Official Title: Effects of SGLT-2 Inhibition on Myocardial Fibrosis and Inflammation as Assessed by Cardiac MRI in Patients with DM2

NCT03782259

Study Protocol and Statistical Analysis Plan - 12JUL2022

Responsible Party:

Principal Investigator

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Drug Substance	Dapagliflozin
Study Number	
Company Reference	ESR 17-13124
Version	8.0
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Effects of SGLT-2 inhibition with dapagliflozin on myocardial fibrosis and inflammation as assessed by cardiac MRI with T1- and T2-mapping in patients with type-2 diabetes

Sponsor: University of Washington

Funding and drug support agency: AstraZeneca

VERSION HISTORY

Version 8.0, 12JUL2022
Amendment to add myocardial strain assessment, global myocardial longitudinal strain (GLS), as one of the primary endpoints.
Version 7.0, 19NOV2020
Amendment to eliminate exclusion criteria #3 - *Currently taking GLP-1 receptor agonists.* This revision does not increase risk or benefit of study participation as concomitant use of SGLT2 inhibitors and GLP-1 receptor agonists is acceptable clinical practice when indicated. The study randomization will be stratified for use of GLP-1 receptor agonists and statistical analysis will be adjusted for this.
Version 6.0, 17JUN2020
Amendment to extend treatment period, if necessary, to accomadate MR service or Clinical space disruptions due to Public Health Emergency such as COVID-19 Pandemic. Description of this extended treatment has been added to section 4.2.
Version 5.0, 29OCT2019
Amendment to safety monitoring in section 9.2 to include Independent Safety Monitor (ISM) and explicit safety responsibilities for PI, ISM and AstraZeneca in section 6.
Version 4.0, 28MAR2019
Amendment to introduce a time window for Cardiac MRI scans. This is necessary due to challenges in scheduling Randomization, Cardiac MR and Mitochondrial Function Assesment bench time for the same day. Another amendment to change eGFR from <60 to <45 mL/min/1.73 m^2 as a study exclusion to be in consistent with the updated PI information.
Version 3.0, 12SEP2018
Amendment version for including: (1) a 2-week telephone follow-up visit; (2) study communication plan with study subjects' Primary Providers; (3) abnormal results communications with study subjects' Primary Providers.

PROTOCOL SYNOPSIS

Effects of SGLT-2 inhibition with dapagliflozin on myocardial fibrosis and inflammation as assessed by cardiac MRI with T1- and T2-mapping in patients with type-2 diabetes

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Study site(s) and number of subjects planned

Single center study with 60 subjects enrolled.

Study period		Phase of development
Estimated date of first subject enrolled	Q-3 2018	Study initiation
Estimated date of last subject completed	Q-2 2020	Study completion

Study design

Randomized, double-blind and placebo controlled cardiac MRI study

Objectives

Primary Objective:	Outcome Measure:
To investigate whether SGLT-2 inhibition with dapagliflozin will improve myocardial strain performance and reduce progression myocardial fibrosis as assessed by cardiac MRI with T1-mapping in patients with type-2 diabetes.	Extracellular volume fraction (ECV) as assessed by T1-mapping. Myocardial strain measurement

Secondary Objective:	Outcome Measure :
To investigate whether SGLT-2 inhibition with dapagliflozin will reduce myocardial inflammation as assessed by T2-mapping.	T2 relaxation time using T2-mapping

Target subject population

Subjects with type-2 diabetes history ≥5 years and HbA1C 7-10%

Duration of treatment

One year

Investigational product, dosage and mode of administration

Dapagliflozin 10mg or matching placebo is taken orally once daily in the morning regardless of meals.

Statistical methods

We will assess changes in myocardial strain and ECV, and T2 relaxation time and their variabilities, any signal on treatment effect with dapagliflozin over at least 1 year using an initial sample size of 60 subjects (n=30 in dapagliflozin group and n=30 in placebo group). Assuming a positive treatment effect on ECV with dapagliflozin at 1 year, a planned "interim" analysis will be performed by an independent group, then, lead to an adaption for a sample size reestimation using variability data generated in this initial group. The investigator(s) will be blinded to subject's treatment status in this process. We will assess the value and feasibility of continuation of the study subject enrollment to reach a sample size suggested by the "interim" analysis and will allowed to include the initial 60 subjects if a larger sample size is needed. On the other hand, if the "interim" analysis indicates no signal of treatment effect on myocardial strain measurement and ECV with dapagliflozin, further study will not be justified.

Given the exploratory nature of the study, we will analyse the data based on if the treatment is truly received, not be limited to intention to treat comparing dapagliflozin with placebo. Furthermore, we will perform the primary analysis based on proportion of time therapy received as needed.

TABLE OF CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

To the Author:

Add terms and abbreviations as required.

Abbreviation or special term	Explanation
T2DM	Type-2 diabetes mellitus
CVD	Cardiovascular diseases
SGLT-2	Sodium-glucose cotransporter-2
ECV	Myocardial extracellular volume
CMRI	Cardiac magnetic resonance imaging
Mit. F	Mitochondrial function
GLS	Global myocardial longitudinal strain

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
International Co-ordinating investigator	If a study is conducted in several countries the International Co-ordinating Investigator is the Investigator co-ordinating the investigators and/or activities internationally.
IP	Investigational Product
IVRS	Interactive Voice Response System

Abbreviation or special term	Explanation
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
LIMS	Laboratory information management system
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
RA	Research Agreement
SAE	Serious adverse event
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Cardiovascular diseases including atherosclerotic vascular disease and heart failure are the most common complications of type-2 diabetes mellitus (T2DM) and the most prevalent cause of morbidity and mortality in T2DM [1-6]. The exact mechanisms involved in the pathogenesis of cardiovascular diseases (CVD) in T2DM are extremely complex and not entirely clear, however, the increased risk of vascular disease and heart failure in T2DM is not surprising since all 3 conditions share common pathogenetic factors related to metabolic disturbances that result in oxidative stress, low-grade inflammation, activation of RAS system, autonomic neuropathy and impaired calcium homeostasis [2,4]. These pathophysiological responses lead to endothelial dysfunction, atherosclerosis and cardiac fibrosis.

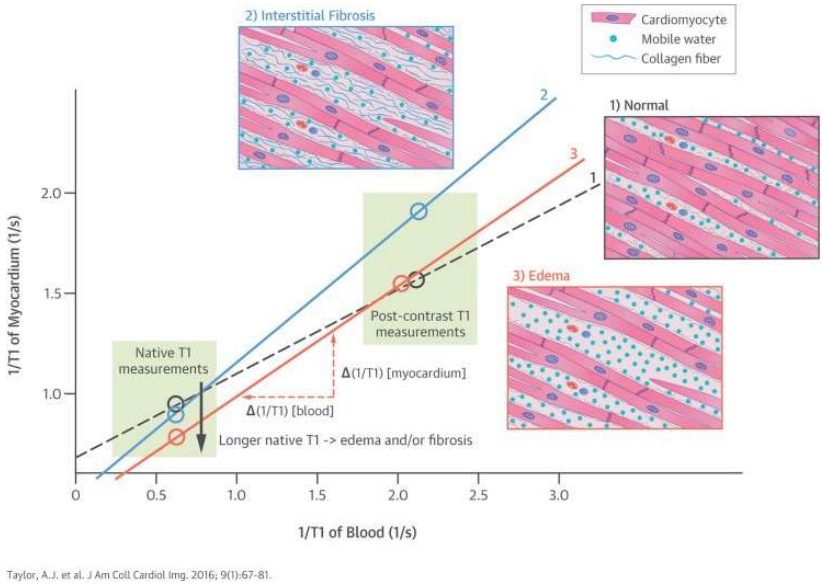
Evidence from clinical trials repeatedly confirms the association of T2DM with heart failure, independent of hypertension, atherosclerosis, coronary artery disease [7-9]. It is well established that T2DM patients with heart failure have reduced function capacity [7] and poor clinical outcomes [8] with significantly increased mortality compared with those without heart failure [9]. In addition to the frequent occurrence of coronary artery disease in T2DM, diabetic cardiomyopathy, which is independent of ischemia, also plays important role in development of heart failure [10,11]. Diabetic cardiomyopathy, although the etiology remains unclear, might include a spectrum of dysfunction, as CHF now does, encompassing mild diastolic dysfunction with LV hypertrophy through to a true dilated cardiomyopathy with important systolic dysfunction. Subclinical myocardial dysfunction is even more common than overt heart failure in diabetic patients. TDI, which can be used to estimate longitudinal LV function (shortening of the heart from apex to base), showed reduced LV strain, and impaired systolic and diastolic velocities in 27% of 120 T2DM subjects without overt heart failure, with 16% demonstrating systolic dysfunction alone and 10% both systolic and diastolic dysfunction [12,13]. Longitudinal LV systolic and diastolic dysfunction as ‘early’ diabetic cardiomyopathy might therefore be extremely common [14-16].

