Official title: Inflammation and coronary endothelial function in patients with coronary artery disease

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1. Abstract

a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

Advances directed at treating systemic risk factors for coronary atherosclerotic disease (CAD), such as hyperlipidemia, hypertension, and tobacco abuse reduce cardiovascular events and are associated with a 50% reduction in cardiovascular mortality over the last 25 years. Despite such remarkable success, cardiovascular disease remains the number one cause of death in the world(1), afflicts close to a million Americans annually, and incurs estimated annual costs of over $300 billion in the US(2). It has been postulated that CAD patients remain at increased risk of cardiovascular events in spite of aggressive current risk factor modification, possibly because conventional treatments do not adequately address some of the inflammatory pathways implicated in the disease.

Coronary atherosclerosis is an inflammatory disease (3). Endothelial cell injury occurs at the earliest stages with expression of endothelial surface molecules (e.g. vascular cell adhesion molecule-1, (VCAM-1)), that attract leukocytes, mostly monocytes, which in turn invade the vessel wall and contribute to the development of fatty streaks (3, 4). In fact, inflammatory cells, cytokines and mediators are involved in all stages of CAD (3, 5). Despite the fact that coronary atherosclerosis was recognized as an inflammatory process over two decades ago, this important concept is still not applied in the management of patients with, or at risk for, the disease.

Inflammation undoubtedly enhances the development and progression of coronary atherosclerosis via several mechanisms, but endothelial dysfunction is believed to be one common result of these mechanisms. Endothelial release of nitric oxide (NO) is a defining characteristic of non-diseased vascular tissue; it inhibits platelet aggregation, attenuates inflammation, decreases cellular proliferation, and induces local vascular smooth muscle vasodilation (6). Most classic and novel cardiovascular risk factors converge to impair endothelial function, including dyslipidemia, insulin resistance, inflammation, tobacco abuse, oxidative stress, as well as hemodynamic and genetic factors (6, 7). Thus, abnormal coronary endothelial function (CEF) represents both a cause and consequence of atherosclerosis. Critically, endothelial dysfunction is a marker for sub-clinical disease, an independent predictor of adverse cardiovascular events, and a potential target for medical interventions (8-15). We recently developed the first noninvasive measures of coronary endothelial function (CEF). These use magnetic resonance imaging (MRI) and isometric handgrip exercise to measure CEF in people noninvasively.

The hypothesis being tested in this application is that anti-inflammatory approaches, namely very low dose methotrexate (VLDM), low dose colchicine (LDC) and/or their combination, improve impaired local CEF in CAD patients with elevated markers of inflammation.

The proposed trial will offer critical new information on the impact of two clinically available anti-inflammatory medications, and their combination, for the first time, on local coronary artery biology in CAD patients. The impact of anti-inflammatory therapy on serum inflammatory markers in stable CAD patients and their relationship to coronary endothelial function, the early assessment (8 weeks) of the effect and of the combination (VLDM+LDC) are additional novel aspects not being studied in ongoing outcomes trials with VLDM or LDC. Importantly, these mechanistic findings using clinically available therapies can guide the design of the next generation of clinical outcome trials and be relatively rapidly translated to practice.

2. Objectives (include all primary and secondary objectives)

To determine whether anti-inflammatory strategies improve coronary endothelial function and/or peripheral vascular endothelial function at 8 and 24 weeks in patients with coronary artery disease, as compared to those receiving placebo.

Primary Endpoint: Coronary segment endothelial function at 8 weeks; specifically, change in coronary cross sectional area (CSA) from rest to that during isometric handgrip exercise (IHE) (as % rest and as mm^2).

Secondary Endpoints:

1. Change in coronary artery CSA from rest to IHE stress (as mm^2 and as % rest) at 24 weeks
2. Change in coronary blood velocity (CBV) from rest to IHE stress (as cm/s and as % rest) at 8 and 24 weeks
3. Change in coronary blood flow (CBF) from rest to IHE stress (as cm/s and as % rest) at 8 and 24 weeks
4. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CSA
5. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CBF
6. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CBV.
7. Serum hs-CRP at 8 and 24 weeks and change in hs-CRP between baseline and 8 and 24 weeks.
8. Serum IL-6 at 8 and 24 weeks and change in IL-6 between baseline and 8 and 24 weeks.
9. Serum TNFα at 8 and 24 and change in TNFα between baseline and 8 and 24 weeks.
10. Brachial flow mediated dilation (FMD) at 8 and 24 weeks and change in brachial FMD between baseline and 8 and 24 weeks.
11. The relationship between change in inflammatory markers (hsCRP, IL-6, TNFα, and others) between baseline and 8 and 24 weeks and change in CEF (IHE-induced change in CSA, CBV, CBF) between baseline and 8 and 24 weeks.
12. Safety endpoints (withdrawal due to side-effects, complete metabolic panel, including liver function tests, and complete blood count.)

