), the patients will stay in the metabolic chamber. During this stay, whole body energy expenditure, sleeping metabolic rate and substrate metabolism will be measured.

On Day 2 of the end of treatment visit when the patients are in the metabolic chamber, blood samples will be drawn at 7 occasions and urine will be collected for 24 hours, volumes will be measured and aliquots may be frozen for later analyses.

On Day 3, the 2-step EHC will be performed to determine whole body and hepatic insulin sensitivity. During this clamp, when tracer infusion has started but before insulin infusion (basal state), 1H-MRS to determine liver lipid content will be done and a muscle biopsy will be taken from the *m. vastus lateralis*. Indirect calorimetry will be performed before and after the start of insulin infusion.

After the assesments the patients can take a break of 2-4 days, which can be spent at home (or in a hotel if needed).

On Day 6, 7 or 8 of the end of treatment visit, a DEXA scan will be performed to determine whole body composition and ^{1}H-MRS will be performed to measure acetylcarnitine levels before and after 30 minutes of cycling (at 70% of maximum work rate (W_{max}) as pre-determined at Visit 2). The study drug accountability will be assessed on this day.

Wash-out Period:

After the last day of the end of treatment visit, treatment with dapagliflozin or placebo will be discontinued and patients will enter a 6–8 weeks wash-out period.

A safety follow- up will be performed about 1 week (5-10 days) after the end of treatment visit for reporting of DAEs and SAEs.

Treatment period 2:

After a 6–8 week wash-out period, the cross over will take place and patients having received dapagliflozin will receive the placebo and patients having received placebo will receive dapagliflozin over a 5-week treatment period.

The study procedures detailed for Visit 2, Visit 3 and Visit 4 from treatment period 1 will be performed again at Visit 5 (with additional physical examination, blood and urine sampling for laboratory safety assessment with the exception of VO_2 cycling test and maximal knee-extension testing), Visit 6 and Visit 7 during the treatment period 2. A safety follow-up will again be performed 5–10 days post-last dose.

Guidelines for individual patient withdrawal are described in Section 4.4. Guidelines for study ending are described in Section 9.10.

Refer to the Study Plan (Table 4) for details on the timing of planned assessments and Section 6.1 for details on the methodology of assessments.

3.2 Criteria for Evaluation of the Study

Refer to the study objectives in Sections 2.1, 0, and 2.3.

3.2.1 Primary Endpoint

Comparison of dapagliflozin versus placebo after 5 weeks treatment in skeletal muscle insulin sensitivity measured as cGDR using a 2-step EHC procedure.

3.2.2 Exploratory endpoints

Comparison of dapagliflozin versus placebo after 5 weeks treatment for the following variables:

- Change of Endogenous Glucose Production (EGP)
- Metabolic flexibility, as determined by the RER from fasted state to insulin stimulated state during EHC
- RER and energy expenditure as well as plasma metabolites such as betahydroxybutyrate and glucose before and after meals, as measured in metabolic chambers
- Maximal capacity to form acetylcarnitine following exercise as measured using ^{1}H-MRS and by CRAT activity (muscle biopsy)
- In vivo mitochondrial function as measured post-exercise by phosphocreatinine recovery using ^{31}P-MRS
- Ex vivo mitochondrial function in permeabilized muscle fibers using high resolution respirometry (muscle biopsy)
- Body composition as measured by DEXA, and skeletal muscle and liver fat content as measured by ^{1}H-MRS
- Change of blood biomarkers such as glucagon, insulin and FGF21
- Change of expression of mRNA and/or proteins involved in metabolic regulation in muscle tissue.
- Changes in body weight, BMI and systolic and diastolic blood pressure.

3.2.3 Safety endpoint

Safety and tolerability of dapagliflozin by assessment of the following:

- Incidence of DAEs and SAEs
- Incidence of independently adjudicated DKA events, if any
- Clinical chemistry/hematology/urine parameters
- Vital signs
- Physical examination

4 STUDY POPULATION

The study population will consist of patients with diagnosed T2DM who have been on stable dose of metformin for at least the last 3 months or are drug naive.

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

4.1 Inclusion Criteria

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

1. Patients are able to provide signed and dated written informed consent prior to any study specific procedures.
2. Women are post-menopausal (defined as at least 1 year post cessation of menses) and aged ≥ 45 and ≤ 70 years. Males are aged ≥ 40 years and ≤ 70 years. Patients should have suitable veins for cannulation or repeated venipuncture.
3. Patients are diagnosed with T2DM for at least the last 6 months, based on American Diabetes Association 2016 standards (Diabetes Care, 2016 [19]).
4. Patients are on no other anti-diabetic drug treatment, or on stable maximum 3000 mg daily dose metformin treatment and/or on stable dose of a DPPIV inhibitor treatment for at least the last 3 months.
5. HbA1c levels ≥6.0% (=42 mmol/mol) and ≤9.0% (75 mmol/mol).
6. Have a body mass index (BMI) ≤ 38 kg/m^2.

4.2 Exclusion Criteria

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

Study-related:

1. 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).
2. Previous enrolment in the present study or participation in another clinical study with an investigational product during the last 3 months or as judged by the Investigator.

General health-related:

3. History of or presence of any clinically significant disease or disorder including a recent (< 3 months) cardiovascular event 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.
4. Clinical diagnosis of Type 1 diabetes, maturity onset diabetes of the young, secondary diabetes or diabetes insipidus.
5. Unstable/rapidly progressing renal disease or estimated Glomerular Filtration Rate < 60 mL/min (Cockcroft-Gault formula).

Males:

$$\text{Creatinine clearance (mL/min)} = \frac{\text{Weight (kg) X (140-Age)}}{\text{Serum creatinine (µmol/l)}} \text{ X } 1.23$$

Females:

$$\text{Creatinine clearance (mL/min)} = \frac{\text{Weight (kg) X (140-Age)}}{\text{Serum creatinine (µmol/l)}} \text{ X } 1.04$$