Myocardial fibrosis is considered as pathological basis for diabetic cardiomyopathy [17]. Detailed pathological examinations reveal myocardial hypertrophy, interstitial fibrosis, capillary endothelial changes, and capillary basal laminae thickening [18]. Microscopic alterations in small intramural coronary arteries include the narrowing of the lumen due to increased proliferation of endothelial cells, increased thickness of the arteriolar wall due to fibrosis and accumulation of mucopolysaccharides, alterations of elastic fibers, myocytolysis, and perivascular fibrosis [19,20].

Cardiac MRI using T1-mapping is capable of quantifying myocardial extracellular volume (ECV), a surrogate of fibrosis, with excellent inter- and intra-observer variability and could, therefore, be potentially employed for investigations in diabetic myopathy [21]. A recent study [22] showed that ECV by T1-mapping increased as the duration of diabetes increased from 3 to 9 months, consistent with the changes in myocardial fibrosis verified by pathology

in diabetic rabbits. Moreover, ECV was strongly correlated with the early diastolic strain rate by Echo ($r = -0.782$, $p < 0.001$). Examples of myocardial fibrosis in dilated cardiomyopathy are shown in **Figures 1-4.** In addition, T2-mapping can help to assess myocardial edema caused by injury and inflammation since the T2 value has been shown significantly higher in the edema area [23,24].

Figure 1. Pre- and post-contrast myocardial T1s in normal, interstitial fibrosis and edema.



Taylor, A.J. et al. J Am Coll Cardiol Img. 2016; 9(1):67-81.

Figure 2. Extracellular volume fraction (ECV) by T1-mapping in dilated cardiomyopathy (DCM).

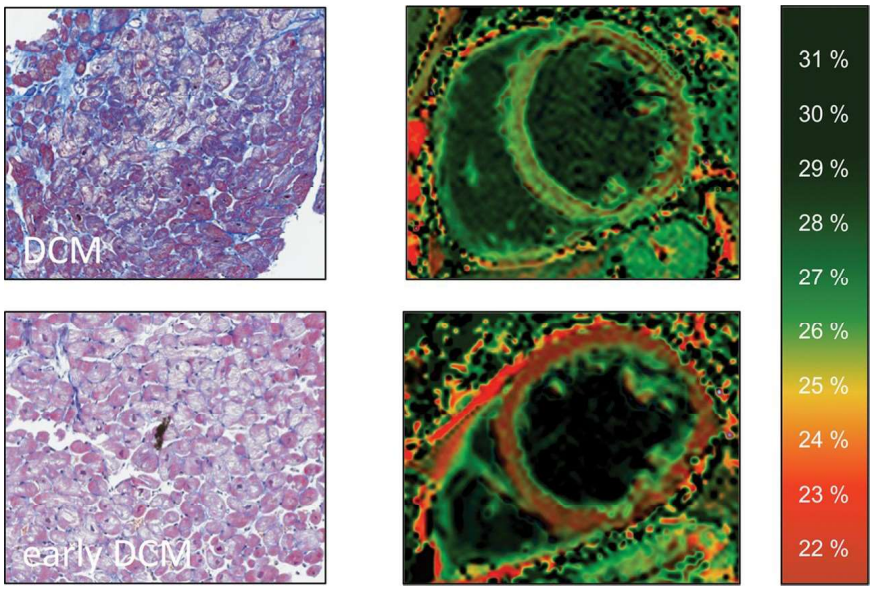


Figure 3. Detectable differences in extracellular volume fraction (ECV) by T1-mapping.

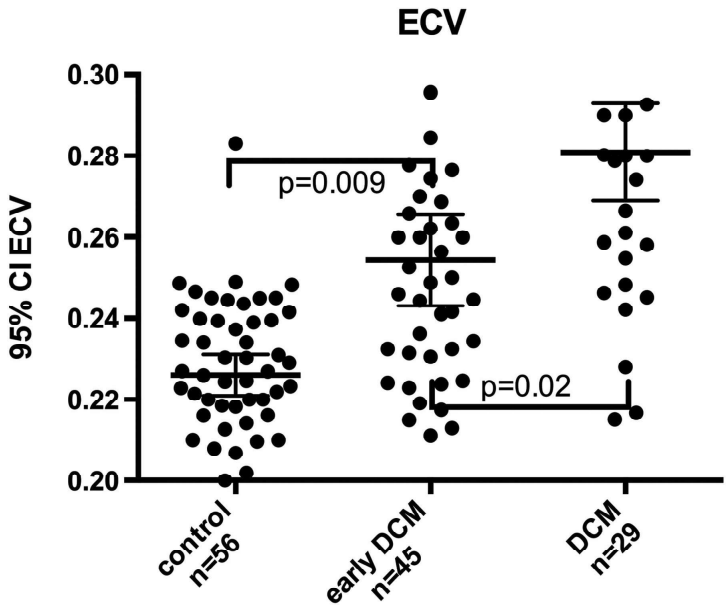
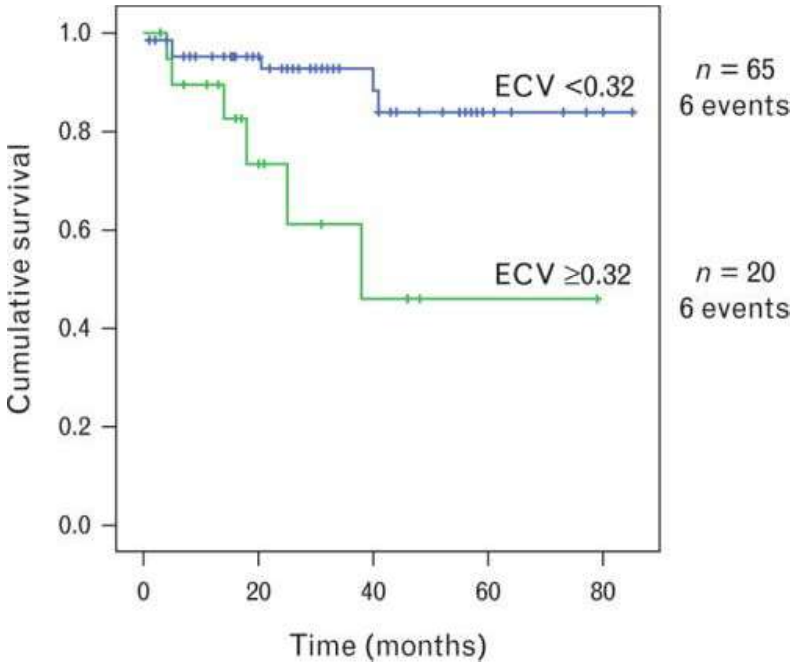


Figure 4. Prognosis of ECV in DCM.



Assessment of myocardial strain has a robust, independent, and incremental predictive value for incident of heart failure in asymptomatic subjects without any history of previous clinical cardiovascular disease [32]. Cardiovascular magnetic resonance feature tracking (CMR-FT) is a novel technique for non-invasive assessment of myocardial motion and deformation [33] that was described by Hor et al in 2011 [32]. Feature-tracking is an analog of speckle tracking in echocardiographic imaging to follow physiological motion [34]. CMR-FT assesses myocardial strain from routinely acquired cine imaging sequences. Feature-tracking algorithms are designed to focus on border displacement, with a stronger weighing of endocardial deformation. CMR-FT has been already shown the excellent inter- and intra-observer reproducibility of different parameters and at different magnetic field strength [35-37].

In the front of treatment of T2DM, sodium-glucose cotransporter-2 (SGLT-2) inhibition not only offers more effective glucose control but also significant weight loss and reductions in blood pressure. Importantly, EMPA-REG OUTCOME [25] demonstrated that a composite of death from CVD causes, nonfatal MI, or nonfatal stroke occurred in a significantly lower percentage of patients on empagliflozin (490 of 4687, 10.5%) than in the placebo group (282 of 2333, 12.1%, HR: 0.86, 95%CI: 0.74-0.99, P<0.001 for non-inferiority and P=0.04 for superiority). Furthermore, EMPA-REG OUTCOME showed that treatment with empagliflozin compared to standard care was associated with significant reduction in hospitalization for heart failure (HR=0.65, 95%CI: 0.50-0.85, p=0.002). Recently reported CVD-REAL Study [26] in 364,828 patients with T2DM in 6 countries showed that treatment with SGLT-2 inhibitor was associated with significant reduction in hospitalization for heart failure (HR=0.61, 95%CI: 0.51-0.73, p<0.001). It was also significantly associated with lower incidence of all-cause mortality (HR=0.49, 95%CI: 0.41-0.57, P<0.001). The mechanism of improvement in heart failure with SGLT-2 inhibitors is not entirely clear. Animal studies have suggested that SGLT-2 inhibition may improve endothelial function and reduce arterial stiffness, and could attenuate the increase in left ventricle mass and left ventricle end diastolic diameter [27,28]. These findings support a testable hypothesis that SGLT-2 inhibition could reduce myocardial fibrosis and inflammation therefore to improve heart function in T2DM.