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Despite advances with contemporary preventive and treatment strategies, coronary atherosclerosis is prevalent and its manifestations have a high personal and societal toll. There is renewed interest in the role of inflammation in coronary atherosclerosis, inflammatory biomarkers and inflammation as a treatment target. Coronary atherosclerosis is an inflammatory disease (3). Endothelial cell injury occurs at the earliest stages with expression of endothelial surface molecules (e.g. vascular cell adhesion molecule-1, (VCAM-1)), that attract leukocytes, mostly monocytes, which in turn invade the vessel wall and contribute to the development of fatty streaks (3, 4). Thus inflammation and endothelial cell injury/dysfunction occur during the initiation of atherosclerosis and the release of cytokines, like interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) formed in endothelial cells, smooth muscle cells or adipocytes, contribute to the inflammatory cascade. In fact, inflammatory cells, cytokines and mediators are involved in all stages of CAD (3, 5).

Despite the fact that coronary atherosclerosis was recognized as an inflammatory process over two decades ago, this important concept is still not applied in the management of patients with, or at risk for, the disease. Two important reasons to date are the lack of an established and easily obtained measure of the effect of inflammation on the processes which result in coronary atherosclerosis and that no clinical trial has established whether an anti-inflammatory strategy, per se, alters these processes. hsCRP and interleukins are markers, though not necessarily mediators,

of inflammation and although individuals with inflammatory autoimmune diseases receiving anti-inflammatory therapies (like colchicine or methotrexate) experience fewer cardiovascular events (16, 17), the impact of anti-inflammatory therapies on the processes which result in coronary atherosclerosis in more general CAD populations are potentially very important, but not yet defined.

Coronary endothelial dysfunction is a critical step in coronary atherosclerosis, an independent predictor of events, and as such, offers an important window into the impact of inflammation on coronary artery biology. Inflammation undoubtedly enhances the development and progression of coronary atherosclerosis via several mechanisms, but endothelial dysfunction is believed to be one common result of these mechanisms (6, 7). Thus, abnormal coronary endothelial function (CEF) represents both a cause and consequence of atherosclerosis. Critically, endothelial dysfunction is a marker for sub-clinical disease, an independent predictor of adverse cardiovascular events, and a potential target for medical interventions (6, 12, 13).

Endothelial-dependent function is assessed by the direction and magnitude of changes in arterial area and flow in response to endothelial-dependent vasomotor interventions (8). The invasive nature of coronary angiography with Doppler flow measures used in traditional measures of CEF limited clinical and research investigations, particularly those benefiting from repeated studies and/or studies in clinically stable participants. We developed a novel, reproducible, validated approach using 3T magnetic resonance imaging (MRI) to assess endothelial-dependent coronary vasomotor function noninvasively which we will use in this trial (18, 19).

There is renewed interest in anti-inflammatory strategies because patients with elevated inflammatory biomarkers (e.g. hsCRP) are at increased risk of primary and secondary cardiovascular events (20-23) and because statins have anti-inflammatory properties (24) (25) (26) and lower cardiovascular mortality (21, 27-29). Nevertheless cardiovascular event rates remain high in statin treated CAD patients (25, 30) and statins alone do not fully suppress inflammation in many patients (25, 31). In addition, the prevalence of subclinical vascular inflammation, as determined by high levels of circulating inflammatory biomarkers, including IL-6(32)(33) is also high in patients with type 2 diabetes and patients with the metabolic syndrome. Furthermore, some have hypothesized that alterations in innate immunity underlie insulin resistance and diabetes (34).

What is needed are well-tolerated anti-inflammatory agents that do not have profound effects on lipids to answer the question of whether inhibiting inflammation per se improves coronary endothelial dysfunction, a driver of atherosclerosis. *Thus it is not known which anti-inflammatory agents are effective in improving CEF (if any), which systemic inflammatory biomarkers best predict the local coronary response (if any), and whether the local CEF response is heterogeneous and related to the extent of underlying atherosclerosis in a given CAD patient.*

Low dose colchicine, methotrexate and their combination represent three appealing choices to suppress inflammation in CAD patients. These agents have been used in clinical practice for decades to treat inflammatory diseases and, in those populations, have reduced inflammatory biomarkers and been associated with fewer cardiac events, albeit with limited data.

Colchicine and cardiac disease: Colchicine is an anti-inflammatory agent used for over 20 years to treat gout, recurrent pericarditis, and post-pericardiotomy syndrome. Low dose colchicine (LDC) (1-2mg/day) is used chronically, sometimes life-long, to treat Familial Mediterranean Fever (FMF), where it is well tolerated (35-37). It is also used to treat Bechet's disease (38). Colchicine is believed to act through inhibition of microtubule assembly in immune cells, including neutrophils and monocytes, which leads to reduced cytokine production, modulation of chemokine and prostanoid production and inhibition and down-regulation of lymphocyte and endothelial cell adhesion molecules surface expression(39, 40). Colchicine is concentrated in leukocytes because of a prolonged half-life in those cells (~60 hours) as compared to plasma (~20 min) resulting in peak concentrations 10x higher than those in plasma(41-43).