6. Clinically significant out of range values of serum levels of either alanine aminotransferase (ALT), aspartate aminotransferase (AST) or alkaline phosphatase (ALP) in the Investigator's opinion.
7. Contraindications to dapagliflozin according to the local label.
8. Use of antidiabetic drugs other than metformin within 3 months prior to screening.
9. Weight gain or loss > 5 kg in the last 3 months, ongoing weight-loss diet (hypocaloric diet) or use of weight loss agents.
10. History of drug abuse or alcohol abuse in the past 12 months. Alcohol abuse is defined as > 14 drinks per week for women and > 21 drinks per week for men (1 drink = 35 cl beer, 14 cl wine or 4 cl hard liquor) or as judged by the Investigator.
11. Any clinically significant abnormalities in clinical chemistry, hematology or urinalysis or other condition the Investigator believes would interfere with the patient's ability to provide informed consent, comply with study instructions, or which might confound the interpretation of the study results or put the patient at undue risk.
12. Plasma donation within one month of screening or any blood donation/blood loss > 500 mL within 3 months prior to screening or during the study.
13. Anemia defined as Hemoglobin (Hb) < 115 g/L (7.1 mM) in women and < 120 g/L (7.5 mM) in men.
14. Use of anti-coagulant treatment such as heparin, warfarin, platelet inhibitors, thrombin and factor X inhibitors.
15. Use of medication such as oral glucocorticoids, anti-estrogens or other medications that are known to markedly influence insulin sensitivity.
16. Use of loop diuretics.
17. Regular smoking and other regular nicotine use.
18. Any contra-indication to magnetic resonance imaging scanning. These contra-indications include patients with following devices:
    - Central nervous system aneurysm clip
    - Implanted neural stimulator
    - Implanted cardiac pacemaker of defibrillator
    - Cochlear implant
    - Metal containing corpora aliena in the eye or brain.

19. Patients, who do not want to be informed about unexpected medical findings, or do not wish that their physician be informed about coincidental findings, cannot participate in the study.

4.3 Discontinuation of Investigational product

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

- Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment.
- Severe non-compliance to protocol, as judged by the Investigator and/or AstraZeneca.
- Safety reasons, e.g., AE that warrants discontinuation, as judged by the Investigator and/or AstraZeneca.
- Patient meets the required exclusion criteria.
- Patient lost to follow-up.
- Pregnancy.

4.4 Criteria for withdrawal

In all cases, the reason(s) for withdrawal, and the primary reason, must be recorded on the electronic case report form (eCRF). If a patient is prematurely withdrawn from the study drug for any reason, the Investigator must make every effort to perform the safety evaluations and refer the patient to further treatment if it becomes necessary.

A patient may be withdrawn from study drug by the Sponsor, Regulatory Authorities, or Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs).

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

A subject may be re-tested during the screening window, provided any inclusion or exclusion criteria the subject fails is expected to resolve prior to the randomisation visit. If the criteria are not expected to resolve, the subject should be screen failed and withdrawn from the study. A subject may be re-screened once if they have failed to meet the inclusion exclusion criteria at a previous attempt. A re-screening can be done if at least 21 days have passed after the initial screening visit. Rescreened subjects should be assigned a different subject number than in the initial screening and sign another ICF. However, rescreening should be documented so that its effect on study results, if any, can be assessed.Patients who are discontinued from the study drug will not be replaced. If the number of discontinued patients exceeds four patients, additional four patients will be randomized. The maximum number of additional patients randomized will be four, i.e., the total number of randomized patients will not exceed 30.

[redacted]

[redacted]

4.6 Patient Identification and Randomization

4.6.1 Patient Identification

Upon enrolment, each patient will receive a unique screening number. Enrolled patients who drop out of the study before randomization will retain their screening number. The enrolment number will be in the following format: E code format (E_Site ID_Patient ID), e.g., E0001001 and will be recorded in the eCRF.

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.

4.6.2 Randomization Scheme

Randomization codes will be assigned strictly sequentially as patients become eligible for randomization, starting from 1001. Randomization will be used to assign each subject to a specific treatment sequence (dapagliflozin: placebo or vice-versa), and the randomization schedule will assign sequences using blocks of equal and fixed size. Blocks as well as sequence will both be randomized within the randomization list.

Once a randomization number has been allocated to a patient, it may not be assigned to another patient.

The randomization scheme will be set-up by the AstraZeneca randomization solution (AZRand).

4.6.3 Allocation/Randomization of Patients to Treatment

Study drug will be prepared and packed by AstraZeneca supply chain (to be unblinded) and then provided to the onsite pharmacy, based on the randomization list. The label will contain the random number and visit associated with the randomization list. The onsite pharmacy (to remain blinded) will coordinate the dispensing of study drug based on the random number and visit.

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 or placebo tablets.

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

Table 1: Identity of the Study Treatment

IMP	Dosage form and strength	Manufacturer
Dapagliflozin	Plain, green, diamond-shaped, film-coated tablet	AstraZeneca
Placebo	Plain, green, diamond-shaped, film-coated tablet	AstraZeneca

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

5.1.2 Non-Investigational Medicinal Product

During the study the following commercial available Non-Investigational Medicinal Products (NIMP) will be sourced from the local pharmacy and used during the study related procedures. All NIMPs will be stored, prepared and handled by the site according to standard practice, with the exception of D-glucose [6,6-D2] infusion (see below).

Euglycemic hyperinsulinemic clamp:

- Glucose Intravenous Infusion BP 20% w/v.
- NovoRapid 100 U/ml solution for injection
- D-Glucose [6,6-D2] infusion 16.8 mg/ml (stable isotope), (prepared by the pharmacy of Radboud University Medical Center, in Nijmegen, the Netherlands and stored and handled by the site).

Muscle biopsy (local anaesthetics):

- Lidocaine Hydrochloride 1% w/v Solution for Injection

5.2 Administration

Patients will be provided with dapagliflozin or matching placebo tablets at Visits 2 and 4 to last for up to 40 days.

Minimum duration of treatment will be 32 days (26 +6 days) and maximum duration of treatment will be 40 days (32 + 8 days).

After a 6–8 week wash-out period, the cross over will take place and patients having received dapagliflozin will receive the placebo tablets and patients having received placebo will receive the dapagliflozin tablets at Visits 5 and 7 for the next 5 weeks of treatment period 2.

The tablet will be taken orally once daily in the morning and at approximately the same time of the day during the treatment periods. On the days of visits for the clamp, the tablet will be taken after fasting blood samples have been collected.

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 study treatment will be taken by the patients the day after randomization.

The patients will be asked to bring their bottles with tablets for the study visits.

5.3 Packaging, Labeling and Storage

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 Unblinding

The study will be performed in a double-blind manner. All study drugs will be supplied in identical bottles and will be similar in color, smell, taste, and appearance, thereby enabling double-blind conditions.

The study blind should not be broken except in a medical emergency (where knowledge of the study drug received would affect the treatment of the emergency) or regulatory requirement.

All attempts must be made to contact the Sponsor/Medical Monitor before the actual treatment dispensed can be provided. These attempts must be documented. If there is an emergency and no contact is possible, an unblinding may be needed.

If unblinded, the date, time and reason must be recorded in the patient's eCRF, and any associated AE report.

If an Investigator, site personnel performing assessments, or patient, is unblinded, the patient must be listed as major protocol deviation.

