Cover Page

Official Title: A single-center, randomized, double-blind, placebo-controlled study to evaluate microvascular disease after 12 weeks of therapy with SGLT2i compared to placebo in symptomatic women with non-obstructive CAD (SMILE)

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PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

BACKGROUND

Heart disease is the leading cause of death for men, women, and people of most racial and ethnic groups in the United States[1]. Coronary heart disease is the most common type of heart disease, killing 360,900 people in 2019[2]. Interestingly, in the NIH-NHLBI-sponsored Women's Ischaemia Syndrome Evaluation (WISE) study, more than 50% of women with angina were found to have either no or minimal IHD on invasive coronary angiography. Women with persistent chest pain but without obstructive IHD had worse cardiac outcomes than asymptomatic women. One year after the index evaluation, nearly 40% of symptomatic women displayed persistent or worsening symptoms[3] . Angina secondary to myocardial ischemia may occur in non-obstructive coronary artery disease (INOCA) patients. INOCA is associated with an increased risk for major adverse cardiac events (MACE) compared with an asymptomatic reference population [13]. Coronary microvascular dysfunction (CMD) and vasospasm of the epicardial arteries are the two most common causes of INOCA [4, 5].

Recent studies have estimated at least 4 million patients currently experience stable INOCA in the United States[6], being more prevalent in women than in men[7]. Recognition of sex differences accentuates the need for sex-specific research. Unfortunately, women have often been excluded from clinical trials, though some recent improvement has been made. A recent publication showed that women had made some headway in representation in clinical trials of conditions such as atrial fibrillation, hypertension, and pulmonary hypertension. However, women remain underrepresented in clinical trials of common diseases like ACS, coronary artery disease (CAD), and heart failure[8].

While traditional cardiovascular risk factors play an important role in the development of IHD in women, women may experience sex-specific IHD risk factors and pathophysiology, and thus female-specific risk stratification is needed for IHD diagnosis and treatment. Emerging data from the past 2 decades have significantly improved the understanding of IHD in women, including mechanism of ischemia with no obstructive coronary arteries and myocardial infarction with no obstructive coronary arteries[9]. In patients with suspected INOCA undergoing invasive evaluation of INOCA, CMD and coronary vasospasm are diagnosed in up to 4 in 5 patients[10]; these patients are often female (50-80%)[11].

Approximately 4 million Americans (>60%) have INOCA[12]. This preponderance of INOCA burden in women, prior work of the NHLBI-sponsored Women's Ischemic Syndrome Evaluation which specified the exclusive study of women, the Congressionally Directed Medical Research Program "Topic Area" of women's heart disease provided the directive for this trial to focus recruitment in women. To date, there is a lack of appropriately designed outcome trials investigating therapeutic strategies targeting women with INOCA and persistent symptoms. Presently, no therapeutic strategies are focused on treating CMD and microcirculation. There is an urgent need to identify novel and specific targets for therapy. Clinical studies in patients with non-obstructive disease are required, specifically in women with suspected or confirmed IHD, especially when young, who have less atherosclerosis than men and less prevalence of obstructive CAD. To address this issue, the multi-site, Women's Ischemia Trial to Reduce Events In No Obstructive CAD (the WARRIOR trial)[13], will

potentially provide data necessary to determine whether intensive statin/Angiotensin-converting-enzyme inhibitor/aspirin therapy will reduce MACE. In our proposed study, we hypothesize that Sodium-glucose cotransporter 2 inhibitors (SGLTi) treatment will improve coronary microvascular dysfunction and reduce the functional impact of this disease process in women with symptoms indicative of ischemia and no evidence of obstructive epicardial CAD.
SGLT2i, a new drug class approved for the treatment of diabetes, has been shown to significantly reduce atherosclerotic events, hospitalization from heart failure, cardiovascular and total mortality, and progression of chronic kidney disease[14]. In preclinical models of MI, dapagliflozin has demonstrated attenuation of cardiac fibrosis and improved cardiac function and remodelin. Furthermore, empagliflozin has been shown to improve coronary microvascular function and increase cardiac output in mice, as evidenced by increases in coronary blood flow and fractional ventricular area change as shown by ultrasound imaging [15]. These findings suggest that SGLTi therapy may have a beneficial impact in CMD.