In addition, a number of studies have shown mitochondrial dysfunction associated with T2DM. However, it is not clear whether the mitochondrial function assessment using peripheral blood mononuclear cell (PBMC) in vivo can reflect specific cell change or damage related to T2DM, for example, myocardial fibrosis to our interest, which is basis for diabetic cardiomyopathy. Another interesting question is whether dapagliflozin can improve mitochondrial function. We propose to address these 2 questions in this study.

1.2 Rationale for study design, doses and control groups

Given the unmet needs for CVD risk reduction in patients with T2DM, the promising findings of more effective glucose management and reductions in overall CVD events and hospitalization for heart failure with SGLT-2 inhibition demonstrated in recent trials, and the capability of cardiac MRI with T1- and T2-mapping in assessments of myocardial fibrosis and inflammation, we propose to conduct a staged research program using **adaptive study design**

[29,30] to investigate the effects of SGLT-2 inhibition with dapagliflozin on myocardial strain, fibrosis and inflammation as assessed by cardiac MRI with T1- and T2-mapping in patients with type-2 diabetes.

A total of 60 subjects with ≥18 years of age, type-2 diabetes history ≥5 years and HbA1C 7-10% will be randomized at 1:1 to Dapagliflozin 10mg or matching placebo once daily for 1 year. All subjects will be followed every 3 months for clinical and laboratory evaluations and assessments. All subjects will undergo CMRI and mitochondrial function (Mit. F) assessement at baseline and at least 1 year.

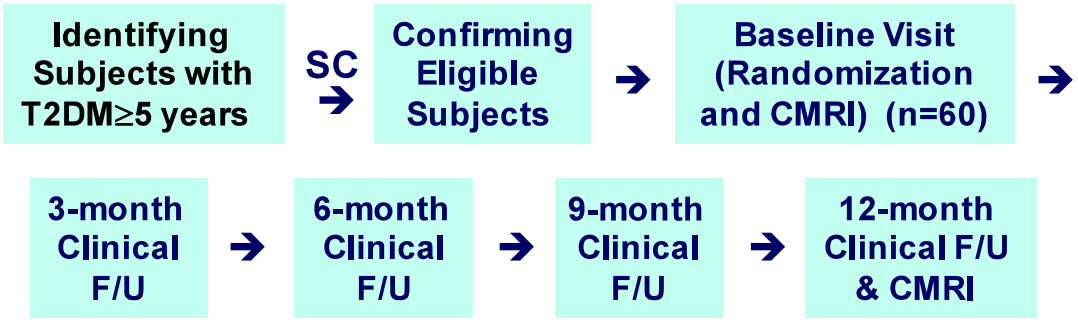
The primary myocardial strain endpoint includes global myocardial longitudinal strain (GLS). Myocardial fibrosis endpoint is change in extracellular volume fraction (ECV) as assessed by T1-mapping over 12 months. ECV combines native and contrast-enhanced T1 mapping. The change of the T1 relaxation rate (i.e., 1/T1) in blood between pre- and post-contrast imaging is converted with the blood hematocrit into a reference for plasma T1, which serves as reference for the T1 changes in tissue as illustrated in **Figure 1.** Endpoint for Mit. F study is monocytes oxygen consumption rates (OCR) using PBMC.

1.3 Benefit/risk and ethical assessment

The anticipated benefits and risks of the study will be described in subject informed consent. These will be complete, accurate and consistent with the most recent evaluation of product benefit/risk. The informed consent will be approved by the University of Washington IRB.

1.4 Study Design

Figure 5. Study flow chart



2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	Outcome Measure:
To investigate whether SGLT-2 inhibition with dapagliflozin will improve myocardial strain and reduce progression of myocardial fibrosis as assessed by cardiac MRI with T1-mapping in patients with type-2 diabetes.	GLS. Extracellular volume fraction (ECV) as assessed by T1-mapping

2.2 Secondary objectives

Secondary Objective:	Outcome Measure :
To investigate whether SGLT-2 inhibition with dapagliflozin will reduce myocardial inflammation as assessed by T2-mapping.	T2 relaxation time using T2-mapping GCS and GRS
To investigate whether SGLT-2 inhibition with dapagliflozin will improve monocytes mitochondrial function.	Oxygen consumption rates (OCR)

2.3 Exploratory objectives

Exploratory Objectie:	Outcome Measure :
To investigate whether SGLT-2 inhibition with dapagliflozin will reduce epicardial fat as assessed by CMRI.	Epicardial fat
To examine associations of epicardial fat, myocardial fibrosis and inflammation.	ECV, T2-relaxation time and epicardial fat
To investigate the effects of SGLT-2 inhibition with dapagliflozin on glucose management, LDL particle size and inflammatory markers.	(1) Fasting glucose and HbA1C levels (2) LDL particle size – performed using stored plasma samples. (3) Inflammatory biomarkers include hsCRP, TNF-α and IL-6 – performed using stored plasma samples.

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

Each subject should meet all of the inclusion criteria and none of the exclusion criteria for this study. Under no circumstances can there be exceptions to this rule.

3.1 Inclusion criteria

For inclusion in the study subjects should fulfil the following criteria based on local regulations:

(1) Men and women ≥18 years of age
(2) Subjects with type-2 diabetes history ≥5 years
(3) HbA1C 7-10% with glucose control medications including insulin, metformin or sullfinuria
(4) Medically stable
(5) Willing to participate and sign informed consent.

3.2 Exclusion criteria

Subjects should not enter the study if any of the following exclusion criteria are fulfilled:

(1) Contraindication to MRI
(2) Currently or within last three months treatment with a SGLT2 inhibitor
(3) eGFR <45 mL/min/1.73 m^2
(4) Unstable or rapidly progressive renal disease
(5) Hypotension with SBP <100 mmHg
(6) Hypersensitivity to dapagliflozin or any excipients
(7) Patients with severe hepatic impairment (Child-Pugh class C)
(8) Patients with active hypetetis B or C infection
(9) Any of the following CV/Vascular Diseases within 3 months prior to signing the consent at enrolment, as assessed by the investigator:
(10) Myocardial infarction
(11) Cardiac surgery or revascularization (CABG/PTCA)
(12) Unstable angina
(13) HF New York Heart Association (NYHA) Class IV
(14) Transient ischemic attack (TIA) or significant cerebrovascular disease
(15) Unstable or previously undiagnosed arrhythmia
(16) Eastablished PAD
(17) Active bladder cancer
(18) Recent episode of DKA, frequent episodes of DKA
(19) High risk of fractures, amputations and fibrosis
(20) Women of child-bearing potential (ie, those who are not chemically or surgically sterilized or who are not post-menopausal) who have a positive pregnancy test at enrolment or randomization, OR women who are not willing to use a medically accepted method of contraception that is considered reliable in the judgment of the investigator,

from the time of signing the informed consent until two weeks after the last dose of IP, OR women who are breast-feeding.

(21) Individuals with a high risk of fractures, known diagnosis of osteoporosis or osteopenia, and/or history of fracture due to degenerative bone disease.

Procedures for withdrawal of incorrectly enrolled subjects see Section 3.4.

3.3 Subject enrolment and randomization

Investigator(s) should keep a record, the subject screening log, of subjects who entered pre-study screening.

The Investigator(s) will:

1. Obtain signed informed consent from the potential subject before any study specific procedures are performed.
2. Assign potential subject a unique enrolment number.
3. Determine subject eligibility. See Section 3.
4. An unique randomisation code will be assigned to an eligible subject.

If a subject withdraws from participation in the study, then his/he enrolment/randomisation code cannot be reused.

Randomisation codes will be assigned strictly sequentially as subjects become eligible for randomisation.

In addition, randomization will be stratified for RAAS blockade and GLP-1 use in both the treatment and placebo groups.

3.4 Procedures for handling incorrectly enrolled or randomized subjects

Subjects who fail to meet the eligibility criteria should not, under any circumstances, be enrolled or receive study medication. There can be no exceptions to this rule. Subjects who are enrolled, but subsequently found not to meet all the eligibility criteria must not be randomized or initiated on treatment, and must be withdrawn from the study.

Where a subject does not meet all the eligibility criteria but is randomized in error, or incorrectly started on treatment, the Investigator should inform the Sponsor immediately, and a discussion should occur between the Sponsor and the Investigator regarding whether to continue or discontinue the patient from treatment. The Sponsor must ensure all decisions are appropriately documented.