The overall randomization code will be broken only for reporting purposes. This will occur once all final clinical data have been entered onto the database and all data queries have been resolved, and the assignment of patients to the analysis sets has been completed.

5.5 Drug Accountability

The study drugs 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 at the end of each treatment period.

The Investigator is responsible for making sure:

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

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

At the termination of the clinical study or at the request of AstraZeneca, the Investigator will either return any unused study drugs to AstraZeneca or its designee, or destroy study drugs at the site depending on local regulations. If the study drugs are destroyed at the site, the site personnel will account for all unused study drugs and for appropriate destruction. If the study drugs are returned to AstraZeneca or its designee, the study site personnel or the AstraZeneca monitor will account for all study drugs received at the site, unused study drugs and for appropriate destruction. Certificates of delivery, destruction and return should be signed and archived.

Each dispensation of the 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). Tablet counts will be recorded in the eCRF.

Compliance with study treatment will be calculated as specified in the statistical analysis plan.

5.7 Previous and Concomitant Medications

Any medication the patient takes other than the study drug, including herbal and other non-traditional remedies, food and vitamin supplements, etc. are 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, dosage, 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, and whether they have taken any other medications since the previous visit.

5.7.1 Permitted concomitant medication

Patients with T2DM will continue on metformin and/or a DPPIV inhibitor therapy at the same dose that they were on prior to start of the study. Metformin treatment should be taken at the same time as the IP if possible.

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 whether the patient has started taking extra concomitant medication. The status of each concomitant medication (i.e., preexisting- or new) will be documented in the eCRF.

Therapies for treatment of other comorbidities are allowed with the exception of medications listed in Section 5.7.2.

All efforts should be made to keep the dose of study medication stable throughout the study.

5.7.2 Prohibited concomitant medication

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

The following medications are prohibited during the study:

- Use of antidiabetic drugs other than metformin or DPPIV inhibitors.
- Use of anti-coagulant treatment such as heparin, warfarin, platelet inhibitors, thrombin and factor X inhibitors.
- Use of medication such as oral glucocorticoids, anti-estrogens or other medications that are known to markedly influence insulin sensitivity.
- Use of loop diuretics.
- NSAIDS should not be used within 3 days of Visit 4 and 7.

6 VARIABLES AND METHODS OF ASSESSMENT

6.1 Efficacy variables

Assessments will be performed in accordance with the Study Plan (Table 4).

6.1.1 Maximal knee-extension test

A maximal knee extension test will be performed at Visit 2 to ensure standardized knee extension intensity during the PCr ^{31}P-MRS measurements scheduled for Visits 4 and 7. This test will be performed on a magnetic-resonance compatible knee-extension exercise devise, with incremental weight put on the left leg (500 g every 30 seconds) till exhaustion is reached. During the actual ^{31}P-MRS test (Visits 4 and 7) 60% of this individual maximal weight will be used.

6.1.2 Maximal VO_2 cycling test

A maximal aerobic cycling test will be performed at Visit 2 to characterize the participant population and to ensure a standardized cycling intensity during the acetylcarnitine ^{1}H-MRS measurements at Visits 4 and 7. Subjects are requested to refrain from strenuous exercise for the last two days before these visits.

Subjects will come to the laboratory in the overnight fasted state and will be provided with a light breakfast before subjects' maximal oxygen uptake (VO_{2max}) will be measured by a cycling test to determine maximal aerobic capacity. An ECG will be performed by the experienced staff at the site during the VO_2 max cycling test. To determine maximal aerobic capacity, VO_{2max} and W_{max} will be measured on an electronically braked cycle ergometer during an incremental exhaustive exercise test. In order to do so, O_2 consumption and CO_2 production will be measured using an Omnical system. In addition, RER will be monitored as a verification of their maximal effort. The following protocol will be used: after a warming-up period of five minutes with a start workload of 55 watt for females and 75 watt for males, the workload will be increased with 50 watt every 2.5 minutes. When the heart rate of patients reaches 80% of their predicted maximal heart rate (220-age), the workload will be increased with 25 watt until exhaustion is reached or until they are no longer able to keep their speed of rotation above 60 times per minute.

6.1.3 Euglycemic hyperinsulinemic clamp, indirect calorimetry and muscle biopsy

Two step 5.5 hour EHC (3 hour 10 mU/m^2/min and 2.5 hour 40 mU/m^2/min insulin infusion) in combination with infusion of D-glucose (6,6-D2) glucose to determine rates of EGP, and whole body GDR and urine glucose excretion to be able to calculate cGDR. The clamp will be performed in the post-absorptive state beginning at about 8 am after an overnight fast (in the respiration chamber), and subjects are asked to refrain from physical exercise for the two days proceeding the clamp test. Study medication will be taken in the morning before the clamp. A teflon cannula will be inserted into the antecubital veins of one arm for the infusion of glucose tracer, insulin and glucose. Another cannula will be inserted in a retrograde manner into a superficial dorsal hand vein. This venous blood will be arterialized by placing the hand into a hotbox, which blows warm air (55ºC). Blood samples will be taken (approximately 167 mL) throughout

the EHC test. Urine volume will be determined during the clamp procedure, and urine aliquots frozen for later analyses of glucose concentrations.

Before the insulin infusion starts, there will be a baseline period of 3 hours including pre-infusion of D-glucose [6,6-D2] (priming dose: 2.4 mg/kg; continuous infusion: 0.04 mg/kg-1/min-1) to reach equilibrium in the plasma pool. This is a naturally occurring isotope, which is not harmful.

Within this baseline period, a muscle biopsy will be taken from the *m. vastus lateralis* (see 6.1.8) and a measurement with indirect calorimetry (ventilated hood) will be performed.

During the indirect calorimetry, respiratory gas exchange will be measured using open air circuit respirometry with an automated ventilated hood system. These indirect calorimetry measurements will be repeated in the last 30 minutes during the 10 mU/m^2/min clamp as well as during the last 30 minutes of the 40 mU/m^2/min clamp. These 30 minute periods are the so-called 'steady-state' periods in which exogenous glucose infusion equals glucose uptake. With the indirect calorimetry measurements, metabolic flexibility will be determined, reflected by the change in RER from baseline to insulin stimulation.

After 150 minutes of isotopic equilibration, four blood samples will be obtained at 10 minute intervals (T=150, 160, 170 and 180 minutes) for the determination of basal blood substrates, whole body glucose disposal and hepatic glucose production.

Indirect calorimetry (ventilated hood) will be performed during this last half hour of the baseline equilibration period to determine substrate oxidation (T150 – 180 minutes).