LDC has been evaluated in a number of inflammatory heart diseases(44). Following cardiac

surgery, the incidence of atrial fibrillation was reduced in patients randomized to LDC (12.0% versus 22.0%, *P*<0.021; relative risk reduction, 45%; number needed to treat, 11) with shorter in-hospital and rehabilitation stays as compared to those receiving placebo(43). The rates of withdrawal and side effects were similar in the placebo and LDC groups. In another trial, LDC (0.5mg twice daily) reduced recurrent atrial fibrillation by about 60% at 3 months in patients undergoing ablation for paroxysmal atrial fibrillation as compared to placebo (odds ratio 0.38, p=0.01; number needed to treat 5.6)(45). Notably, *the inflammatory biomarkers, hsCRP and IL-6, were reduced by 35% and 19%, respectively (both p<0.01) after only 3 days of LDC in these cardiac patients*. In a recent randomized placebo-controlled acute pericarditis trial, LDC reduced symptoms, hospitalizations and 3 month relapse rates, yet with adverse event and discontinuation rates similar to those taking placebo(46).

Several observational studies in the last 2 years suggest a positive effect of LDC on cardiovascular disease. Gout patients taking colchicine had a lower incidence of MI (1.2% vs 2.6%, p<0.03) and lower CRP levels versus those who did not take colchicine, despite similar baseline risk factors(47). In stable CAD patients with hsCRP>2mg L^{-1}, LDC (0.5mg twice daily) reduced hsCRP levels in four weeks by about 60%(48). Importantly, last year the Low Dose Colchicine for Secondary Prevention of Coronary Artery Disease (LoDoCo) was reported(49). 532 stable CAD patients on aggressive background cardiovascular therapy were randomized to placebo or colchicine (0.5mg/day) in addition to usual care and followed for a median of 3 years. LDC significantly reduced the primary composite endpoint of acute coronary syndrome, out-of-hospital cardiac arrest, and stroke by 67% (hazard ratio: 0.33; 95% confidence interval [CI] 0.18 to 0.59; p<0.001; number needed to treat: 11). The LDC benefit was observed early, continued to accrue over time, and was largely driven by a reduction in coronary events(49). 11% of colchicine patients withdrew during the first 30 days and another 11% over the ensuing 3 years, mostly due to the well-known gastrointestinal side effects. An accompanying editorial commented that the 67% reduction in coronary events is about twice that seen with statins(5). Taken together, these studies demonstrate that LDC reduces inflammatory biomarkers rapidly in patients with cardiac disease (possibly as early as 3 days), is safely tolerated in the vast majority of CAD patients for several years, and appears to reduce cardiac events (pending confirmation) by an unidentified mechanism. <u>This trial proposes to mechanistically probe the effect of anti-inflammatory agents on coronary and systemic endothelial function in CAD patients and determine which biomarker, if any, best predicts the vascular response.</u>

Methotrexate and cardiac disease: Methotrexate is an anti-inflammatory agent that has been used in low doses (15-20mg/week) for over 20 years to treat rheumatoid arthritis, psoriasis, and psoriatic arthritis. In addition, well-established guidelines are available regarding very low dose methotrexate (VLDM) monitoring and safety precautions (50). Methotrexate has minimal interaction with most concomitant medications, including statins, aspirin, beta-blockers, and inhibitors of the renin-angiotensin system that are commonly used in CAD patients. The use of VLDM in clinical practice further reduces the potential for unanticipated off-target toxicity (51). VLDM reduces hsCRP and IL-6 with little effect on blood pressure or lipids in patients with rheumatoid arthritis (51). In several observational and prospective studies in patients with rheumatoid arthritis, VLDM use was associated with lower rates of myocardial infarction and death than in those not receiving VLDM, in spite of the fact that VLDM subjects had worse CAD risk profiles (52, 53). In such studies VLDM was associated with 20%-80% reductions in cardiovascular events(51). At low doses, with a favorable safety profile, methotrexate reduces hsCRP rapidly (<1 week)(54) with clinical effects on rheumatologic disease activity in as little as 4 weeks(55). Therefore, VLDM is an anti-inflammatory agent with considerable appeal for evaluating the role of inflammation, per se, in CAD. Unfortunately, there are almost no reports of the effect of VLDM on inflammatory biomarkers in CAD patients and none on CEF. Of note, the NIH-sponsored trial, Cardiovascular Inflammation Reduction Trial (CIRT), is an <u>ongoing</u> randomized, double-blind, placebo-controlled trial of VLDM

(15-20 mg per week) over the course of 4 years in the secondary prevention of myocardial infarction, stroke, and cardiovascular death among 7000 patients with known prior cardiovascular disease and an elevated CRP (>2 mg L^{-1}) despite usual therapy (56). All study participants additionally receive folic acid (1 mg daily). It is important to again note that it is not known whether or not VLDM reduces low level inflammation in CAD patients and/or whether it impacts the mechanisms affecting coronary atherosclerosis, like CEF, in CAD patients. This trial will use the same VLDM dosing scheme as the CIRT trial.

Combination of colchicine and methotrexate to reduce inflammation

The combination of low dose colchicine and methotrexate has not been studied in CAD patients. Because multiple innate and acquired inflammatory responses contribute to atherosclerosis, it seems likely that one anti-inflammatory agent alone may not adequately suppress all of the inflammatory processes adversely affecting atherosclerotic coronary arteries. Because colchicine and methotrexate do not operate through the same mechanism and because both are associated with reduced cardiac events in observational studies, there is considerable appeal to evaluating their combined effects on biomarkers and CEF.