3.5 Methods for assigning treatment groups

A randomization software will be used by a designated investigator to perform the randomization scheme. There will be no stratification factors used in the randomization. This randomization scheme will be linked to study medication dispensing at the University of Washington Investigational Drug Services (IDS).

3.6 Methods for ensuring blinding

Investigators and study subjects will be blinded in the study. Matching placebo will be used to ensure the blinding.

3.7 Methods for unblinding

Individual treatment codes, indicating the treatment randomisation for each randomised subject, will be available to the Investigator(s) or pharmacists at the study site, and the personnel who are independent to the study evaluation at the Sponsor.

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomisation. The Investigator documents and reports the action to Company, without revealing the treatment given to subject to the Company staff.

The Company retains the right to break the code for SAEs that are unexpected and are suspected to be causally related to an investigational product and that potentially require expedited reporting to regulatory authorities. Treatment codes will not be broken for the planned analyses of data until all decisions on the evaluability of the data from each individual subject have been made and documented.

3.8 Restrictions

None.

3.9 Discontinuation of investigational product

Subjects may be discontinued from the study medication due to adverse events.

3.9.1 Procedures for discontinuation of a subject from investigational product

A subject that discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. The Principal Investigator/Investigator will perform the best possible observation(s), test(s) and evaluation(s) as well as give appropriate medication and all possible measures for the safety of the subject. They will also immediately inform Sponsor of the withdrawal. Adverse events will be followed up (See Section 6); events will be recorded using CRFs and all unused study drug should be returned by the subject.

If an adverse event possibly related to the study drug is resolved, subjects will be allowed to re-start the study medication.

If a subject is withdrawn from study, see Section 3.10.

3.10 Criteria for withdrawal

If investigational product is stopped permanently, the subject will be withdrawn from further study procedures/visits (except follow up of adverse events).

3.10.1 Screen failures

Screening failures are patients who do not fulfil the eligibility criteria for the study, and therefore must not be randomized. These patients should have the reason for study withdrawal recorded as 'Incorrect Enrolment' (i.e., patient does not meet the required inclusion/exclusion criteria). This reason for study withdrawal is only valid for screen failures (not randomized patients).

3.10.2 Withdrawal of the informed consent

Patients are free to withdraw from the study at any time (investigational product and assessments), without prejudice to further treatment.

A patient who withdraws consent will always be asked about the reason(s) and the presence of any adverse events (AE). The Investigator will follow up AEs outside of the clinical study. All unused study drug will be returned.

If a subject withdraws from participation in the study, then his/her enrolment/randomisation code cannot be reused. Withdrawn subjects will not be replaced.

3.11 Discontinuation of the study

The study may be stopped if, in the judgment of the Sponsor, trial subjects are placed at undue risk because of clinically significant findings that:

- meet individual stopping criteria or are otherwise considered significant
- are assessed as causally related to study drug
- are not considered to be consistent with continuation of the study

Regardless of the reason for termination, all data available for the subject at the time of discontinuation of follow-up must be recorded in the CRF. All reasons for discontinuation of treatment must be documented.

In terminating the study, the Sponsor will ensure that adequate consideration is given to the protection of the subjects' interests.

4. STUDY PLAN AND TIMING OF PROCEDURES

Table 1 Study Plan detailing the procedures

Visit Visit window Days –28 to 0 for Visit 1 ±14 day for Visit 3-7	**1** **Screening**	**2** **Baseline**	**3** **2-week Telephone follow-up**	**4** **3-month follow-up**	**5** **6-month follow-up**	**6** **9-month follow-up**	**7** **12-month follow-up**	**8** **2-4 week Telephone follow-up**	**Un-scheduled** **AEs or SAEs**
Months		**0**	**0.5**	**3**	**6**	**9**	**12**	**12.5**	
Written informed consent *(including tissue samples, pharmacogenetics*)	X								
Demographics	X								
Physical examination, height, and weight	X								X
Medical/surgical history	X								
Inclusion/exclusion criteria	X	X							
Vital signs	X	X		X	X	X	X		X
Randomisation to study treatment		X							
Treatment dispensed/returned		X		X	X	X	X		X
Concomitant medication	X	X	X	X	X	X	X	X	X
Adverse event review (AEs and SAEs)	X	X	X	X	X	X	X	X	X
Blood samples for haematology and clinical chemistry	X	X		X	X	X	X		X
Plasma sample storage		X					X		
Mit. F assessment		X					X		
Cardiac MRI scan*		X					X		

*Cardiac MRI Scan may occur ±14 days from Baseline/12-Month clinical visit and data collection.

4.1 Enrolment/screening period

With IRB approval, potential qualified subjects will be identified using patient electronic medical records system at the University of Washington. Subjects who appear to meet the study entry requirement will be invited for participating in the screening visit.

At screening, consenting subjects are assessed to ensure that they meet eligibility criteria. Subjects who do not meet these criteria must not be enrolled in the study.

Following Screening, a Physician Introduction Notification Letter will notify that the subject is considering participation once subject has consented and authorized discussion.

For the Mit. F study, 30 age, gender and CVD risk matched non-diabetic subjects will be included ONLY for baseline monocytes Mit. F assessment.

4.2 Treatment period

All eligible subjects will be randomized at 1:1 to Dapagliflozin 10mg or matching placebo orally once daily at baseline visit. We anticipate <10% of drop out from study enrollmen to randomization. The treatment duration is at least 1 year. All study participants will be followed by telephone at 2 weeks and clinically every 3 months, a total of 4 follow-up visits. We will also perform a telephone visit at 2-4 weeks post the study completion to collect information on AE or SAE.

Following Randomization, Physician Randomization Letter will be sent to the subjects' physician(s) describing the study design, subject's participation, medication limitations and contact information for the study PI for any questions.

In the case of abnormal test results, we will contact healthcare providers by phone as soon as the results are available to inform them of the abnormal test results.

Additional glucose management, as needed, using any non-SGLT2 inhibitors or GLP-1 receptor agonists, will be allowed during the study.

In the event of a Public Health Emergency, such as COVID-19 Pandemic, MR service or Clinical space disruptions will be unavoidable. In this case, subjects will be invited to extend study treatment phase until a time that MR service or Clinical space disruptions have ended. This will allow study endpoint measures to coincide with the end of treatment. If treatment phase is extended, subjects will contine on randomized therapy with the same quarterly schedule described in Table 1 with procedures modelled on "3-Month follow-up" and "6-Month follow-up".

4.3 Follow-up period

Same as in 4.2.

In addition, Physician Exit Letter will be sent to notify the subjects' physician(s) of study exit to explicitly indicate that the study drug/placebo has been stopped.

5. STUDY ASSESSMENTS

The Investigator will ensure that data are recorded on the paper Case Report Forms and are recorded in an electronic database.

The Investigator ensures the accuracy, completeness, legibility and timeliness of the data recorded and of the provision of answers to data queries according to the Research Agreement.

The Principal Investigator/Investigator will record data on the observations, tests and assessments specified in the protocol on the paper CRFs provided by Sponsor. The CRF will be accompanied with 'Instructions for the Investigator', which should be followed. These instructions provide guidance for the recording of study data in the CRF including how to change data incorrectly recorded.

5.1 Efficacy assessments

Subjects will be scanned on a Philips Achieva 3T scanner at the University of Washington. Cardiac coils will be applied in the scan session. There will be pre-contrast and post-contrast scans. ECG leads will be placed on the subject to obtain cardiac gating signal for the cardiac scans. Subject will be instructed to hold their breath in end-expiratory position for breath-hold scans.

The heart will be identified using 3-plane gradient echo scan followed by additional planes to identify the short axis. A pre-contrast T1 mapping scan (MOLLI) will be obtained at a single location oriented in the short axis scan plane of the heart. Next gadolinium contrast bolus will be injected intravenously at a dose of 0.05 mmol/kg using a power injector at the rate of 1ml/sec. Then a 3D phase-sensitive inversion recovery (3D PSIR) of the heart will be obtained followed by a post-contrast MOLLI T1 mapping scan and T2 mapping scan. Total scan time for the protocol is <1 hour including patient setup time.

Myocardial strain measurements with feature tracking will be performed using Circle Cardiovascular Software (cvi-42, Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) to measure myocardial strain and strain rate from the bSSFP short-axis and long-axis cine images. Long-axis cine images will be further used to compute global myocardial longitudinal strain (GLS). Short-axis images will be used to compute circumferential (GCS) and radial strain (GRS) and strain rate. The global values will be obtained through averaging the values according to an AHA 17-segment model [38].