At T=180 minutes, a 3-hour low primed constant infusion of insulin will be initiated (10 mU/m^2/min) to asses hepatic insulin resistance. Plasma glucose levels will be clamped at ~5 mmol/L by variable co-infusion of 20% glucose. At regular time points (every 5 to 10 minutes), a small volume of blood (0.9 mL) will be sampled for immediate determination of plasma glucose concentration. When necessary, glucose infusion rate will be adjusted to obtain plasma glucose levels of ~5 mmol/L (euglycemia).

Indirect calorimetry will be performed at T=330-360 minutes. During this time period, four blood samples will be obtained at 10 minute intervals for the determination of whole body glucose disposal and hepatic glucose production (T=330, 340, 350 and 360 minutes). At T=360 minutes, the 2.5 hour high primed constant insulin infusion will take place (40 mU/m^2/min), in order to fully stop the hepatic glucose production and only study the rate of disappearance (Rd) as a measure for skeletal muscle insulin sensitivity. Plasma glucose levels will be clamped at ~5 mmol/L by variable co-infusion of 20% glucose. At regular time points (every 5 to 10 minutes), a small volume of blood (0.9 mL) will be sampled for immediate determination of plasma glucose concentrations. When necessary, glucose infusion rate will be adjusted to obtain plasma glucose levels of

~5 mmol/L (euglycemia). Indirect calorimetry will be performed at T=480–510 minutes. During this time period, four blood samples will again be drawn at 10 minute intervals (T=480, 490, 500 and 510 minutes).

6.1.4 Body composition

DEXA scan will be used to determine body composition. DEXA assesses total body bone mineral density and accurately measures body soft tissue composition (muscle mass and fat mass). By measuring the body's muscle mass, fat mass and bone mineral density, total body fat percentage can be determined.

6.1.5 24h energy expenditure, 24h RER

This will involve a 36-hour stay in a whole-body room calorimetry chamber, of which the last 24 hours will be used for measurements (to determine sleeping metabolic rate as well as energy expenditure, 24-hour RER fasting-fed state and substrate metabolism). Diurnal blood samples will be taken at 7 occasions during the stay in the chamber (before and after meals as well as before bedtime) to determine a.o. plasma levels of glucose, Non-Esterified Fatty Acids (NEFA), beta-hydroxybutyrate, insulin, glucagon and Fibroblast Growth Factor 21 (FGF21).

6.1.6 ^{1}H-MRS

Acetylcarnitine levels are measured in the muscle using ^{1}H-MRS (before and after exercise at 70% of Wmax). Lipid quantification of the liver and skeletal muscles will also be performed using ^{1}H-MRS. The post-exercise PCr recovery measurement will be performed using phosphor-MRS (^{31}P-MRS) to assess in vivo mitochondrial function.

MRS and MRI are modern diagnostic tools that do not imply significant risks (no ionizing radiation). MRI will be used to guide the spectroscopic measurements to the correct location. All scans will be performed on a 3.0 T clinical MRI scanner.

All patients will be in the supine position. For the intramyocellular lipid content, acetylcarnitine and PCr recovery measurements, the patients will be shifted by the legs into the bore of the magnet and the head will stay outside the bore. For the intrahepatic lipid measurement, the patients will again be in a supine position, but the other way around (head first). The patients will be asked to lie still during the whole measurement, unless otherwise instructed during the post-exercise PCr recovery measurement. During this measurement, knee-extension exercise will be performed for 5 minutes, while being in the magnet. During the scans, the patients will receive a buzzer to let the Investigator know when they are uncomfortable. Additionally, they will stay in contact with the Investigator via an intercom communication system. As the acquisition of MRI images is accompanied by a 'clanging' noise, the patients will be provided with protective headphones. There is a chance that the MRI may reveal an unexpected medical condition, of which the patient and the treating physician will be informed.

Hepatic lipid accumulation:

An 18 cm^{3} volume will be selected within the right lobe of the liver as per the MRI images of the upper abdomen and the ^{1}H-MRS spectra will be acquired from this region. To prevent motion artefacts, patients will be asked to breathe in the rhythm of the spectroscopic measurement. In the ^{1}H-MRS spectra, the water signal that is dominating

the proton spectra will be suppressed using frequency-selective pre-pulses and the spectra will be fitted to quantify the lipid peak. The lipid/water ratio will be used as the parameter of intrahepatic lipid content. Patients will need to breath as per the 4s-rhythm for about 6 minutes. Total acquisition time (with positioning of patient and imaging) should be about 45 minutes.

Intramyocellular lipid content:

A volume of interest will be selected within the *tibialis anterior* muscle as per the MRI images of the leg and the ^{1}H-MRS spectra will be acquired from this region. Patients will be able to breathe freely during the whole measurement. In the ^{1}H-MRS spectra, the water signal that is dominating the proton spectra will be suppressed using frequencyselective- pre-pulses and the spectra will be fitted to quantify the lipid peak. The lipid/water ratio will be used as the parameter of intramyocellular lipid content. Total acquisition time is estimated to be about 30 minutes.

Muscle acetylcarnitine levels:

Acetylcarnitine quantification will be performed by ^{1}H-MRS during rest and directly after exercise. Exercise is well known to increase acetylcarnitine concentrations and a high-intensity cycling protocol for 30 minutes would result in quasi-maximal acetylcarnitine levels. Therefore, this will enable investigation of the maximal capacity to form acetylcarnitine. During the 30 min cycling test blood will be drawn at 3 time-points: T=0 min, T=15 min and T=30 min for measurement of lactate.

A volume of interest will be selected within the *vastus lateralis* as per the MRI images and the ^{1}H-MRS spectra will be acquired from this region. The ^{1}H-MRS spectra will allow quantification of the amount of acetylcarnitine in the muscle relative to creatine. A 30-minute cycling protocol will be used where patients will be required to cycle at 70% of their individual maximal wattage (determined during the maximal aerobic capacity test). The exercise intensity is relatively high to maximize the acetylcarnitine production. A fixed duration and wattage will be chosen to prevent variation in exercise intensity and duration among patients. The cycling will be performed on an ergometer located in the room next to the MRI scanner. The total scanning time including exercise would be about 1.5 hours.

6.1.7 ^{31}P-MRS

In vivo mitochondrial function using ^{31}P-MRS methodology will be performed. Level of PCr will be measured for 5 minutes during rest, 5 minutes during kneeextension- exercise (at 60% of maximal weight as pre-determined during Visit 3) and during 5 minutes after exercise, during the recovery phase.

6.1.8 Muscle biopsy from the m. vastus lateralis

A percutaneous muscle biopsy will be obtained from the *vastus lateralis* muscle during the EHC procedure once every treatment period. After local anaesthesia, a side-cutting needle of 5 mm diameter will be passed through a 7 mm skin incision, as per the technique by Bergström J (Bergström et al, 1967[20]).