Chronic LDC and VLDM treatments are sometimes used together in patients with primary biliary cirrhosis (PBC). In a study of PBC patients who failed to respond to conventional therapy, colchicine (0.6mg BID) and methotrexate (0.25mg/kg lean body weight/week) were added and patients were followed for improvement in liver function tests and liver biopsy as well as for safety over a median 3.4 years. Overall there was improvement in alkaline phosphatase and AST results and reduced hepatic fibrosis at biopsy with LDC+VLDM with approximately 80% of the 93 patients responding to therapy(57). The authors reported four cases of methotrexate toxicity (4% of population) with 1 case of interstitial fibrosis (reversed with VLDM withdrawal) and 3 cases of oral apthous ulcers (that responded to treatment with leucovorin). Note that these rates were over 3.4 years and so we can expect lower rates over 24 weeks. Thus these two drugs that are commonly used separately in practice for decades have also been used in combination and found to chronically reduce inflammatory complications of PBC while being reasonably well tolerated(57, 58). Thus we propose below to also study the effects of the combination of LDC and VLDM on inflammatory biomarkers, systemic and CEF over 8 and 24 weeks in stable CAD patients. This will provide the first biomarker and coronary biology data on a combination of anti-inflammatory agents as well as safety data in CAD patients who will undergo frequent clinical and biochemical surveillance for side effects.

4. Study Procedures

a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

The following are research procedures:

Randomized, double-blind, 2x2 factorial design trial of (1.) LDC, (2.) VLDM (3.) LDC+VLDM and (4.) placebo to determine their effects on coronary endothelial function (CEF), peripheral vascular endothelial function (brachial FMD) and inflammatory biomarkers in 170 patients with stable CAD.

More specifically, stable CAD patients on conventional therapy (statin, ACE/ARB, BB, ASA) will undergo baseline assessment of inflammatory markers, lipids, MRI quantification of CEF and brachial ultrasound measurement of FMD. Those with hsCRP >2mg L^{-1} or those with metabolic syndrome or diabetes will undergo screening MRI and those with abnormal CEF (change in coronary CSA during IHE ≤0% of the resting value) will be randomized, to 24 weeks of either:

1) methotrexate (15mg wk-1) +placebo for colchicine (daily) + folate (1mg daily);
2) colchicine (0.6mg daily) + placebo for methotrexate (weekly) + folate (1mg daily);
3) methotrexate (15mg wk-1) + colchicine (0.6mg daily) + folate (1mg daily); or

4) placebo for methotrexate (weekly) + placebo for colchicine (daily) + folate (1mg daily).

Please note that we will now recruit CAD patients with a clinical diagnosis of diabetes or with metabolic syndrome. Metabolic syndrome will be defined, as three or more of the following:
1. Abdominal obesity (waist circumference: Men>102 cm (>40 in), Women >88 cm (>35 in)),
2. Serum triglycerides ≥150 mg/dL (or taking medication to treat high triglycerides),
3. HDL cholesterol: Men<40 mg/dL, Women<50 mg/dL (or taking medication to treat low HDL cholesterol),
4. High Blood pressure: ≥130/≥85 mm Hg (or taking medication to treat high blood pressure), and
5. Fasting glucose: ≥100 mg/dL (or taking medication to treat high fasting glucose).
The rationale and these criteria are based on the NIH-sponsored CIRT trial where diabetes/metabolic syndrome are used as entry criteria for that trial studying whether the anti-inflammatory methotrexate reduces cardiovascular events (56).

Note also that to assess for tolerance, during week 1, the methotrexate dose will be 5mg weekly; during weeks 2-3, the methotrexate dose will be 10mg weekly; and thereafter the methotrexate dose will be 15 mg weekly. Study staff will make telephone contact with the participant at the end of weeks 1 and 3 to assess tolerance to methotrexate. Subjects will have blood samples obtained at week 2 and the dose would not be increased further for 4000>WBC≥3000, 75,000>platelets≥50,000, 40>GFR≥30, or LFT>2x upper limit of normal. Note that for those taking placebo instead of methotrexate, their dosages will also be incremented on the same initial schedule.

The Johns Hopkins Investigational Drug Service will conduct the randomization procedure, prepare medications (colchicine, methotrexate and respective placebos) in similar appearing formulations, and maintain group assignment documentation, keeping investigators blinded.

Initial evaluation: A careful history including whether angina is present and its severity by the Canadian Cardiovascular Society Angina Classification will be obtained, and a physical examination performed in a private examination room. Blood samples will be acquired for complete blood cell count, routine chemistry panel including hepatic transaminases and serum creatinine, LDL and HDL cholesterol and triglycerides, as well as serum hs-CRP, and certain biomarkers (IL-6, IL-1β, TNFα, IFN-γ, ICAM-1, sEselectin, sPselectin, Thrombo, modulin, vWF, PAI, endothel in-1, and adiponectin). Patients will also be tested for hepatitis B and C.

Systemic Endothelial Function: Brachial Flow Mediated Dilatation (FMD) and Velocity: These studies will be conducted at the JHU ICTR on participants in the fasting state (>10 hours) asked to refrain from drinking alcohol or beverages containing caffeine in the prior 24 h using a standardized protocol (59, 60). Supine participants will have the brachial artery visualized with a high-resolution ultrasound probe proximal to the antecubital fossa. After baseline images and flow measurements, a pressure cuff on the upper arm will be inflated to 200–250 mmHg for 5 min. Blood flow will be measured during the 15 s following cuff release and vessel diameter between 60 and 90 s after cuff deflation. Flow-mediated dilation (FMD) and velocity time interval (VTI) will be calculated (59, 60). Images will be coded and analyzed in blinded fashion.