Coronary microvascular dysfunction (CMD) is defined as epicardial and/or microvascular endothelial and/or non-endothelial dysfunction that limits myocardial perfusion, most often detected as reduced coronary flow reserve (CFR) [17]. Coronary flow reserve (CFR), which is calculated as the ratio of hyperemic to rest absolute myocardial blood flow, is an indicator of the ability of the myocardium to increase flow in response to stress or vasodilation. CFR is an integrated marker of coronary vasomotor dysfunction that measures the hemodynamic effects of focal, diffuse, and small-vessel coronary artery disease on myocardial tissue perfusion][18]. Because the coronary microcirculation is beyond the resolution of invasive or noninvasive coronary angiography, direct interrogation of coronary microvascular function is necessary to establish the diagnosis of CMD [19]. Positron Emission Tomography (PET) studies have shown that even in visually normal PET Myocardial Perfusion Imaging, impaired CFR adds essential prognostic value, and in addition, abnormalities in the microcirculation could explain inducible myocardial ischemia beyond the effects of epicardial coronary obstruction and identify individuals with a high risk of MACE [19]. In patients with very low CFR (<1.6), women showed a higher frequency of non-obstructive CAD, whereas men showed a higher frequency of severely obstructive CAD (P=0.002)[20]. A differential effect on outcomes between women and men is noted in individuals with very low CFR (CFR <1.6) (hazard estimated from the linear interaction of CFR and sex in the final model, model P<0.001, interaction P=0.04). High-resolution CMR stress perfusion imaging can quantify MPR both globally and between the endocardium and epicardium[21]. A multicenter study of 1049 patients with suspected and known CAD showed that lower myocardial stress MBF and MPR by CMR perfusion mapping were associated with death and MACE independent of other clinical risk factors, [62] even in patients who had normal qualitative perfusion and no known obstructive CAD[22].

Although small trials have suggested benefits from Angiotensin-converting enzyme (ACE) inhibitors, aspirin, and statins, there is a lack of appropriately designed clinical outcome trials to inform evidence- based therapeutic strategies.
Sodium-glucose cotransporter 2 inhibitors (SGLT2i), a new drug approved for the treatment of diabetes, have been shown to have a significant impact on cardiovascular events. Recent large-scale clinical trialshave shown that SGLT2i reduce MACE, prevent heart failure (HF) hospitalizations, and are nephroprotective [23-26]. Many of these findings were unanticipated and have stimulated substantial mechanistic research to improve the understanding of this class of drug [27]. Currently, four SGLT2i agents (empagliflozin, canagliflozin, dapagliflozin, and ertugliflozin) have been approved by regulatory agencies in

the United States and in Europe for the treatment of patients with diabetes.. Dapagliflozin has been approved for the treatment of HF. The U.S. Food and Drug Administration has also approved canagliflozin

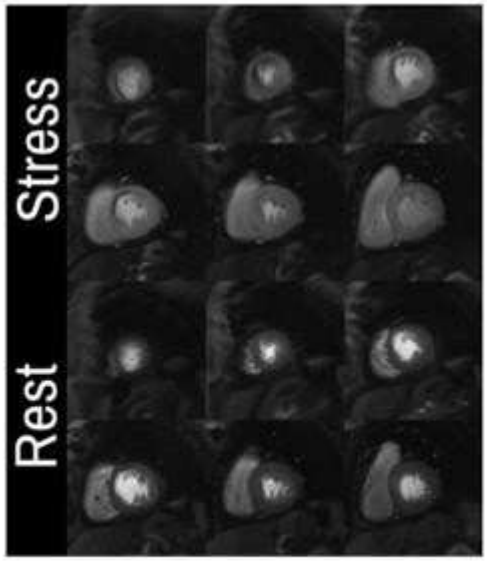


Figure 1. Stress and Rest myocardial Perfusion images from a representative subject demonstrated stress- induced myocardial perfusion defects.

Figure 1. Stress and Rest myocardial Perfusion images from a representative subject demonstrated stress- induced myocardial perfusion defects.

to reduce the risk of end-stage kidney disease and worsening kidney function in adults with diabetic kidney disease.

In preclinical models of myocardial infarction, dapagliflozin has demonstrated attenuation of cardiac fibrosis and improved cardiac function and remodeling[28] . Furthermore, empagliflozin has been shown to improve coronary microvascular function and increase cardiac output in mice, as evidenced by increases in coronary blood flow and fractional ventricular area change as demonstrated by ultrasound imaging [29]. Therefore, it might be expected that treatment with SGLT2i would improve MPR; however, this has not been studied to date.