Nonsteroidal anti-inflammatory drugs (NSAIDs) should not be used 3 days before muscle biopsies.

Biopsies will be performed for assessment of the following:

- Ex vivo mitochondrial function in permeabilized muscle fibers (using high resolution respirometry)
- CRAT and CS activities and acylcarnitine levels
- Ribonucleic acid/protein extraction and measurements of relevant mRNA and/or proteins involved in metabolic regulation [such as but not limited to FGF21, PCG1 alpha, Mitofusin (Mtfn) 1 and 2, AMP-activated protein kinase (AMPK)/ mammalian target of rapamycin (mTOR) pathway]
- For assessment of size and appearance of the mitochondria, a small fragment of muscle will be processed for electron microscopy (EM)provided that muscle biopsy size allows doing so.

6.1.9 Laboratory blood assessments during Visits 4 and 7

- Fasting plasma/serum samples (+ one extra vial of serum and plasma Biobank samples)
- Insulin, glucagon, glucose, NEFA, betahydroxybutyrate and FGF21 (7 different occasions during 24-hours in the metabolic chamber)
- HbA1c (assessed also at Visit 1 (screening) and 5 (restart treatment))
- Uric acid
- Acylcarnitines and amino acids
- Lactate (during ^{1}H-MRS acetylcarnitine exercise test)
- High-sensitivity Creactive protein (hsCRP-)

6.1.10 Laboratory urine assessments

Urine collection during clamp and 24-hour urine collection (in 6 hour aliquots) in the metabolic chamber will be followed by measurement of volume and glucose. The urine samples from the metabolic chamber will also be analyzed for nitrogen and creatinine. Samples for measurement of glucose, nitrogen, creatinine and a sample for later exploratory measurements will be stored frozen until analysis. Analysis of glucose in urine collected during the clamp in the metabolic chamber must be performed after the randomization code has been broken to avoid unblinding.

6.2 Safety variables

6.2.1 Laboratory safety assessments

Blood and urine samples for determination of clinical chemistry, hematology, and pregnancy testing will be taken in accordance with the Study Plan (Table 4) and sent to a local Laboratory for analysis.

Self-Monitoring of Blood Glucose will be allowed but not reported.

Additional safety samples may be collected if clinically indicated at the discretion of the Investigator. A retest performed by the Central Laboratory is preferred. However, in case of safety concerns and urgency, the Investigator may decide to perform the retest locally according to local rules. 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.

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

Table 2 specifies the laboratory safety assessments to be determined.

Table 2 Laboratory safety assessments

Hematology/Hemostasis (whole blood)	**Clinical Chemistry [serum (S) or plasma (P)]**
B-Hemoglobin (Hb)	S-Aspartate transaminase (AST)
B-Hematocrit	S-Alanine transaminase (ALT)
	S-Alkaline phosphatase (ALP)
	S-Bilirubin, total
	S-Creatinine
	S/P-Glucose
Urine analysis (dipstick)	S/P-Potassium
Pregnancy test	

For information on how AEs based on laboratory tests should be recorded and reported, see Section 6.3.3.6.

For information regarding labelling, storage and shipment of the laboratory samples, see the Laboratory Manual.

6.2.2 Physical examinations

Physical examinations will be performed in accordance with the Study Plan (Table 4).

A physical examination will be performed at the baseline and at the end of treatment visits of both treatment periods and will include an assessment of the following: general appearance, abdomen, cardiovascular and respiratory systems.

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 only if they are SAEs or lead to discontinuation of the study treatment(see Section 6.3.3.6).

6.2.3 Vital signs

The following vital signs will be assessed in accordance with the Study Plan (Table 4):

- Pulse (beats per 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 lying 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 6.3.3.6.

Abnormal vital signs values should be reported as AEs only if they are SAEs or lead to discontinuation of the study treatment (see Section 6.3.3.6).

6.2.4 Other safety assessments

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 < 14 mmol/l (250 mg/dl). If ketoacidosis is suspected, discontinuation or temporary interruption of study drug should be considered and the patient should be promptly evaluated. All potential events of DKA will be reported in the eCRF and sent to an independent DKA committee.

A separate DKA manual will define and describe the procedures for the collection of DKA information, handling, adjudication criteria and reporting of these events/cases.

6.3 Safety reporting

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

6.3.1 Definition of AEs

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 (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

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

6.3.2 Definitions of serious adverse event

An SAE is an AE occurring during any study phase (i.e., 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 hospitalization or prolongation of existing hospitalization
- 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 10.1.

6.3.3 Recording of AEs

6.3.3.1 Time period for collection of AEs

Discontinuation AEs (DAEs) will be collected throughout the study, from the time of enrolment excluding the wash-out period. SAEs will be collected from the time of signing the informed consent form, throughout the treatment period and including the follow-up period (see Table 4).

6.3.3.2 Follow-up of unresolved AEs

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 subject with ongoing DAE(s)/SAE(s) at the end of the study, if judged necessary.

6.3.3.3 Variables

The following variables will be collect for each DAE/SAE;

- AE (verbatim)
- The date when the AE started and stopped
- Maximum 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 hospitalization (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 (e.g., 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 6.3.2. 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 6.3.2. 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 6.3.2.

6.3.3.4 Causality collection

The Investigator will assess causal relationship between study drug and each DAE/SAE, 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 study drug?'

For SAEs 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.

6.3.3.5 AEs based on signs and symptoms

All DAEs/SAEs 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 DAEs/SAEs, 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.

6.3.3.6 AEs based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarized in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, or vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria 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 (e.g., anaemia versus low hemoglobin 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 only if they are SAEs or lead to discontinuation of the study treatment.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the study drug, 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 within one day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative 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 of any follow-up information on a previously reported SAE within one calendar day i.e., 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.

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

The AstraZeneca representative will advise the Investigator/study site personnel how to proceed.

The reference document for definition of expectedness/listedness is in the Investigator's brochure for the AstraZeneca drug.

6.3.5 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 T2DM. Suspected single intake of more than 50 tablets of 10 mg dapagliflozin tablets or repeated intake of more than 10 tablets of 10 mg dapagliflozin tablets should 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/DAEs/SAEs 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, the Investigator or other site personnel inform appropriate AstraZeneca representatives immediately, or **no later than 24 hours** after he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is provided to the AstraZeneca Patient Safety data entry site.

For overdoses associated with a SAE, the standard reporting timelines apply, see Section 6.3.4 For other overdoses, reporting must occur within 30 days.

6.3.6 Pregnancy

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

6.3.6.1 Maternal Exposure

Women of childbearing potential are not allowed to be included in this study. Should a pregnancy still occur, the investigational product should be discontinued immediately and the pregnancy reported to AstraZeneca.