MRI methods for Coronary Vasoreactivity: an index of CEF: Participants will undergo a detailed baseline MRI study of CEF in the fasting state using MRI methodology at rest and during continuous IHE as previously described (18). The MRI will be used to measure cross-sectional area (CSA), coronary flow velocity (CFV), and coronary blood flow (CBF) changes in response to IHE stress (continuous isometric handgrip for ~5 min at 30% of each subject's maximum, determined prior to entering the MRI), as previously reported (18, 61, 62). Patients who qualify,

based on the baseline MRI and other entry criteria, will be randomized and undergo repeat MRI at 8 and 24 weeks.
We plan to image segments of two coronary arteries in the same subject during IHE to obtain multiple CEF measures, ideally of segments with different amounts of atherosclerosis, as measured by coronary wall thickness (CWT). Coronary black blood imaging will also be performed in the same coronary slices for vessel wall measurements: CWT, vessel wall area and normalized wall index (NWI), an index of arterial remodeling calculated as: vessel wall area divided by the total vessel area. In the event of technical or other difficulties in obtaining adequate images, the MRI may be repeated with the participant's permission.

Fasting: Subjects will be asked to fast after midnight and delay eating breakfast and taking morning cardiovascular medicines until after the study procedure, which will be completed by noon. In insulin-dependent diabetics, we will ask the subjects to delay their morning insulin dose until after the study procedure and will complete their studies by 10am. In addition, we will have a snack available and access to a finger-stick glucometer for any insulin-dependent diabetics who become symptomatic.

MRI analysis: Coronary MR images will be analyzed in blinded fashion without operator knowledge of time or treatment group for CEF (eg change in CBF, CBV, and CSA) as previously validated and described (18). For determining the effects of anti-inflammatory interventions, as described below, in participants in whom adequate image quality permits the acquisition of CEF in multiple coronary segments, the results will be averaged for all segments meeting entry criteria (e.g. CSA change during IHE ≤0% of resting values) for a given participant so that the results for participants with multiple acquisitions are not more heavily weighted than those with fewer acquisitions. If the participant withdraws from the study before week 24, we will request participant permission to perform an MRI and FMD at termination of study.

Blood Draw and Biomarker Analysis: Blood samples will be obtained from a peripheral vein using standard venipuncture techniques. Blood specimens will be collected in collection tubes without anticoagulant and centrifuged 30 min after collection (time allowed for clotting) using a centrifuge with an integrated refrigeration system (at 4°C/1000 *g* for 15 min) and kept at -80°C (63). Analysis will be performed with ELISA or multiplexed ELISA for the biomarkers of inflammation, activation, and clotting listed above.

Safety surveillance: Participants will undergo surveillance safety monitoring every 4 weeks as recommended in the American College of Rheumatology Guidelines and will include complete blood counts, liver function tests, and serum BUN and creatinine levels. Dr. Lisa Christopher Stein, a Rheumatologist with considerable experience with methotrexate and colchicine and an NIH funded clinical investigator, will serve as the Chair of the DSMB. She and Dr. Weiss will review laboratory results from a safety standpoint. She will not be involved in other primary- or secondary data analysis or interpretation. Participants will also return 4 weeks after the last dose of study drug (i.e. 28 weeks after randomization and initial dosing) for a safety evaluation.

If any of the following criteria are met (CBC<3000, platelets<50,000, HCt<27%, GFR <30ml/min or a >50% reduction in GFR compared to baseline values, and LFTs >3x upper limit of normal), the test would be repeated. If confirmed on repeat, there would be a temporary stop of study drugs. If the abnormality resolves, study drugs would be resumed at the same or lower dose. If the abnormality does not resolve or the temporary stop occurs on three consecutive measures, then the case will be reviewed for discontinuation from the study.

Follow up End-point Evaluation: After 8 and 24 weeks of study-drug administration, participants will undergo repeat clinical evaluation, lipid and inflammatory biomarker analysis, as well as brachial ultrasound FMD and MRI for CEF with the same protocols used at baseline, prior to study drug administration. In particular, coronary MRI will be repeated with an identical protocol with special attention taken to interrogate the same coronary segments in follow-up as those studied at baseline, using anatomic landmarks of coronary ostia and branch vessels- as we have done in the past(19) and to replicate the identical MRI IHE protocol. Study drug compliance will be assessed by questionnaire and pill count at the 8, 16 and 24 week follow-up visits.

b. Study duration and number of study visits required of research participants.

Each participant will be followed for a 7-8 month period with the possibility of 8 – 9 study visits.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

Participants will be randomly assigned to one of four study groups (placebo, LDC, VLDM, LDC+VLDM) after providing informed consent and meeting all entry criteria. To keep the investigators blinded, the Johns Hopkins research pharmacy will conduct the randomization procedure, prepare medications (colchicine, methotrexate and respective placebos) in similar appearing formulations, and maintain group assignment documentation. The Johns Hopkins Investigational Drug Service has many decades of experience in this process including randomization for study group assignment, blinding of medications, and maintenance of appropriate records for group assignment. Blinding is important to avoid potential bias of participants and/or investigators.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

All participants will receive routine care and will not have current therapy stopped because of participating in the study.

e. Justification for inclusion of a placebo or non-treatment group.