Presently, there are no therapeutic strategies focused on specifically treating CMD and the microcirculation. There is an urgent need to identify novel and specific targets for therapy. Clinical studies in patients with non-obstructive disease, specifically in women designed to assess novel therapies, are required. Measuring myocardial blood flow quantitatively with stress CMR is a particular expertise of the Cardiovascular Imaging Center at UVA and our research group.

SIGNIFICANCE

Cardiovascular disease is the leading cause of death in American women, predominantly due to Ischemic heart disease[1]. Women's ischemic heart disease is different from men's, and women have a unique phenotype of less calcified lesions and more non-obstructive disease. A significant number of patients with symptoms and signs of IHD do not have significant coronary artery stenosis (>50%). This condition is termed INOCA[30]. Patients with INOCA have a high symptom burden and reduced quality of life. Patients with INOCA reported frequent time missed from work and work limitations in one study, suggesting a substantial economic impact[31].

One proposed mechanism contributing to INOCA is CMD. In addition, there is an increasing recognition that coronary microvascular dysfunction (CMD) with or without epicardial CAD is more prevalent in women [19, 23].

Small size, short-term follow-up studies with INOCA using intense medical therapies targeting endothelial, microvascular, and/or atherosclerosis mechanisms suggest symptoms, ischemia, and coronary functional improvement[32]. However, randomized, controlled outcome trials testing treatment strategies have not been performed. There are currently no therapeutic strategies focused on specifically treating CMD and the microvasculature proposed studies are

consistent with an unmet urgent need for new novel therapies to target the microcirculation, specifically in women with suspected or confirmed IHD, especially when young, who have less atherosclerosis than men and less prevalence of obstructive CAD. Our proposal will also contribute to understanding how SGLT2i improves coronary microvascular function seen in animal models.

INNOVATION

Our study will focus on a **novel study population and a novel therapy**. CVD is the leading cause of death in American women. Currently, cardiovascular disease claims more women's lives each year than cancer and chronic lung disease combined . Women under investigation for myocardial ischemia are more likely to have non-obstructive CAD, and here CMD is relevant. Targeting symptomatic women with ischemia and non-obstructive CAD is clearly an urgent need. This group of patients with a disabling problem, as we have mentioned , also incur health-care costs similar to many with obstructive CAD . CMD has historically been under-recognized and under-treated. Accordingly, CMD has generated substantial interest in the clinical and basic science research community in recent years.

Innovative treatment option. At present**,** the management strategy for INOCA/CMD remains unclear due to the absence of randomized trials comparing therapies to reduce adverse cardiac events, and available data are limited to cohort studies. There are no therapeutic options specifically to treat CMD. Our study will test whether SGLT2i therapy improves coronary blood flow and quality of life in women with INOCA. Results will also contribute to understanding the mechanistic effect of this novel drug.

INCLUSION OF WOMEN, MINORITIES, AND CHILDREN

Inclusion of Women: We propose to study the effect of SGLT2 inhibitor therapy in women with symptoms concerning for ischemia and no evidence of obstructive CAD (INOCA) and possible microvascular disease. INOCA and microvascular disease are more common in women than in men. Given that women constitute the majority of the population with this condition. Our study plans to enroll a study exclusively of women older than 18 years old. Urine pregnancy testing will be performed on all women unless they are surgically sterile or unless there is a documented history of their postmenopausal status. A urine pregnancy test will be performed at the baseline visit unless a serum pregnancy test is preferred at the discretion of the investigator or if required by local regulations (if a serum pregnancy test is required, it will be performed at the screening visit). Additional serum or urine pregnancy tests may be performed, as determined necessary by the investigator or required by local regulation, to establish the absence of pregnancy at any time during the study.

Inclusion of Minorities: Minority subjects will be included in this research study, and every effort will be made to ensure proportional representation based on the population demographics for the University of Virginia Health System primary service area.

Inclusion of Children: No children will be recruited in this study.