Pregnancy itself is not regarded as an adverse event 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 subject was discontinued from the study.

If any pregnancy occurs during the course of the study, then the Investigator or other site personnel informs the appropriate AstraZeneca representatives within 1 day i.e., immediately but **no later than 24 hours** after he or she becomes aware of it.

The designated AstraZeneca representative 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 and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

6.4 Medication Error

For the purposes of this clinical study a medication error is an unintended failure or mistake in the treatment process for an AstraZeneca study drug that either causes harm to the subject or has the potential to cause harm to the subject.

A medication error is not due to lack of efficacy of the drug, but rather a human or process related failure while the drug is in control of the study site staff or subject.

Medication error includes situations where an error:

- Occurred
- Was identified and intercepted before the subject received the drug
- Did not occur, but circumstances were recognize that could have led to an error.

Examples of events to be reported in clinical studies as medication errors:

- Drug name confusion
- Dispensing error e.g. medication prepared incorrectly, even if it was not actually given to the subject
- Drug not administered as indicated, for example, wrong route or wrong site of administration

- Drug not taken as indicated e.g. tablet dissolved in water when it should be taken as a solid tablet
- Drug not stored as instructed e.g. kept in the fridge when it should be at room temperature
- Wrong subject received the medication
- Wrong drug administered to subject.

Examples of events that **do not** require reporting as medication errors in clinical studies:

- Subject accidentally missed drug dose(s) e.g. forgot to take medication
- Accidental overdose (will be captured as an overdose)
- Subject failed to return unused medication or empty packaging
- Errors related to background and rescue medication, or standard of care medication in open label studies, even if an AZ product.

Medication errors are not regarded as AEs but AEs may occur as a consequence of the medication error.

If an medication error occurs in the course of the study, then the Investigator or other site personnel informs the appropriate AstraZeneca representatives within 1 day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the Investigator to ensure that all relevant information is completed within 1 or 5 calendar days if there is an SAE associated with the medication error (see Section 6.4) and within 30 days for all other medication errors.

6.5 Demographic and Baseline Characteristics

Demographic and baseline characteristics are assessed at screening. These include:

- Demographics (as appropriate to country regulations): sex, date of birth, race, ethnic group
- Height (in cm) and weight (in kg)
- Previous and concomitant medications at Visit 1 (see Section 5.7).

6.6 Medical and surgical history:

- Confirmation of diagnosis of T2DM prior to 6 months
- Other concomitant diseases within the last 3 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.

6.7 Volume of blood to be collected

The total blood volume to be collected from each patient during the study will be approximately 624 mL, as summarized in Table 3.

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

Table 3: Total blood volume to be collected per Visit

Visit	Description	Total blood volume per visit (mL)
Visit 1- screening	Fasting blood	21
Visit 4	Fasting blood	36
	Metabolic chamber	84
	EHC	167
	Cycling	6
Visit 5 – day 1 period 2	Fasting blood	17
Visit 7	Fasting blood	36
	Metabolic chamber	84
	EHC	167
	Cycling	6
Total blood volume (maximum)	**624**	

6.8 Storage and destruction of Biological Samples

Biological samples (i.e. urine, serum and plasma) for future research will be retained at AstraZeneca or its designee for a maximum of 15 years following the last visit of the last patient in the study, after which they will be destroyed. The results of this biomarker research may be reported in the clinical study report itself, as an addendum, or separately in a scientific report or publication. The results of this biomarker research may be pooled with biomarker data from other studies involving the study drug to generate hypotheses to be tested in future research.

6.9 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 analyzed and the action documented. As collection of donated biological samples is an integral part of the study, these patients 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 4.

Table 4: Study procedures

Visit	**1 Screening**	**2 Randomization**	**3 Safety**	**4 End of treatment[a]**	**Wash Out period**	**5 Re-start treatment**	**6 Safety**	**7 End of treatment[a]**	**Safety Follow-up[b] 5–10 days post last IP dose**
Treatment period	**1**				**–**	**2**			
Week	**(-3) -0**	**0**	**2**	**5**	**6-8 weeks**	**0**	**2**	**5**	**6**
Day		**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**Safety Follow-up[b] 5–10 days post last IP dose**	**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**5–10 days post last IP dose**
Signing informed consent	X								
Inclusion/exclusion criteria	X	X							
Demographic data	X								
Medical and surgical history	X								
Electrocardiogram	X								
Physical examination	X			X		X		X	
Vital signs, body weight and other assessments [c]	X	X	X	X		X	X	X	
HbA1c, Clinical chemistry, hematology, urine dip-stick (pregnancy test)[d,g]	X [e]			X		X		X	
The lifestyle card release	X								
Maximal VO_2 cycling test		X[i]							

Visit	**1 Screening**	**2 Randomization**	**3 Safety**	**4 End of treatment[a]**	**Wash Out period**		**5 Re-start treatment**	**6 Safety**	**7 End of treatment[a]**	**Safety Follow-up[b] 5–10 days post last IP dose**
Treatment period	**1**				**–**		**2**			
Week	**(-3) -0**	**0**	**2**	**5**	**6-8 weeks**		**0**	**2**	**5**	**6**
Day		**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**Safety Follow-up[b] 5–10 days post last IP dose**		**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**5–10 days post last IP dose**
Drug dispensation		X		X			X		X	
Maximal kneeextension- test		X[i]								
Body composition (DEXA)				X					X	
Euglycemic hyperinsulinemic clamp (incl. urine collection)[g]				X					X	
36-hour metabolic chamber (incl. 24-hour collection of blood samples and urine)[h]				X					X	
^{1}H-MRS (lipids) and ^{31}P-MRS (PCr recovery) (60% maximal kneeextension-)				X					X	
^{1}H-MRS acetylcarnitine at 70% VO_2 max (and plasma lactate [g])				X					X	

Visit	**1 Screening**	**2 Randomization**	**3 Safety**	**4 End of treatment[a]**	**Wash Out period**		**5 Re-start treatment**	**6 Safety**	**7 End of treatment[a]**	**Safety Follow-up[b] 5–10 days post last IP dose**
Treatment period	**1**				**–**		**2**			
Week	**(-3) -0**	**0**	**2**	**5**	**6-8 weeks**		**0**	**2**	**5**	**6**
Day		**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**Safety Follow-up[b] 5–10 days post last IP dose**		**1**	**15 ± 3 days**	**29 ± 3 (+6-8 days for final assessments)**	**5–10 days post last IP dose**
Muscle biopsy				X					X	
Biomarkers[f]				X					X	
Exploratory samples for future analysis (serum, plasma and urine)[g]				X					X	
Current and concomitant medication	X	X	X	X			X	X	X	
Discontinuation adverse events		X	X	X	X		X	X	X	X
Serious adverse events	X	X	X	X	X	X	X	X	X	X
Study drug accountability				X					X	

IP= Investigational Product, VO_2= Volume Oxygen, ^{31}P-MRS=^{31}P-magnetic resonance spectroscopy, PCr= Phospho Creatinine, ^{1}H-MRS= ^{1}H-Magnetic Resonance Spectroscopy, DEXA= Dual-Energy X-ray Absorptiometry, AEs= Adverse Events

[a] The end of treatment visits (Visit 4 and Visit 7) will need to take place over 6–8 days. Four days (Day 1–3 and Day 6, 7 or 8) will be spent at the site and 2-4 days after Day 3 will be spent at home or at the hotel. The last dose will be given on the last day of the end of treatment visit (Visit 4 and Visit 7). Minimum duration of treatment will therefore be 32 days (26 +6 days) and maximum duration of treatment will be 40 days (32 + 8 days).