The placebo group is included as a separate control group and will be used as a comparator group. Anti-inflammatory strategies like colchicine and methotrexate are not a standard part of contemporary therapy for patients with coronary artery disease thus there is no ethical problem with withholding them in a placebo group. Because it is not known whether these anti-inflammatory agents improve coronary endothelial function and/or limit atherosclerosis in CAD patients it is critical to have a placebo group to determine the efficacy of the anti-inflammatory drugs and to evaluate their safety.

f. Definition of treatment failure or participant removal criteria.

Participants who experience significant symptoms will be evaluated and those or others who develop laboratory abnormalities will have the study drug withheld. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance) or the participant withdrawn from the study. Participants who wish to end participation in the trial at any time may do so upon request without penalty.

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants withdrawn from receiving additional study drug due to an adverse experience will be followed by the Investigator until the outcome is determined. Every effort will be made to follow the subject for the full study period as per the schedule of study visits.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

A. Participants of either gender who are ≥21 years of age (no upper age limit),
B. History of prior MI, coronary revascularization, or coronary angiography or MDCT demonstrating at least one coronary artery with ≥50% luminal stenosis and no plans for revascularization,
C. Clinically stable for 3 months,
D. Vascular inflammation based on elevated hsCRP (>2mg L^{-1}), or a clinical diagnosis of diabetes mellitus or metabolic syndrome. Metabolic syndrome is defined by three or more of the following:
1. Abdominal obesity (waist circumference: Men>102 cm (>40 in), Women >88 cm (>35 in)),
2. Serum triglycerides ≥150 mg/dL (or taking medication to treat high triglycerides),
3. HDL cholesterol: Men<40 mg/dL, Women<50 mg/dL (or taking medication to treat low HDL cholesterol),
4. High blood pressure: ≥130/≥85 mm Hg (or taking medication to treat high blood pressure), or
5. Fasting glucose: ≥100 mg/dL (or taking medication to treat high fasting glucose).

E. Abnormal CEF (change in CSA during IHE of ≤0% of the resting value: by this we mean any decrease in CSA or no change (0%) from baseline during IHE),
F. Permission of patient's clinical attending physician,
G. Patients being treated with a statin.

Exclusion criteria:

A. Patients unable to understand the risks, benefits, and alternatives of participation and give meaningful consent,
B. Patients with contraindications to MRI such as implanted metallic objects (pre-existing cardiac pacemakers, cerebral clips) or indwelling metallic projectiles,
C. Acute coronary syndrome within the prior three months,
D. Pregnant women,
E. Contraindications to methotrexate or colchicine as outlined by the American College of Rheumatology; including active bacterial infection, tuberculosis, or herpes zoster infection, leukopenia (<4000/mm^3), thrombocytopenia (<135,000/mm^3), elevation in hepatic transaminases (>2x upper limit of normal), hepatitis B or C, moderate renal disease (estimated creatine clearance <45ml/min), or planned surgery,
F. Chronic inflammatory condition such as lupus or rheumatoid arthritis, ulcerative colitis or Crohn's disease,
G. Interstitial lung disease or pulmonary fibrosis,
H. HIV positive,
I. Requirement for, or intolerance to, methotrexate or colchicine ,
J. Intolerance to methotrexate, colchicine or folate,
K. History of non-basal cell malignancy or treatment for lymphoproliferative disease in the past 5 years,
L. Requirement for use of drugs that alter folate metabolism,
M. History of alcohol abuse or unwillingness to limit consumption to < 4 drinks per week,
N. Women of childbearing potential or intention to breastfeed.
O. Men who plan to father children during the study period; men who have sexual intercourse with women of childbearing potential must agree to use a condom,
P. Chronic use of oral or IV steroid therapy or other immunosuppressive or biologic response modifiers,
Q. History of chronic pericardial effusion, pleural effusion or ascites,