Inclusion of participants having Type 2 DM: Sodium-glucose cotransporter 2 inhibitors (SGLT2i), a drug class approved for the treatment of diabetes, has been shown to significantly reduce atherosclerotic events, hospitalization from heart failure (HF), cardiovascular and total mortality, and progression of chronic kidney disease[26]. New recommendations suggest the

use of SGLT2i in symptomatic patients with chronic HF with reduced ejection fraction to reduce hospitalization and cardiovascular mortality, regardless of the presence of type 2 diabetes. The guidelines also suggest a benefit in patients with HF with mildly reduced and preserved ejection fraction[34]. The study population will include patients both with and without type 2 diabetes, as the benefits of SGLT2i appear to be independent of the glycemic effect.

Monitoring of HbA1c in study participants: Recruitment to three categories of Type 2 diabetes, pre-DM or no DM will be monitored. DM in this context is defined as active treatment with antidiabetic medication (for indication of DM) or screening HbA1c ≥6.5% or history of DM. Pre-DM is defined as screening HbA1c ≥5.7% and < 6.5 % without any intake of antidiabetic medication (unless taken for a non-DM indication) and no history of DM. We will collect information of HbA1c at baseline and at the end of the study. We will not change therapy based on the levels of HbA1c.The clinical studies in healthy subjects at high multiple doses showed, that due to mechanism of action, dapagliflozin does not induce hypoglycemia in nondiabetic subjects[33].

Monitoring plan and hypoglycemia prevention strategy in patients with established diagnosis of type 2 diabetes: Diabetes medications at baseline and during the study will be recorded. Participants with T2DM at randomisation will continue their T2DM treatment. Patients treated with insulin or insulin secretagogues have a higher risk of experiencing hypoglycemic events compared with those treated with other antidiabetic agents. Participants with T2DM taking insulin or insulin secretagogues will be instructed to closely monitor their glucose for the first 4 weeks of the study. If needed, T2DM treatments may be adjusted at the discretion of the Investigator or diabetes health care provider.

Management of euglycemic ketoacidosis: Dapagliflozin has not been shown to induce hypoglycaemia in non-diabetes patients. In clinical pharmacology studies, healthy subjects have been treated with single oral doses up to 500 mg and multiple oral doses of 100 mg up to 14 days without any hypoglycaemic events. There have been post-marketing reports of ketoacidosis, including diabetic ketoacidosis, in patients with T2D taking dapagliflozin and other SGLT2 inhibitors, although a causal relationship has not been established. Patients treated with dapagliflozin who present with signs and symptoms consistent with ketoacidosis, including nausea, vomiting, abdominal pain, malaise, and shortness of breath, should be assessed for ketoacidosis, even if blood glucose levels are below 14 mmol/L (250 mg/dL). If ketoacidosis is suspected interruption of dapagliflozin treatment should be considered and the patient should be promptly evaluated.
Predisposing factors to ketoacidosis include a low beta-cell function reserve resulting from pancreatic disorders (e.g., T1D, history of pancreatitis, or pancreatic surgery), insulin dose reduction, reduced caloric intake, or increased insulin requirements due to infections, illness or surgery and alcohol abuse. Dapagliflozin should be used with caution in patients in these circumstances. Dapagliflozin is currently not indicated for the treatment of patients with T1D; these patients are excluded from this study.

Objectives/Hypothesis

We hypothesize that SGLTi treatment will improve coronary microvascular dysfunction and reduce the functional impact of this disease in symptomatic women with INOCA. We will address this hypothesis through the following two aims:

AIM 1: Determine whether SGLT2i improves coronary blood flow in women. We hypothesize that SGLT2i therapy will improve coronary microvascular function. Coronary flow reserve (CFR; assesses both the epicardial and microvascular compartments and is defined as the ratio between hyperemic and resting flows) will be assessed using stress Cardiac Magnetic Resonance (CMR) before and after 12 weeks of SGLT2i vs. control (placebo treatment) in women with symptoms concerning for ischemia and no evidence of obstructive epicardial CAD (stenosis >50%). The primary endpoint is between-group change in CFR from baseline at week 12. Food/medications restrictions described in this protocol have been chosen to optimize quantitative assessment of myocardial perfusion and are in accordance with current clinical guidance and routine practice (35).

AIM 2: Determine whether SGLT2i treatments improve patient quality of life. We hypothesize that SGLT2i therapy will improve the quality of life by reducing symptoms and improving functional status. We will measure the quality of life in women provided SGLT2i vs. control group (placebo treatment) by using the following questionnaires: EQ-5D-3L, Duke Activity Status Inventory, Modified Morisky Medicine Scale, Seattle Angina Questionnaires, GAD 7, and PHQ8 metrics at baseline, 6 weeks, and at 12 weeks post-treatment.