[b] Telephone meeting for safety follow-up should be made about 1 week (5–10 days) after each end of treatment visit. The visit can be made by a nurse and if needed, this visit can also take place at the site and include physical examination and laboratory assessments.
[c] Blood pressure, pulse rate, weight, height (height only at screening).
[d] U-pregnancy test using dip-stick should only be done at Visits 1 and 5.
[e] C-peptide is measured at Visit 1.
[f] HbA1c, uric acid, hsCRP and acylcarnitines will be measured in a lab to be determined
[g] To be stored at the central lab
[h] To be analyzed in blood:glucose, insulin, glucagon, betahydroxybutyrate, NEFA, FGF21 and to be analysed in urine: glucose, nitrogen and creatinine
[i] In case it is needed Maximal kneeextension- test and Maximal VO_2 cycling test can be performed at the Visit 3

7.2 Procedures by Visit

Visit 1 can take place up to three weeks before Visit 2 (i.e. randomization and start of treatment). All times should be recorded using the 24-hour clock (e.g., 23:20, not 11:20 pm).

Patients are advised to continue their normal eating and drinking pattern during the entire study period and to maintain their normal activity and sleeping pattern throughout the study. Before specific test days, restrictions in physical activity apply.

7.2.1 Treatment period 1

7.2.1.1 Screening (Visit 1)

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 assessments for Visit 1 will be performed as listed in Table 4.

All SAEs are collected from time of signing the informed consent and throughout the study, including the wash-out period and cross-over period.

The following procedures are performed at Visit 1:

- Signing of informed consent
- Check of eligibility criteria
- Assignment of enrolment number
- Demographic data collection
- Medical and surgical history
- ECG
- Physical examination
- Vital signs (blood pressure and pulse), weight and height
- Blood and urine dip-stick sampling for laboratory safety assessment
- Prior and current medication
- SAEs.

Patients will be advised to bring extra clothes and equipment needed for the maximal VO_2 cycling test and the maximal knee-extension test to be performed at Visit 2.

7.2.1.2 Randomization (Visit 2)

Patients meeting all eligibility criteria will be randomized into the study.

The following procedures will be performed at Visit 2 (Day 1):

- Check of eligibility criteria
- Vital signs (blood pressure and pulse) and weight
- Maximal knee-extension test
- Maximal VO_2 cycling test

- Randomization into two sequences to receive either treatment with dapagliflozin 10 mg/day or placebo for 5 weeks starting from the day after randomization
- Drug dispensation
- Concomitant medication
- DAEs and SAEs.

7.2.1.3 Safety (Visit 3)

The following procedures will be performed at Visit 3 (Day 15 ± 3):

- Vital signs (blood pressure and pulse) and weight
- Concomitant medication
- DAEs and SAEs.

Patients will be asked to perform no physical activity like exercise training or other extra physical activity beyond their routine daily activity and will be given diet instructions three days before Visit 4.

7.2.1.4 End of treatment (Visit 4)

The end of treatment visit (Visit 4) will need to take place over 6–8 days, of which four days (Day 1–3 and Day 6, 7 or 8) will be spent at the site and 2–4 days after Day 3 will be spent at home or at the hotel.

At the site, meals will be served according to a standardized schedule. At about 8 am, patients will be required to consume a regular breakfast with cheese and/or ham sandwiches, glass of orange juice and/or a cup of coffee or tea, provided at the site. A similar meal will be provided during lunch at about 12 pm, after which the patients will be required to fast until the acetylcarnitine measurement has been finalized.

The following procedures will be performed during Visit 4 [Day 29 ± 3 (+ 6–8 days for final assessments)]:

Day 1

- Physical examination
- Vital signs (blood pressure and pulse) and weight
- Drug dispensation
- Blood and urine sampling for laboratory safety assessment
- ^{1}H-MRS (muscle fat)
- ^{31}P-MRS (PCr recovery)
- Concomitant medication
- DAEs and SAEs
- Entering the metabolic chamber.

Day 2

- 36-hour stay in a whole-body room calorimetry chamber (to determine 24-hour energy expenditure, 24 hour RER fasting-fed state). Diurnal blood samples will be taken during the stay in the chamber (before and after meals and before bedtime, in total 7 occasions) to determine a.o. plasma levels of glucose, NEFA,

betahydroxybutyrate, insulin, glucagon and FGF21. A cannula may be used to draw these blood samples.

- Urine collection during clamp and 24-hour urine collection (in 6 hour aliquots) in the whole-body room calorimetry chamber for measurement of glucose, nitrogen and creatinine
- DAEs and SAEs.

Day 3

- Fasting plasma/serum samples (+ one extra vial of serum and plasma)
  - HbA1c
  - Uric acid
  - Acylcarnitines and amino acids
  - hsCRP
- Muscle biopsy from the m. vastus lateralis
- Indirect calorimetry
- ^{1}H-MRS (liver fat)
- EHC
- DAEs and SAEs.

Day 6, 7 or 8

- Body composition using DEXA
- ^{1}H-MRS (acetylcarnitine) including lactate
- DAEs and SAEs
- Drug accountability.

Refer to Figure 2 for an overview of the examinations taking place at Visit 4 and 7.

The last treatment dose will be given on the last day of the end of treatment visit (Visit 4).

7.2.1.5 Wash out period

A safety followup will be performed in Week 7 (5–10 days -postlast- assessment).

A telephone follow-up should be performed, else a visit may be made by a nurse or study personel and if needed, this visit can also take place at the site and include physical examination and laboratory assessments as specified in Section 6.2.1, at the discretion of the Investigator.

After the last day of the end of treatment visit, treatment with dapagliflozin or placebo will be discontinued and patients will enter a 6–8 weeks wash out period.