Quantitative measurements of Extracellular Volume Fraction (ECV), the primary study measure, will be performed using MASS research software (Department of Radiology,

Leiden University Medical Center, Leiden, the Netherlands). T1 maps will be constructed offline. On each T1 map (pre- and post-contrast), a region of interest will be manually drawn around the core myocardium to exclude the blood pool and epicardial fat to calculate the myocardial T1 time for each subject. The partition coefficient will be determined by the slope of the linear relationship of ($1/T1_{myo}$ vs. $1/T1_{blood}$). If the change of $1/T1_{myo}$ and $1/T1_{blood}$ is expressed as $\Delta R1_{myo}$ and $\Delta R1_{blood}$, fraction of extracellular volume (ECV [%]) will be derived using the formula: $ECV = \Delta R1_{myo} / \Delta R1_{blood}$ X (1 − hematocrit).

PBMC monocyte oxygen consumption rates (OCR) will be assessed at the mitochondrial research center of the University of Washington using the established methods [31].

5.2 Laboratory assessments

5.2.1 Laboratory assessments

The following laboratory variables will be measured. Fasting glucose, creatinine, and HbA1C will be measured at every 3 months and other variables will be assessed at every 6 months.

Table 2 Laboratory Variables

Whole blood	**Clinical Chemistry (serum or plasma)**
HbA1C	S/P-Creatinine
	S/P-Fasting glucose
Clinical Chemistry (serum or plasma)	S/P-Alkaline phosphatise (ALP)
S/P-Total cholesterol	S/P-Aspartate transaminase (AST)
S/P-Triglycerides	S/P-Alanine transaminase (ALT)
S/P-LDL cholesterol	S/P-Albumin
S/P-HDL cholesterol	S/P-Potassium
S/P-Total cholesterol	S/P-Calcium, total
S/P-Creatine kinase (CK)	S/P-Sodium
S/P-ProBNP	S/P-HsCRP
S/P-Uric acid	**Urinalysis**
S/P-Potassium	Pregnancy test

5.2.2 Physical examination

A targeted physical examination will be performed at baseline and 12-month follow-up visits and at any visits related to AEs. It will include an assessment of the following: general appearance, respiratory, cardiovascular, abdomen and lower extremities.

5.2.3 ECG

Not required for the study.

5.2.3.1 Resting 12-lead ECG

Not required for the study.

5.2.4 Vital signs

5.2.4.1 Pulse and blood pressure

Pulse and blood pressure will be taken at each of the clinical visits at screening, baseline/randomization, 3-month, 6-month, 9-month and 12-month follow-up visits. These measures will be taken at sitting position after 15 minutes of resting. They will be an average of 2 readings 5 minutes apart.

5.2.4.2 Body temperature

It will be taken at each of the clinical visits at screening, baseline/randomization, 3-month, 6-month, 9-month and 12-month follow-up visits.

5.2.5 Other safety assessments

Body weight in Kg will be assessed at each of the clinical visits at screening, baseline/randomization, 3-month, 6-month, 9-month and 12-month follow-up visits.

5.3 Other assessments

5.3.1 Patient reported outcomes

Only adverse events reported by the study participants will be recorded using an AEs log.

5.4 Pharmacokinetics

5.4.1 Collection of samples

Pharmacokinetic samples will not be taken during the study.

5.4.2 Determination of drug concentration

Drug concentration will not be assessed in this study.

5.4.3 Storage and destruction of pharmacokinetic samples

There will be no PK study.

5.5 Pharmacodynamics

5.5.1 Collection of samples

Pharmacodynamic samples will not be taken during the study.

5.5.2 Storage, re-use and destruction of pharmacodynamic samples

There will be no PD study.

5.6 Pharmacogenetics

Pharmacogenetic samples will not be taken during the study.

5.6.1 Collection of pharmacogenetic samples

5.6.2 Storage, re-use and destruction of pharmacogenetic samples

5.7 Biomarker analysis

The subject's consent to the use of donated biological samples is mandatory.

Additional plasma will be stored at -80 degree and may be analysed for exploratory biomarkers to assess correlations with disease activity, effects of study drug, clinical outcomes and toxicity.

5.7.1 Storage, re-use and destruction of biological samples

Samples will be stored for a maximum of 15 years from the date of the Last Subject's Last Visit, after which they will be destroyed. The results of this biomarker research will be reported either in the Clinical Study Report itself or 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 with the study drug to generate hypotheses to be tested in future research.

5.7.2 Labelling and shipment of biological samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual, see Appendix B International Airline Transportation Association (IATA) 6.2 Guidance Document.

5.7.3 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator will keep full traceability of collected biological samples from the subjects while in storage at the centre until shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.

Sponsor keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

5.7.4 Withdrawal of Informed Consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, Sponsor is not obliged to destroy the results of this research.

As collection of the biological samples is an optional part of the study, then the subject may continue in the study.

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to Sponsor
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study site
- Ensures that the subject and Sponsor are informed about the sample disposal.

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

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

6.1 Definition of adverse events

An adverse event 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.

6.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, 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 hospitalisation
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the Investigator(s) and communicated to the Independent Safety Monitor for review and to the funding agent, AstraZeneca, to support post-marketing safety surveillance data..

For further guidance on the definition of a SAE, see Appendix A Additional Safety Information to the Clinical Study Protocol.

6.3 Recording of adverse events

6.3.1 Time period for collection of adverse events

Adverse Events including serious and non-serious will be collected from the time of informed consent signature throughout the treatment period of 1 year, visit 6, or last contact.

SAEs will be recorded from the time of informed consent throughout the treatment period and including the follow-up period.

6.3.2 Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last AE assessment or other assessment/visit as appropriate in the study are followed up by the Investigator for as long as medically indicated, but without further recording in the CRF. Company retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

6.3.3 Variables

The following variables will be collect for each AE;

- AE (verbatim)
- The date when the AE started and stopped
- Maximum intensity or intensity or changes in intensity

- CTCAE grade/max CTCAE grade/changes in CTCAE grade
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study *(*yes or no*)*
- Outcome.

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

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment in relation to the investigational product
- Causality assessment in relation to Other medication
- Causality assessment in relation to Additional Study Drug
- Description of SAE.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE unless it meets the criteria shown in Section 6.2. On the other hand, a stroke that results in only a limited degree of

disability may be considered a mild stroke but would be a SAE when it satisfies the criteria shown in Section 6.2.

6.3.4 Causality collection

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

For SAEs 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'.

The causality of AEs/SAEs as assessed by the Investigator(s) will be communicated to the Independent Safety Monitor for secondary review.

6.3.5 Adverse events based on signs and symptoms

All AEs spontaneously reported by the subject 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 tool that the study personnel will be using.

When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

6.3.6 Adverse events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated glucose levels, HbA1C, liver and renal functions should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting Investigator uses the clinical, rather than the laboratory term (e.g., anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.3.7 Hy's Law

Cases where a patient shows elevations in liver biochemistry may require further evaluation and occurrences of AST or ALT ≥3 × ULN together with total bilirubin ≥2 × ULN may need to be reported as SAEsReporting of serious adverse events to the IRB and/or the Regulatory Authority

6.3.8 Urinary Tract Infections (UTI)

All enrolled patients will be counseled at every clinical visit for risk of UTI and genital mycotic infection. Adequate hydration, frequent visits to the bathroom and appropriate perineal hygiene (washing with water or wipes) will be recommended.

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

If any SAE occurs in the course of the study, then Investigators or other site personnel inform the appropriate funding agent, AstraZeneca, represntatives within one day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The Investigator will ensure that all the necessary information is provided to the database **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 adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform funding agent, 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.

6.5. Reporting of serious adverse events

The PI must inform the FDA, via a MedWatch form, of any serious or unexpected adverse events that occur in accordance with the reporting obligations of 21 CFR 312.32 , and will concurrently forward all such reports to AstraZeneca. A copy of the MedWatch report must be emailed to AstraZeneca (TCS vendor) at the time the event is reported to the FDA. It is the responsibility of the PI to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time.

- **** PI*** must also indicate, either in the SAE report or the cover page, the ***causality*** of events ***in relation to all study medications***, as determined by the principal investigator.

- **** Send SAE report and accompanying cover page by way of email to AstraZeneca's designated mailbox:*** AEMailboxClinicalTrialTCS@astrazeneca.com
- If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

Serious adverse events that do not require expedited reporting to the Regulatory Authority/IRB/IEC still need to be reported to AstraZeneca . In the case of blinded trials, AstraZeneca will request that the Investigator either provide a copy of the randomization code/ code break information or unblind those SAEs (because of a critical patient safety concern related to that SAE), which require expedited reporting.

6.6. Overdose

An overdose is defined as a patient receiving a dose of IP in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.

If an overdose on the study drug occurs in the course of the study, then the Investigator is required to inform AstraZeneca Patietn Safety within 24 hours of the knowledge of the event using the designated safety mailbox

If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE

For overdoses associated with a SAE, the standard reporting timelines apply, see Section 0. For other overdoses, reporting must occur within 30 days.