Study Design: Biomedical

1. Will controls be used?YES

2. What is the study design?

Prospective, randomized, double-blind.

3. Does the study involve a placebo?

Answer/Response: Yes

► IF YES, provide a justification for the use of a placebo

Answer/Response: Dapagliflozin has not been proven safe or effective in the population in which the trial is planned, and thus the use of placebo does not constitute treatment withheld. Methodologically, the use of placebo enhances the quality of the information to be gained by conducting the trial. Thus we feel that the use of placebo in this study is justified.

Human Participants

Ages: ≥18 years

Sex: F
Race: Any

Subjects- see below

> **INSTRUCTIONS:** For question 1-4 below insert an exact #. Ranges or OPEN is not allowed. This # should be the maximum # you expect to need to enroll (i.e. sign consent) If you are only collecting specimens the number of participants should equate to the # of specimens you need. If you are collecting only data from a chart review the number should designate the number of subjects whose medical records you plan to review. Age/ Sex/Race criteria should designate the demographics of participants from whom you will obtain the specimen/data.

1. Provide target # of subjects (at all sites) needed to complete protocol. 40

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites. 10-25%

3. How many subjects will be enrolled at all sites?

50 to account for screen failure and withdrawal rates.

4. How many subjects will sign a consent form under this UVA protocol?

50

Inclusion/Exclusion Criteria

1. List the criteria for inclusion

- Female sex
- Age≥18 years
- Willing to provide written informed consent.
- Signs and symptoms of suspected ischemia that prompted referral for further evaluation by cardiac catheterization or CCTA within two years of consent.
- No evidence of obstructive epicardial CAD (stenosis >50%) of a major epicardial vessel (>3mm) or an FFR>0.80 by invasive catheterization or CCTA. (Participants who have not undergone cardiac catheterization or CT angiogram within the last 2 years for their clinical care will be screened with CT angiogram of the coronary arteries to confirm eligibility).
- eGFR≥30ml/min/1.73m2 at enrollment
- For subjects having a history of Type 2 DM: approval of diabetes care/prescribing provider

2. List the criteria for exclusion

- History of non-ischemic cardiomyopathy LVEF <40% or hypertrophic cardiomyopathy.
- History of congestive heart failure, severe pulmonary disease, liver disease
- History of Acute coronary syndrome (ACS) within previous 30 days
- Stroke within the last 180 days or intracranial hemorrhage at any time.

- Severe Valvular disease
- Life expectancy <3 years, due to non-cardiovascular comorbidity.
- Pregnancy or women who are breast-feeding
- Type 1 diabetes mellitus
- History of diabetic ketoacidosis in subjects with Type 2 Diabetes Mellitus
- Symptomatic hypotension or systolic BP<95 mmHg on 2 consecutive measurements
- Active malignancy requiring treatment at the time of visit
- Severe (eGFR <30 mL/min/1.73 m2 by CKD-EPI), unstable, or rapidly progressing renal disease at the time of randomization
- History of recurrent UTI/bladder/kidney infections
- Asthma with ongoing wheezing
- Known or suspected bronchoconstrictive or bronchospastic lung disease (ARDS, emphysema)
- Greater than first degree heart block
- Implanted cardiac device
- Profound sinus bradycardia (heart rates<40 beats per minute)
- Atrial fibrillation or supraventricular arrhythmias at time of imaging
- Known intolerance of nitrates (other than hypotension)
- History of reaction to iodinated contrast agents

3. List any restrictions on use of other drugs or treatments.
No drug restrictions apart from holding cardiac medications prior to the cardiac imaging described in this protocol.

Statistical Considerations

1. Is stratification/randomization involved?

Yes (randomization). The trial will be performed as a double-blind design with respect to placebo and dapagliflozin. Subjects will be randomized to the trial treatment in a 1:1 ratio. The randomization will not use other stratified factors

► IF YES, who will generate the randomization scheme?