7.2.2 Treatment period 2

After a 6–8 week wash-out period, the cross over will take place and patients having received dapagliflozin will receive the placebo and patients having received placebo will receive dapagliflozin over a 5 week treatment period.

Re-start treatment (Visit 5):

- Physical examination
- Vital signs (blood pressure and pulse) and weight
- Blood sampling for laboratory safety assessment and urine dip-stick sampling for pregnancy testing
- Drug dispensation
- Concomitant medication
- DAEs and SAEs.

The study procedures detailed for Visit 3 and Visit 4 from treatment period 1 will be performed again at Visit 6 (with the exception of maximal oxygen consumption test and maximal knee-extension testing) and Visit 7 during the treatment period 2.

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 the study drug/as soon as possible after it was learned that the patient will not be able to complete followup- (See also Section 4.4). If the patient is in agreement, the safety profile and vital signs will be assessed and physical examination will be performed.

8 STATISTICAL ANALYSES

8.1 Statistical considerations

- All personnel involved with the analyses of the study will remain blinded until database lock and identification of protocol violators.
- Analyses will be performed by AstraZeneca or its representatives.

The Statistical Analysis Plan (SAP) will include further details of the analyses.

8.2.1 Definition of 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.

- The Enrolled Analysis Set will consist of all patients who sign the informed consent form.
- The Randomized Analysis Set will consist of all randomized patients. This is also known as the intent to treat (ITT) population. In analyses of the Randomized Analysis Set, patients will be represented using the treatment to which they were randomized (even if the treatment they received is different).
- The Safety Analysis Set will consist of all patients who received at least one dose of study drug.
- The Evaluable Analysis Set will be the primary analysis set for efficacy, and is a subset of the Randomized Analysis Set. This is also known as the PerProtocol- population. Relevant protocol deviations may lead to certain data points to be excluded from this analysis. Important protocol deviations are defined as deviations which could potentially affect the interpretability of the study results. Patients' data will be represented using the treatment they received. All

decisions to exclude data from the Evaluable Analysis Set will be made prior to the database lock of the study.

8.3 Outcome measures for analyses

Refer to Section 3.2.

8.4 Methods for statistical analyses

8.4.1 Primary Analysis

The analysis of efficacy will be based on the evaluable analysis set. Comparison of cGDR between dapagliflozin and placebo after 5 weeks of treatment will be performed using a mixed effect Analysis Of Variance (ANOVA) model. The model will include terms for treatment, sequence and period. A term representing patient nested within sequence will be included as a random effect. Model assumptions will be checked using conventional methods (residual plots and tests for the normal distribution of model residuals).

Least Square Mean (LSM) estimates and 95% confidence limits will be generated from the fitted model for each treatment. For the primary comparison, the difference in LSM estimate of dapagliflozin versus placebo treatment will be generated for the corresponding 95% confidence interval and p-value. Statistical significance will be inferred at a (twosided-) 0.05 level.

8.4.2 Exploratory Analyses

Analysis of exploratory variables will be performed using the evaluable analysis set. The same random effect ANOVA model as described for the primary variable will be applied, where appropriate. Statistical significance will be inferred at a (two-sided) 0.05 level, and nominal p-values will be reported.

Benjamini and Hochberg (Devan VM and Joseph FH, 2004[21]) false discovery rate may be applied to interpret p-values from exploratory analyses.

8.4.3 Baseline characteristics

Demographic and other baseline characteristics will be summarized by the treatment sequence group and overall. For qualitative variables, frequency counts (number of patients [n] and percentages) will be made. For quantitative variables, descriptive statistics that include n, mean, standard deviation, median, minimum and maximum will be presented, if not otherwise specified in the SAP. The statistics will be presented for the Evaluable Analysis Set. No formal comparison of the baseline characteristics using statistical testing procedures is planned.

8.5 Safety Analyses

The analysis of safety will be based on the safety analysis set. Safety data during the treatment period will be evaluated and variables will only be summarized descriptively. DAEs and SAEs will be summarized by system organ class and preferred term and will be listed for each patient. The number and percent of subjects that discontinue due to

adverse events (DAE) and, separately, that experience at least one SAE will be summarized for each treatment.

Safety laboratory, vital signs, physical examination and ECG findings will be listed by patient. Descriptive statistics will be presented by treatment for both absolute values and changes from baseline.

8.6 Interim Analyses

No interim analysis is planned for this study.

9 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 Data Quality Assurance

The Sponsor or Sponsor's 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 (i.e., medical records).

9.1.1 Database Management and Quality Control

All data generated by the site personnel will be captured electronically at the study center 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 center, 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, medical files, etc.

Data that will be entered directly into the eCRF (i.e., 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 site monitor.

After review by the site monitor, completed eCRF entries will be uploaded. 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 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 authorized staff) will be given access to an online web-based 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 authorized 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.

Computerized 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 on-line. All discrepancies will be solved online directly by the Investigator or by authorized 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 drug dispensed to the patient and any dosage changes will be tracked on the eCRF.

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, IRBs, 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 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 adverse events will be coded using the 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 Harmonization (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 the Sponsor and Investigator abide by the principles of the Good Clinical Practice 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 and according to WMO (Wet Medisch-wetenschappelijk Onderzoek met mensen, i.e. scientific medical research).

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 consent form 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 informed consent form may need to be revised to reflect the changes to the protocol. If the consent form 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. The Sponsor and the Investigator 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, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate).

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 for each of the 2 treatment periods will be 40 days, separated by a wash out period of 6–8 weeks.

The study will close when all patients have completed the final safety follow-up.

9.10 Premature Termination of the Study

9.10.1 Stopping Rules for an Individual Patient

Withdrawal criteria for an individual patient are specified in Section 4.4.

9.10.2 Stopping Rules for the Study

If the Investigator or AstraZeneca 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 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 eCRF and other documents submitted to the Sponsor by their patient number and/or birth date, not by name. Documents not to be submitted to Sponsor that identify the patient (e.g., the signed informed consent) must be maintained in confidence by the Investigator.

9.12 Other Ethical and Regulatory Issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the Sponsor will issue prompt notification to all parties – Regulatory Authorities, Investigators, and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical study or program (including the potential for suspension of the development program or amendments to protocols) or warrants immediate update of informed consent.

9.13 Publication Policy

By signing the study protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, Regulatory Authorities will be notified of the Investigator's name, address, qualifications and extent of involvement.

An Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with AstraZeneca in advance. Details are provided in a separate document.

10 APPENDICES

10.1 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 (e.g., hepatitis that resolved without hepatic failure).

Hospitalization

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (e.g., 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, hospitalization, 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 iv 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 (e.g., neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalization.
- 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 recognized 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 judgment. 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 REFERENCE LIST

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