The investigator will use clinical judgment to treat any overdose

6.7. Pregnancy

All pregnancies and outcomes of pregnancy should be reported to UW HSD and the funding agent, AstraZeneca,.

6.7.1. 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 the UW HSD and the funding agent, 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 <<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 Investigator will ensure that all relevant information is provided to AstraZeneca Patietn Safety within 1 or 5 calendar days for SAEs (see Section 0) and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

6.7.2. Paternal exposure

There is no restriction on fathering children or donating sperm during the study.

6.8. Management of IP related toxicities

Study drug will be discontinued. Appropriate supportive treatment should be initiated as dictated by the patient's clinical status.

6.9. Study governance and oversight

Data Monitoring Committee (DMC) is not required for the proposed study, because the study drug has established safety profiles in T2DM.

6.9.1. Steering Committee

NA.

6.9.2. Data Monitoring Committee

NA.

6.9.3. Scientific Advisory Committee

Investigators and ESR team numbers.

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1. Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Dapagliflozin	10mg	AstraZeneca

7.2. Dose and treatment regimens

Dapagliflozin 10mg or matching placebo is taken orally once daily in the morning regardless of meals.

7.3. Labelling

Details are specified in the document explaining the reconstitution procedures and other handling procedures for the investigational products.

The label will include the following information and manufacturing number:

- Name of Sponsor
- Investigational product/study drug dosage form, route of administration, and quantity of dosage units
- Storage conditions
- Study code
- Enrolment code or Randomisation Code
- Directions for use
- The name of the Principal Investigator, where applicable (this may be pre-printed or to be added on the label when the investigational product / study drug is dispensed)
- The period of use e.g., expiry date
- 'for clinical study use only'
- 'keep out of reach of children'.

7.4. Storage

A description of the appropriate storage conditions is specified in the document explaining the reconstitution procedures and other handling procedures for the investigational products. The study drug will be stored at the University of Washington Investigational Drug Services using the standard procedures.

7.5. Compliance

The administration of all study drugs (including investigational products) should be recorded in the appropriate sections of the Case Report Form. The study drug compliance will be calculated based on number of days dispensed vs. number of days that study drug taken.

7.6. Accountability

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

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

Study drug will not be distributed to the study site until the contract is concluded between the study site and Company. The Investigational Product Storage Manager is responsible for managing the study drug from receipt by the study site until the return of all unused study drug to Company. Company will provide the study documents 'Procedures for drug accountability' and 'Procedures for drug storage' which describes the specific requirements. The Investigator(s) is responsible for ensuring that the subject has returned all unused study drug.

7.7. Concomitant and other treatments

Restricted Medication/Class of drug:	**Usage:**
None	

Prohibited Medication/Class of drug:	
None	

Rescue/Supportive Medication/Class of drug:	**Usage:**
None	

7.7.1. Other concomitant treatment

Other medication other than that described above, which is considered necessary for the subject's safety and well being, may be given at the discretion of the Investigator and recorded in the appropriate sections of the Case Report Form.

7.8. Post Study Access to Study Treatment

NA.

8. STATISTICAL ANALYSES

8.1. Statistical considerations

- All personnel involved with the analysis of the study will remain blinded until database lock and protocol violators identified.
- Analyses will be performed by Principal Investigator or designees.

- Refer to Statistical Analysis Plan for details (if such a document exists and more details will be added).

A comprehensive Statistical Analysis Plan (SAP) will be prepared prior to first subject randomised and any subsequent amendments will be documented, with final amendments completed prior to unblinding of the data (this is the expectation for the pivotal studies).

8.2. Sample size estimate

Although a sample size calculation is not possible due lack of available data of changes in myocardial strain, ECV and T2 relaxation time and their variabilities in diabetic patients, we will start a group of 60 qualified subjects for a randomization to dapagliflozin 10 mg (n=30) or matching placebo (n=30) for 1 year. All subjects will undergo cardiac MRI scan at baseline and 1 year. We will assess changes in myocardial strain, ECV and T2 relaxation time and their variabilities, any signal on treatment effect with dapagliflozin. This planned "interim" analysis will be performed by an independent group, then, lead to an adaption for a sample size reestimation using variability data generated in this initial study. The investigator(s) will be blinded to subject's treatment status in this process. We will assess the value and feasibility of continuation of the study subject enrollment to reach a sample size suggested by the "interim" analysis and will allowed to include the initial 60 subjects if a larger sample size is needed. On the other hand, if the "interim" analysis indicates no signal of treatment effect on ECV with dapagliflozin, further study will not be justified.

The tested hypothesis is that SGLT-2 inhibition with dapagliflozin, in comparison to placebo, will improve myocardial strain, reduce progression of myocadial fibrosis and inflammation in patients with type-2 diabetes.

8.3. Definitions of analysis sets

8.3.1. Efficacy analysis set

This is primary analysis set for the proposed study and will include any subjects who have both baseline and 1-year follow-up cardiac MRI scans and actually take study medication or placebo.

8.3.2. Safety analysis set

This will be secondary analysis set given the small number of subjects in the study. Subjects who take at least 1 dose of study medication or placebo will be incldued.

8.3.3. PK analysis set

NA.

8.3.4. PRO analysis set

NA.

8.4. Outcome measures for analyses

The primary outcome measures include GLS and Extracellular Volume Fraction (ECV) as assessed by T1-mapping using cardiac MRI.

The secondary outcome measures are T2 relaxation time as assessed T2-mapping, GCS and GRS, and Mit. F assessment. Other biomarkers include fasting glucose and HbA1C levels, LDL particle size and hsCRP, TNF-α and IL-6.

8.5. Methods for statistical analyses

We will first perform graphical and tabular descriptive analyses. These methods include scatterplots, boxplots, histograms, frequency listings for categorical variables, and descriptive statistics (mean, median, SD, maximum, minimum, skewness, coefficient of variation and percent missing). We will screen each variable for plausible values and range; outlier detection; inadequate variation; sparse categories that need to be combined or dropped; skewness or other distribution problems for continuous variables, suggesting use of a transformation (such as log) or conversion from a continuous variable to a categorical variable; and, finally; an excessive fraction of missing values.

Primary and secondary endpoints will be compared based on actural treatment is received.

8.5.1. Analysis of the primary variable (s)

Ancova test with adjusted for baseline GLS and ECV will be used to compare change in GLS and ECV over 12 months between 2 treatment groups.

8.5.2. Analysis of the secondary variable(s)

Ancova test will be used for comparisons of changes in secondary endpoints between 2 treatment groups.

8.5.3. Subgroup analysis (if applicable)

NA.

8.5.4. Interim analysis

NA.

8.5.5. Sensitivity analysis (if applicable)

NA.

8.5.6. Exploratory analysis (if applicable))

Ancova test will also be used for comparisons of epicardial fat between 2 different timepoints within each treatment group, at between 2 treatments at the same time point, and changes during the study. In addition, we will examine associations between epicardial fat and ECV and between epicardial fat and T2 relaxization time.

9. STUDY AND DATA MANAGEMENT

9.1. Training of study site personnel

Before the first subject is entered into the study, an Sponsor representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and data collection system both CRFs and database.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.2. Monitoring of the study

Monitor

Monitoring for the study is provided by an Independent Safety Monitor (ISM).

An Independent Safety Monitor (ISM) will serve in this study to ensure impartial review of subject safety during the course of the study. The study intervention, dapagliflozin, has a well-established safety profile and is in use by many patients with diabetes. It is appropriate to have an ISM with clinical experience and endocrinology training as the safety monitor.

MONITORING PROCEDURES

The PI reviews adverse events (AEs and SAEs) at the time they are discovered.

Quarterly Monitoring Process:

ISM will be provided with quarterly database report for all enrolled subjects to include data points and clinical events (AEs and SAEs) collected in the quarterly interval:

Visit vital signs, drug compliance percentages, laboratory values, any AEs and SAEs will be provided. At ISM's request additional supporting documents and access to the source materials will be provided. If necessary, to know the treatment assignment of a particular subject, either 10mg dapagliflozin daily or matching placebo, ISM will contact Harborview Investigational Pharmacy which can provide drug assignment to maintain the double-blind of PI, the study team and the subjects.

ISM will review data, clinical reports and medical records as necessary to make an overall recommendation for study modification, continuation or discontinuation as relevant. This determination will be submitted in writing to the study PI and will be submitted to UW HSD.

This review will take place for all subjects including those randomized to active dapagliflozin and matching placebo. Any AEs/SAEs encountered during Mitochondrial Function control subjects' single visit will also be presented for ISM review.

MONITORING REPORT

The Independent Safety Monitor provides a written report to the study team with recommendations for study modification, study continuation/discontinuation as relevant.