__X___ UVA Statistician.:Wendy Novicoff, PhD

2. What are the statistical considerations for the protocol?
For the primary aim, the outcome measure is change of myocardial perfusion reserve (MPR) in response to 12 weeks of treatment assessed by stress CMR. The effect of SGLT2i will be assessed by comparing mean changes of CMR at the end of 12 weeks from the baseline between the SGLT2i and placebo groups using a two-way repeated-measures ANOVA. As a secondary aim, we will examine whether changes in myocardial perfusion correlate with improvement in quality of life measurements. In the analysis of AIM 2, we will examine changes over time from baseline and identify the major determinants of those changes using regression analysis.

3. Provide a justification for the sample size used in this protocol.
Because there is no preliminary data in the literature, our study is a pilot study to obtain preliminary data. The formal sample size calculation is not performed. The study will include 40 subjects at risk for Microvascular disease based on recruitment feasibility. We believe this number will be adequate to obtain reliable preliminary data. Detailed inclusion criteria are listed previously.

4. What is your plan for primary variable analysis?
Myocardial microvascular perfusion, defined as myocardial perfusion reserve (MPR) as assessed by stress CMR, will be compared before and after study intervention in each group (treatment and control). Comparisons will be done using a two-way repeated-measures ANOVA within each individual group using data accrued at the beginning and end of the 12-week intervention interval (placebo vs. SGLT2i) with each individual serving as her own baseline versus the end of 12-week intervention.
In the analysis of AIM 2, we will examine changes over time from baseline and identify the major determinants of those changes using regression analysis

5. What is your plan for secondary variable analysis?
We will explore whether improvement in myocardial perfusion correlate with improvement in patient-reported quality of life surveys (PROs). We will examine changes over time from baseline and identify the major determinants of those changes using regression analysis.

6. Have you been working with a statistician in designing this protocol? Yes
IF YES, what is their name? Wendy Novicoff, PhD

7. Will data from multiple sites be combined during analysis? No

Study Procedures-Biomedical Research

1. What will be done in this protocol?

Overview of Study: All participants will undergo eligibility screening, informed consent, randomization procedures by the study coordinator. Randomized participants will follow up at 6 and 12 weeks as described below (see **Table 1** for complete assessment schedule). At every study visit, information about all health care utilization (doctor visits, ER, hospital, testing), as well as medical status, medication, and clinically performed blood pressure, heart rate, hemoglobin A1C, blood lipid profile and urine analysis will be collected at baseline and in the 6 week and 12 week visits. Stress CMR studies will be done at baseline and after 12 weeks of completed therapy with SGLT2i or placebo. The baseline CMR study should be performed before starting the study drug. The window to perform follow-up stress CMR after 12 weeks of treatment is completed is +/- 15 days.

Once eligibility has been confirmed, subjects will be randomized to receive either Dapagliflozin (10mg, oral, once daily) or placebo for a total of 12 weeks. The dapagliflozin will be encapsulated to look identical to placebo to facilitate blinding. Capsules will be packaged by the UVA Investigational Drug Services and labeled according to institutional guidelines for investigational product. Subjects will receive enough study drug to complete 12 weeks plus/minus 15 days of treatment to facilitate scheduling of endpoint stress CMR completion. After the stress CMR is completed the study drug be will discontinued.

Study Assessments/Procedures

Screening/Baseline visit: The subject will fast overnight (starting at midnight) on the first visit, and abstain from medications as detailed in the CTA and MRI procedures below. The consent form will be signed prior to any study procedures. Vital signs, height, and weight will be obtained. Pulse oximetry will measure the percentage of oxygen in the blood. Blood will be drawn forlaboratory testing as described in Table 1. History and brief physical examination will be obtained. Subjects in whom obstructive epcardial coronary artery disease has not been excluded by clinical testing within the 2 years prior to screening will undergo coronary CTA. Once cleared for participation, subjects will be randomized assigned to one of two groups: Placebo or dapagliflozin 10mg for 12 weeks. Baseline cardiac MRI will be done at the end of the visit. To facilitate scheduling, the screening procedures may be completed within 14 days prior to Baseline assessments.