R. New York Heart Association Class IV heart failure.

6. Drugs/ Substances/ Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

Some of the background for choosing these drugs is articulated above on pages 3-5. Briefly, the chosen low doses of colchicine and methotrexate are based on those used in clinical practice and recent studies showing that those doses reduce inflammation and improve endothelial function in patients with rheumatoid arthritis and other conditions. Colchicine is an anti-inflammatory agent used for over 20 years to treat gout, recurrent pericarditis, and post-pericardiotomy syndrome. Low dose colchicine (LDC) (1-2mg/day) is used chronically, sometimes life-long, to treat Familial Mediterranean Fever (FMF), where it is well tolerated (35-37). It is also used to treat Bechet's disease(38). We have chosen the colchicine dose of 0.6mg QD not only because it is the dose used in practice, but also because it is similar to the dose (0.5mg QD) that reduced inflammatory biomarkers and the incidence of atrial fibrillation in patients with heart disease (43) (45) and reduced recurrent cardiac events in CAD patients in the Low Dose Colchicine for Secondary Prevention of Coronary Artery Disease (LoDoCo) trial(49). In these studies the drug was reasonably well tolerated. We have been told by the Investigational Drug Service that the 0.5mg colchicine dose is not available in the US, only the 0.6mg dose that we will use here is available. Methotrexate is an anti-inflammatory agent that has been used in low doses (15-20mg/day) for over 20 years to treat rheumatoid arthritis, psoriasis, and psoriatic arthritis. In addition, well-established guidelines are available regarding very low dose methotrexate (VLDM) monitoring and safety precautions(50). Methotrexate has minimal interaction with most concomitant medications, including statins, aspirin, beta-blockers, and inhibitors of the renin-angiotensin system that are commonly used in CAD patients. In several observational and prospective studies in patients with rheumatoid arthritis, VLDM use was associated with lower rates of myocardial infarction and death than in those not receiving VLDM, in spite of the fact that VLDM participants had worse CAD risk profiles(52, 53). The proposed dose of methotrexate (15mg per week), and the ramped dosing to enhance tolerance (5mg per week for first week, 10mg per week for weeks 2-3, and then 15mg per week for weeks 4-24) are consistent with clinical use and precisely in line with those of the ongoing NIH-sponsored Cardiovascular Inflammation Reduction Trial (CIRT) in 7000 individuals (56).

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

These drugs (low dose colchicine and methotrexate) are FDA approved medications that are, however, not approved for this indication. We received an exemption from the FDA that an IND is not needed. Safety information on the use of these drugs is detailed on pages 3-5 above.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered. **NA**

7. Study Statistics

Primary Endpoint: Coronary segment endothelial function at 8 weeks; specifically, change in coronary cross sectional area (CSA) from rest to that during isometric handgrip exercise (IHE) (as % rest and as mm^2).

Secondary Endpoints:

1. Change in coronary artery CSA from rest to IHE stress (as mm^2 and as % rest) at 24 weeks
2. Change in coronary blood velocity (CBV) from rest to IHE stress (as cm/s and as % rest) at 8 and 24 weeks

3. Change in coronary blood flow (CBF) from rest to IHE stress (as cm/s and as % rest) at 8 and 24 weeks
4. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CSA
5. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CBF
6. Difference between baseline and 8 and 24 weeks in IHE-induced changes in coronary CBV.
7. Serum hs-CRP at 8 and 24 weeks and change in hs-CRP between baseline and 8 and 24 weeks.
8. Serum IL-6 at 8 and 24 weeks and change in IL-6 between baseline and 8 and 24 weeks.
9. Serum TNFα at 8 and 24 and change in TNFα between baseline and 8 and 24 weeks.
10. Brachial flow mediated dilation (FMD) at 8 and 24 weeks and change in brachial FMD between baseline and 8 and 24 weeks.
11. The relationship between change in inflammatory markers (hsCRP, IL-6, TNFα, and others) between baseline and 8 and 24 weeks and change in CEF (IHE-induced change in CSA, CBV, CBF) between baseline and 8 and 24 weeks.
12. Safety endpoints (withdrawal due to side-effects, complete metabolic panel, including liver function tests, and complete blood count.)

Additional variables for evaluation: local coronary *plaque burden (CWT, NWI, and percent luminal stenosis) and LGE (measured as CNR) at site of CEF measurement.*

a. Statistical plan including sample size justification and interim data analysis.

Power/sample size justification: To answer the question of whether any of the anti-inflammatory strategies (i.e. LDC, VLDM, and/or their combination) improves CEF in stable CAD patients with increased inflammation and abnormal CEF as compared to that of patients receiving placebo, this 2x2 factorial trial was designed with the primary endpoint of change in [coronary cross-sectional area (CSA) from rest to that during IHE at 8 weeks. We chose this parameter because it reflects macrovascular coronary changes related to the endothelial-dependent stressor and because this parameter has been shown to be reproducible over at least 8 weeks. CSA increases during IHE by 18%±13% (mean±SD) in healthy participants and declines by -6%±11% (mean±SD) in patients with coronary atherosclerosis (18). We believe that a mean IHE-induced increase in CSA to +6% with IHE in the LDC or VLDM groups would be physiologically significant. As the normal mean CSA response with IHE in healthy individuals is +18% and that in those with coronary atherosclerosis is -6%, then a mean change from -6% in placebo to +6% in the LDC or VLDM groups represents an improvement of one-half of the difference between CAD and normal (i.e. 12 is one-half of the difference between normal and CAD or -6 to +18= 24 units). Such a difference is in line with methotrexate-induced changes in FMD in RA patients (64, 65). We will also measure CBF and CFV which have microvascular components and an endothelial dependent component (66).

Therefore, we assume that at 8 weeks:
(1) Mean CSA will decrease by 6% from rest to IHE in CAD patients on placebo (**Δ =** -6%)
(2) Mean CSA will increase by +6% from rest to IHE in CAD patients on LDC (**Δ =** +6%)
(3) Mean CSA will increase by +6%from rest to IHE in CAD patients on VLDM (**Δ =** +6%)
(4) Mean CSA will increase by +12% from rest to IHE in CAD patients on LDC+VLDM (**Δ =** +12%)
(5) The pooled standard deviation for all four groups will be 11%.