The study team is responsible for forwarding the report to the IRB. The Independent Safety Monitor will be available between visits if the Investigator(s) or other staff at the centre needs information and advice about the study conduct.

Data Monitoring

PI and staff perform audits of the data entered into the electronic database to ensure integrity, completeness and conformity. This is done as required for dataset preparation. Any deficiencies are resolved after confirming with source documentation.

PI and staff perform routine audits of charts and safety profile at subject milestones. For example when a subject completes Randomization, the chart is reviewed to ensure all supporting documentation is included and that subject has no outstanding issues. The subject record would again be reviewed after the 3Month visit, 6month visit and at study completion.

Study team provides data reports by database query at PI's request.

9.2.1. Source data

Source data are any data generated as a result of the subject's inclusion in the study (including run-in and/or follow up related to the study) and includes all related medical examinations and other records.

9.2.2. Research agreement(s)

The Principal Investigator at each centre should comply with all the terms, conditions, and obligations of the Research Agreement with the Sponsor, or equivalent, for this study. In the event of any inconsistency between this CSP and the Research Agreement with Sponsor, the terms of CSP shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Research Agreement with Sponsor shall prevail. Agreements between Sponsor and the Principal Investigator should be in place before any study-related procedures can take place, or any subjects are enrolled.

9.2.3. Archiving of study documents

Study files. Sponsor will provide the Principal Investigator with a file in which to organise and retain all study-related documents. All study documents (including letters from Sponsor) should be retained in this file by the Principal Investigator. The monitor will regularly check the file to ensure that all relevant documents are retained. The contents of the file may be audited/inspected by Sponsor's auditor, regulatory authorities, or IRB.

Period of record retention. The study site (and the Principal Investigator) will retain the essential documents specified in the ICH GCP (e.g., source document such as medical records, contract, signed consent form). Essential documents should be retained at the study site for at least 15 years following completion of the study, or per regulatory obligations if longer, and thereafter destroyed only after agreement with Sponsor. However this is not always applied to those that are not preservable such as blood samples. In the event of any inconsistency between the above-mentioned contents and the contract with the study site, the contract shall prevail. These documents should be retained for a longer period however if needed by Sponsor, and the specific period and method of retention will be separately discussed between the study site and Sponsor. Sponsor should notify the head of the study site in writing when the study related records are no longer needed. The records should be managed by a responsible person appointed by the head of the study site.

9.2.4. Deviation from the clinical study protocol

The Investigator(s) must not deviate from or make any changes to the protocol without documented agreement between the Principal Investigator and Sponsor or the IRB approval based on its deliberations. However, this shall not apply to cases where the deviation or change is necessary to avoid an immediate hazard to the subjects or for other compelling medical reasons, or where the changes involve only logistical or administrative aspects of the clinical study (e.g., changes to the organisation/structure of the Sponsor, the name/department name of the study site, the address or phone number of the study site or Sponsor, the job title of the Investigator, and monitors).

The Investigator(s) should document any deviation from the protocol regardless of their reasons. Only when the protocol was not followed in order to avoid an immediate hazard to the subjects or for other medically compelling reason, the Investigator should prepare and submit the records explaining the reasons thereof to Sponsor and the head of study site, and retain a copy of the records.

The Investigator(s) may deviate from or make a change to the protocol without documented agreement between the Principal Investigator and Sponsor or the IRB approval, only in the event of a medical emergency, e.g., it is only way to avoid an immediate hazard to the subjects. In such case, the Principal Investigator must notify details of the deviation or change, the reason, and a proposed revision in the protocol if required, to Sponsor and the head of the study site and IRB via the head of the study site as soon as possible, in order to obtain their approval. A certificate of approval by the head of the study site as well as Sponsor should be obtained via the head of the study site.

9.3. Study timetable and end of study

The end of the study is defined as 'the last visit of the last subject undergoing the study'.

The study is expected to start subject enrolment in Q-3 2018 and to end the last visit of the last subject by Q-2 2020.

The study may be terminated at individual centres if the study procedures are not being performed according to GCP, or if recruitment is slow. Sponsor may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with dapagliflozin.

Planned duration of the study: 3 years

Study period: April, 2018 - March, 2021

If Sponsor decides to prematurely terminate or suspend the study, the Principal Investigator/Investigator, the head of the study site, and regulatory authorities should receive written notification of the reasons for the premature termination or suspension.

The Principal Investigator/Investigator will immediately notify the decision to the subjects, give appropriate medical treatment; take necessary measures, and record treatment or measures provided on the source documents.

Completion of the study

Upon terminating the study, the Principal Investigator/Investigator will report in writing the completion of the study as well as the summary of the results to the head of the study site in accordance with the study site's rules. The head of the study site, who is informed of the termination by the Investigator, will provide a written notification of the results to the IRB and Sponsor.

9.4. Data management

The Clinical Atherosclerosis Research Lab in Division of Cardiology at the University of Washington will be responsible for data management of this cardiac MRI study. All patient demographic information, clinical and laboratory characteristics and MRI data at baseline and/during 12 months will be collected and stored in a multi-dimensional database located at the University of Washington Computing Center, with appropriate backups and security. This database has been used in our previous studies. The database system can export data into statistical applications for generating study progress reports and for performing statistical analysis. The unique subject ID will be used for all data linking.

Adverse events and medical/surgical history will be classified according to the terminology of the latest version the Medical Dictionary for Regulatory Activities (MedDRA). Medications will be classified according to disease conditions.

Data queries will be raised for inconsistent, impossible or missing data. All entries to the study database will be available in an audit trail.

The data will be validated as defined in the Data Management Plan. Quality control procedures will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly. The Data Management Plan will also clarify the roles and responsibilities of the various functions and personnel involved in the data management process

When all data have been coded, validated, and locked, clean file will be declared. Any treatment revealing data may thereafter be added and the final database will be locked.

Serious Adverse Event (SAE) Reconciliation

SAE reconciliation reports are produced and reconciled with the study database and/or the investigational site.

Data Management of genotype data

NA.

Data associated with human biological samples

Data associated with biological samples will be transferred from laboratory (ies) internal or external to Sponsor.

Management of external data

NA

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1. Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the Sponsor policy on Bioethics and Human Biological Samples.

Study will be reviewed and approved by the IRB at the University of Washington Human subjects division. Continuing review reports will be submitted as required by the IRB. The annual review should be submitted in time to assure no lapse in approval.

10.2. Subject data protection

The Master Informed Consent Form will explain that:

- Study data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation

- Patient data will be maintaining confidentiality in accordance with national data legislation

- For data verification purposes, authorised representatives of Sponsor, a regulatory authority, an IRB may require direct access to parts of the hospital or practice source records relevant to the study, including subjects' medical history

- All data computer processed by Sponsor will be identified by study code and enrolment code

10.3. Ethics and regulatory review

An Ethics Committee should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The Investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee should be given in writing. The Investigator should submit the written approval to Sponsor before enrolment of any subject into the study.

The Ethics Committee should approve all advertising used to recruit subjects for the study.

Sponsor should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the protocol should be re-approved by the Ethics Committee annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

Sponsor will handle the distribution of any of these documents to the national regulatory authorities.

Sponsor will provide Regulatory Authorities, Ethics Committees and Principal Investigators with safety updates/reports according to local requirements.

An IRB should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The head of the study site will ensure the distribution of these documents to the applicable IRB, and the Principal Investigator to the Investigator and study site staff.

The opinion of the IRB should be given in writing. The head of the study site should submit a notification of direction/determination as well as a copy of the IRB written approval to Sponsor and the Principal Investigator before enrolment of any subject should into the study.

The IRB should approve all advertising used to recruit subjects for the study.

Sponsor should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

The protocol should be re-approved by the IRB annually.

The head of the study site should seek the opinion of the IRB with respect to the appropriateness of continuing the study at the study site at least once a year when the duration of the study exceeds one year. The Principal Investigator should submit progress reports to the IRB via the head of the study site.

Before enrolment of any subject into the study, the final study protocol, including the final version of the ICF, should be approved by the national regulatory authority with notification provided, according to local regulations. Sponsor will handle the distribution of any of these documents to the national regulatory authorities.

Sponsor will provide Regulatory Authorities, IRB, the head of the study site and the Principal Investigator with safety updates/reports according to local requirements.

The head of the study site should submit a written report to the IRB providing the details of all safety relative information reported by Sponsor.

10.4. Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject

- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by IRB.

10.5. Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal Investigator and Sponsor. If it is necessary for the study protocol to be amended, the amendment should be submitted to the Head of the Study Site and be approved by its IRB. If applicable, Sponsor should submit a notification to the regulatory authority before it is implemented. If a protocol amendment requires a change to a particular centre's Informed Consent Form, then Sponsor and the centre's IRB should be notified. Approval of the revised Informed Consent Form by Sponsor and by the IRB is required before the revised form is used. If an administrative change is required, such a change should be notified to or approved by each IRB according to local requirements.