Table 1. Schedule of Study Procedures

Study Procedures	**Visit 1 (Screening and Baseline)**	**Visit 2 (Week 6)**	**Visit 3 (Week 12)**
	(-14 days to Day 1)	(6 +/- 1 weeks) (42±7 days)	(12 +/- 2 weeks) (84±15 days)
Informed Consent	X		
Review study eligibility	X		
Randomization	X		
Complete blood count, Lipid Panel, HbA1c	X		X
Basic metabolic panel	X	X	X
Pregnancy test (if applicable)	X	X	X
Urinalysis	X	X	X
blood collection for Inflammatory biomarkers	X	X	X
C-Reactive Protein (CRP)	X		X

Cardiac biomarkers (Troponin-I, NT-pro-BNP)	X		X
Optional specimen banking	X		
Medical History/health status	X	X	X
Vitals signs	X	X	X
Physical exam	X	X	X
Study Medication Dispensed	X	X	X
Study Medication Collected		X	X
NYHA and CCS class	X	X	X
Patient Reported Outcome Questionnaires	X	X	X
Concomitant Medication	X	X	X
Cardiac MRI w/Adenosine Stress (w/12-lead ECG)	X		X
Coronary CTA (if not done within 2 years)	X		
Assessment of Adverse Events		X	X
Volume of blood drawn (mL)	127	110	117

Coronary CTA Procedure:

Study participants in whom obstructive CAD has not been assessed (with either invasive cardiac catheterization or cCTA) within the 2 years prior to study participation will undergo a research coronary CTA. All studies will be obtained at the UVA Department of Radiology in the main hospital. These studies will be read by a radiologist and a report placed in the subject's medical chart.

Coronary CTA may be performed on the same day or a different day than the stress cardiac MRI. Study subjects will be instructed to adhere to the following restrictions prior to coronary CTA:

- Phosphodiesterase inhibitors stopped 48 hours prior
- NPO for 4 hours prior to procedure

Abstain from dietary sources of methylated xanthines for 24 hours prior to the study procedures:

- Coffee (even decaffeinated)
- Tea (even decaffeinated)
- Colas (even decaffeinated)
- Chocolate

Vital signs will be obtained pre-procedure. Creatinine will be collected if not available within 30 days; urine pregnancy test for WOCBP. An 18G IV will be placed in the arm. ECG monitoring leads will be placed on chest and remain for duration of study. Subjects with a heart rate

greater than 60 BPM will receive metoprolol for heart rate control. Metoprolol may be administered orally (at 25-50mg PO) or intravenously (at 5mg q5min for a maximum of 3 doses). All subjects will receive 0.4 mg of sublingual nitroglycerin prior to CTA for coronary vasodilatation. For CTA acquisition 50-80 mL of iohexol (Omnipaque 350 mgI/mL, GE Healthcare) will be injected at a flow rate of 5 mL/s, followed by 60 mL saline chasing.

Imaging protocol with 192-slice Siemens SOMATOM Force scanner:

- AP Topogram covering the chest
- Ca score
- Bolus tracking
- Coronary CT angiogram with an appropriate protocol based on heart rate and regularity (FLASH/prospective/retrospective)

Stress Cardiac MRI Procedure:

Study subjects will be instructed to adhere to the following restrictions prior to stress cardiac MRI:

1. Fast from food and nicotine for 4 hours prior to the scheduled MRI

2. Abstain from dietary sources of methylated xanthines for 24 hours prior to the study procedures:
    - Coffee (even decaffeinated)
    - Tea (even decaffeinated)
    - Colas (even decaffeinated)
    - Chocolate

3. Hold the following medications for 48 hours:
    - Beta blockers
    - Alpha blockers
    - Calcium-channel blockers (CCB)
    - Angiotensin-converting ezyme (ACE) inhibitors
    - Angiotensin receptor antagonists (ARAs), and
    - Diuretics

MRI Safety Screening:

All participants will complete a standard MRI safety screening questionnaire as administered by the Department of Radiology. An MRI technologist will review the participant's responses. If indicated, screening orbit films will be performed to ensure the absence of metallic foreign bodies in the eye area. Women of child-bearing potential must have a negative urine pregnancy test the day of the MRI.

12-lead ECG

Prior to cardiac MRI, a 12 lead ECG performed within 30 days will be reviewed by the PI or sub-investigator. This assessment will be reviewed in subjects' chart or performed at both baseline and 12 week visit. Subjects with contraindication to adenosine noted on this assessment will be discontinued from study participation.

Cardiac MRI Procedure

- After peripheral intravenous access is obtained (2 sites) and ECG leads for gating and monitoring are placed, the patient will be placed in the center of the magnet with a phased array surface coil overlying the chest.
- Scout imaging will be performed followed by images of heart morphology, function, tissue characterization, and blood flow.
- Vasodilator stress perfusion imaging will be performed with adenosine or regadenoson as the pharmacological agent. Heart rate and blood pressure will be monitored continuously.
    - Adenosine: 140 mcg/kg/min will be infused for 3-4 minutes. Should the patient experience any significant problems during the infusion of adenosine, the adenosine will be immediately discontinued, and due to the short half-life, the effects of adenosine resolve within 30 seconds. During the last minute of the adenosine infusion a bolus of 0.075 mmol/kg of macrocyclic Gadolinium-based contrast agent will be injected at 4-5 ml/sec with a power injector while images of myocardial perfusion are obtained.
    - Regadenoson: 0.4 mg in 5 mL will be administered over ~10 seconds, followed immediately by a 5 mL saline flush. Stress images will be obtained after a bolus of 0.075 mmol/kg of Gadoteric acid is administered as above. Vasodilator stress will be reversed after images have been collected by up to 100 mg of aminophylline administered by slow IV injection over 60 seconds.
- Rest perfusion imaging:When adosine is the vasodilator agent, a second set of perfusion images will be obtained 10 minutes post-adenosine stress, to allow for adequate contrast agent washout. When regadenoson is used as the vasodilator, resting images will be collected first.
- A second injection of 0.1 mmol/kg of macrocyclic Gadolinium-based contrast agent will be injected as described above.
- Additional CMR imaging of the heart will be used to evaluate for myocardial scarring, tissue characterization, and infarction.
- Additional images of the heart without contrast may be obtained. The total study procedure should take <90 minutes.

Follow-up visits: Subjects will return at 6 weeks and 12 weeks of treatment. They will have blood drawn for blood chemistry and complete the quality of life questionnaires. WOCBP will undergo urine or serum pregnancy testing. At the end of 12 weeks, a final visit will be

scheduled, screening labs will be repeated, and history and physical will be made. Cardiac MRI will be done at the end of the visit.

Inflammatory Biomarkers:
100mL (6.5 T) of blood will be collected at the Baseline and Week 12 visits for inflammatory biomarker analysis. Specifically, methylation of cell-free DNA will be analysed, and plasma will undergo quantification of 276 cytokines using the Olink Target 96 Cardiovascular Panels I, II, and III.

Optional Specimen Banking:
For Subjects who consent to the optional specimen banking sub-study, an additional sample will be collected and banked for unspecified future analysis. Banked samples will be labelled with a code containing no HIPAA identifiers; the link to subjects will be retained by Dr. Rodriguez. The samples will be analyzed and stored under the leadership of Jonathan Lindner. The specimen bank will be stored in a locked room and generator backed-up -80 freezer. The laboratory is registered with the Institutional Biosafety Committee under IB#4383-16.

A sub-aliquot of blood collected for the Specimen Banking will be sent to the Eniola-Adefeso lab at the University of Michigan. The impact of SGLT2i treatment on neutrophils and NETosis in study participants will be assayed by exposing neutrophils isolated from treated patients' blood to LPS and PMA and evaluating the extent of NETing relative to non-treated patient cells and healthy donor cells using assays in the Eniola-Adefeso lab. Secondary assays will evaluate the expression level of inflammation markers on cells harvested from patient blood.

The specimen banking sub-study is not funded by iTHRIV.

Procedures for Discontinuation of Study Intervention

A study subject's participation will be stopped in the following circumstances:

- Contraindication to further dosing with IP, in the opinion of the Investigator, such as adverse event or other safety reasons.
- Severe/continued non-compliance with the study protocol.
- Diabetic ketoacidosis (DKA). Consider temporarily interrupting IP if DKA is suspected. If DKA is confirmed, Investigational product should be discontinued permanently.
- Positive pregnancy test.
- Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment.

2. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.**

   Study participants will remain on medical management as overseen by their clinician during their participation in the study. Once their 12-week treatment period is complete, they will be discontinued from study drug.

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.**
   Study procedures will be administered by study staff at subject visits. Participant knowledge of and motivation to adhere to medication regimen will be assessed with the Modified Morisky Scale at baseline, 6 and 12 week visits. Participants will bring their study drug containers with them to study visits for IP dose reconciliation.

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**
   Participants whose capsule counts suggest >20% missed doses will be deemed non-compliant with the protocol.

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