10.6. Audits and inspections

Authorised representatives of Sponsor, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The Investigator will contact Sponsor immediately if contacted by a regulatory agency about an inspection at the centre.

All study data may undergo a reliability review and onsite-GCP inspection by the regulatory authorities.

11. LIST OF REFERENCES

1. Garcia MJ, McNamara PM, Gordon T, Kannel WB. Mor- bidity and mortality in diabetics in the Framingham population: sixteen year follow-up study. Diabetes 1974; 23: 105-11.
2. Beckman JA, Creager MA, Libby P. Diabetes and Atherosclerosis – Epidemiology, pathophysiology, and Management. JAMA 2002;287:2570-2581.
3. Bell DSH. Diabetic cardiomyopathy: a unique entity or a complication of coronary artery disease? Diabetes Care 1995; 18: 708-14.
4. Kasznicki J and Drzewoski J. Heart failure in the diabetic population – pathophysiology, diagnosis and management. Arch Med Sci 2014;10,3:546-556.
5. Kemp TM, Barr ELM, Zimmet PZ, et al. Glucose, lipid, and blood pressure control in Australian adults with type 2 diabetes: the 1999–2000 AusDiab. Diabetes Care 2005;28(6):1490–1492.
6. Laing SP, Swerdlow AJ, Slater SD, et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 2003;46(6):760–765.

7. Suskin N, McKelvie RS, Burns RJ, et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. Eur Heart J 2000;21:1368–75.
8. Berry C, Clark AL. Catabolism in chronic heart failure. Eur Heart J 2000;21:521–32.
9. Bertoni AG, Hundley WG, Massing MW, Bonds DE, Burke GL, Goff DC Jr. Heart failure prevalence, incidence and mortality in the elderly with diabetes. Diabetes Care 2004;27:699–703.
10. Bell, DS. Diabetic cardiomyopathy. Diabetes Care 2003;26: 2949–51.
11. Hayat SA, Patel B, Khattar RS, Malik RA. Diabetic cardiomyopathy: mechanisms, diagnosis and treatment. Clinical Science 2004;107: 539–57.
12. Fang ZY, Schull-Meade R, Downey M, Prins J, Marwick TH. Determinants of subclinical diabetic heart disease. Diabetologia 2005;48:394–402.
13. Fang ZY, Yuda S, Anderson V, Short L, Case C, Marwick TH. Echocardiographic detection of early diabetic myocardial disease. J Am Coll Cardiol 2003;41:611–17.
14. Vinereanu D, Nicolaides E, Tweddel AC, et al. Sub- clinical left ventricular dysfunction in asymptomatic patients with Type II diabetes mellitus, related to serum lipids and glycated haemoglobin. Clin Sci 2003;105:591–99.
15. Andersen NH, Poulsen SH, Eiskjaer H, Poulsen PL, Mogensen CE. Decreased left ventricular longitudinal contraction in normotensive and normoalbuminuric patients with Type II diabetes mellitus: a Doppler tissue tracking and strain rate echocardiography study. Clin Sci 2003;105:59–66.
16. Cosson S, Kevorkian JP. Left ventricular diastolic dysfunction: an early sign of diabetic cardiomyopathy? Dia- betes Metab 2003;29:455–66.
17. Asbun J, Villarreal FJ. The Pathogenesis of Myocardial Fibrosis in the Setting of Diabetic Cardiomyopathy. JACC 2006;47(4):693-700.
18. Fischer VW, Barner HB, Larose LS. Pathomorphologic aspects of muscular tissue in diabetes mellitus. Hum Pathol 1984;15:1127-1136.
19. Nunoda S, Genda A, Sugihara N, Nakayama A, Mizuno S, Takeda R. Quantitative approach to the histopathology of the biopsied right ventricular myocardium in patients with diabetes mellitus. Heart Vessels 1985;1:43-47.
20. Uusitupa MI, Mustonen JN, Airaksinen KE. Diabetic heart muscle disease. Ann Med 1990;22:377-386.
21. Taylor AJ, Salemo M, Dharmakumar R, Jerosch-Herold M. T1 Mapping. JACC Cardiovascular Imaging 2016;9(1):67-81.
22. Mu Zeng, Yingyan Qiao, Zhaoying Wen, Jun Liu, Enhua Xiao, Changlian Tan, Yibin Xie, Jing An, Zishu Zhang, Zhanming Fan, and Debiao Li. The Association between Diffuse Myocardial Fibrosis on Cardiac Magnetic Resonance T1 Mapping and Myocardial Dysfunction in Diabetic Rabbits. Sci Rep 2017;7:44937.
23. Bohnen S. Radunski UK, Lund GK, Kandolf R, Stehning C, Schnackenburg B, Adam G, Blankenberg S, Muellerleile K. Performance of T1 and T2 Mapping Cardiovascular Magnetic Resonance to Detect Active Myocarditis in Patients With Recent-Onset Heart Failure. Circ Cardiovasc Imaging. 2015;8: e003073.
24. Montanta P, Sigovanb M, Revelc D, Douekc P. MR imaging assessment of myocardial

edema with T2 mapping. Diagnostic and Interventional Imaging 2015;96:885—890.
25. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Biomath D, Devins T, Johansen OE, Woerle HJ, Broedl UC, and Inzucchi SE, for the EMPA-REG OUTCOME Investigators. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-2128.
26. Kosiborod M, Cavender MA, Fu AZ, Wilding JP, Khunti K, Holl RW, Norhammar A, Birkeland KI, Jørgensen M, Thuresson M, Arya N, Bodegård J, Hammar N, Fenici P; CVD-REAL Investigators and Study Group. Lower Risk of Heart Failure and Death in Patients Initiated on SGLT-2 Inhibitors Versus Other Glucose-Lowering Drugs: The CVD-REAL Study. Circulation. 2017;136:249-259.
27. Verma S, Garg A, Yan AT, et al. Effect of empagliflozin on left ventricular mass and diastolic function in individuals with diabetes: an important clue to the EMPA-REG OUTCOME Trial? Diabetes Care 2016;39:e212–3.
28. Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, Espadero R-M, Woerle H-J, Broedl UC and Johansen OE. SGLT-2 inhibitors and cardiovascular risk: Proposed pathways and review of ongoing outcome trials. Diabetes & Vascular Disease Research 2015;12(2): 90–100.
29. Mahajan R and Gupta K. Adaptive design clinical trials: Methodology, challenges and prospect. Indian J Pharmacol. 2010; 42(4):201–207.
30. Guidance for Industry – FDA. https://www.fda.gov/downloads/drugs/guidances/ucm201790.pdf
31. Chacho BK, Kramer PA, Ravi S, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. Laboratory Investigation 2013;93:690–700.
32. Choi E-Y, Rosen BD, Fernandes VRS, et al. Prognostic value of myocardial circumferential strain for incident heart failure and cardiovascular events in asymptomatic individuals: the Multi-Ethnic Study of Atherosclerosis. European Heart Journal 2013; 34, 2354–2361.
33. Hor KN, Baumann R, Pedrizzetti G, et al. Magnetic Resonance Derived Myocardial Strain Assessment Using Feature Tracking. J Vis Exp JoVE 2011. https://doi. org/10.3791/2356
34. Pedrizzetti G, Claus P, Kilner PJ et al. Principles of cardiovascular magnetic resonance feature tracking and echocardiographic speckle tracking for informed clinical use. J Cardiovasc Magn Res; 2016; 18:51.
35. Schmidt B, Dick A, Treutlein M et al. Intra- and interobserver reproducibility of global and regional magnetic resonance feature tracking derived strain parameters of the left and right ventricle. Eur J Radiol 2017; 89:97–105.
36. Morton G, Schuster A, Jogiya R et al. Inter-study reproducibility of cardiovascular magnetic resonance myocardial feature tracking. J Cardiovasc Magn Res 2012; 14:43.
37. Schuster A, Morton G, Hussain ST et al. The intra-observer reproducibility of cardiovascular magnetic resonance myocardial feature tracking strain assessment is independent of field strength. Eur J Radiol 2013; 82:296–301.
38. Jung J, Kim YH, Kim N, Yang DH. Patient-specific 17-segment myocardial modeling on a bull's eye map. J Appl Clin Med Phys. 2016;17(5):453-465.

Appendix A Additional Safety Information

Further Guidance on the Definition of a Serious Adverse Event (SAE)

Life threatening

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

Hospitalisation

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

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize 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 (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation

Development of drug dependency or drug abuse

A Guide to Interpreting the Causality Question

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

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

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? Or could the AE be anticipated from its pharmacological properties?

- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Re-challenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? Sponsor 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.