With a sample of 88 (22 in each cell), the power will be 0.83 (alpha=0.05, two-sided test) to detect a difference between the response in the placebo group and the response in each of the three groups receiving the anti-inflammation intervention (LDC, VLDM, LDC+VLDM) (17). We believe this is a conservative estimate as it does not assume that a medication will improve CEF to normal values but instead will increase it by about 50% of the difference between healthy and CAD, a physiologically relevant difference in line with changes observed in other settings (64, 65,

67). We will assume a 10%-15% dropout rate over 8 weeks due to withdrawal and/or side effects from LDC, VLDM, LDC+VLDM (68), and unwillingness to repeat MRI. Thus we plan to randomize 25 in each group for a total of 100 CAD patients. Based on our preliminary studies in statin-treated CAD patients, we expect that ~20% of CAD patients undergoing the initial screening MRI will not meet the CEF inclusion criterion of an IHE-induced change in CSA of ≤0% of baseline. Thus we anticipate enrolling 170 stable CAD patients on statins with hsCRP>2mg L^{-1}, in order for 100-104 to meet entry criteria for randomization and to ultimately have 22 in each of the 4 groups complete the eight week follow-up MRI examinations. We plan to follow patients and to re-evaluate CEF and systemic endothelial function at 24 weeks (and should have 20 in each group then based on a 10% dropout in the LoDoCo trial), giving us a second, longer-term time point, as suggested in initial review. This 24 week time will provide longer-term safety data for LDC+VLDM and be in line with some prior studies of endothelial function and inflammatory markers (69-71).

Statistical Analysis:

Demographic and baseline characteristics (e.g. age, race, gender, height, weight, etc) will be summarized using descriptive statistics for all participants. Unless otherwise specified, descriptive statistics on continuous variables will consist of the number of participants, mean, standard deviation, median, minimum and maximum.

The primary analysis will use an intent-to-treat approach. To answer the question of whether CEF is different at 8 weeks with anti-inflammatory interventions as compared to placebo, comparisons for the primary outcome variable, change in CSA from rest to IHE stress at 8 weeks, will be made (placebo vs LDC, placebo vs VLDM, placebo vs LDC+VLDM) using ANOVA. Comparisons will also be made among those groups for the change between baseline and 8 weeks in IHE CSA using a generalized estimating equation (GEE) analysis to examine whether CEF changes between baseline and 8 weeks are associated with treatment type (group assignment). Three dummy variables will be created as the independent variables of interest: LDC vs. otherwise; VLDM vs. otherwise and LDC+VLDM vs. otherwise. The analysis will be repeated for 24 week data with correction for multiple comparisons. To answer the questions of whether changes between baseline and 8 and 24 weeks in other indices of CEF (such as CBV or CBF) or in inflammatory biomarkers (hsCRP, IL-6, TNFα, etc.) differ among the groups (placebo vs LDC, placebo vs VLDM, placebo vs LDC+VLDM) a GEE approach will be used. The p-value, least square (LS) treatment means, difference between the LS treatment means, and 95% confidence intervals for the treatment differences will be determined. A similar approach will be used to compare brachial ultrasound FMD among groups. Multiple regression analysis will be used to examine whether there is a relationship between inflammatory biomarkers and CEF at 8 and 24 weeks, as well as between changes in inflammatory biomarkers and changes in CEF between baseline and 8 and 24 weeks. To determine whether there is a relationship between coronary atherosclerosis (CWT) and CEF at baseline and at 8 and 24 weeks we will use multiple regression analysis combining all 4 groups and also for the placebo and the LDC, VLDM, LDC+VLDM groups separately. To determine whether there is a difference among groups in the proportion of participants who experience abnormal laboratory values or withdrawal from the study a Chi-square analysis will be performed. As an exploratory analysis, to determine whether there are differences in CEF or inflammatory biomarkers among the 3 treatment groups, the endpoints at 8 weeks will be compared using ANOVA and the changes from baseline to 8 and 24 weeks will be compared with GEE.

b. Early stopping rules.

Participants who experience significant symptoms will be evaluated and those, or others who develop laboratory abnormalities will have the study drug withheld. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance)

or the patient withdrawn from the study. Participants who wish to end participation in the trial at any time may do so upon request without penalty.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

The MRI procedures do not expose patients to ionizing radiation and are not considered a significant risk(72). No long term side effects have been noted at these magnetic field strengths. The potential risks of the MRI study are few and primarily relate to injury from metallic objects attracted to the MR scanner or the isometric handgrip exercise (see below). A blood pressure cuff is inflated and maintained for a few minutes during brachial endothelial function testing and this is associated with transient discomfort but no known major risks or long term problems. The likelihood of MRI-related risks is minimized by thoroughly screening participants for metal prior to entering the scanner, and limiting access to the scanner area. A minority of individuals develop a claustrophobic sensation inside the magnet, which ceases when the subject exits the scanner.

Isometric handgrip exercise performed in the MRI scanner results in modest increases blood pressure and heart rate and can result in paradoxical vasoconstriction in atherosclerotic coronary vessels, and is relatively safe (73). The cited side effect in patients with coronary disease of handgrip exercise is non-sustained ventricular ectopy, which occurred in one percent of patients and which spontaneously resolved after termination of the exercise (74). The IRB-approved consent form states that myocardial infarction and cardiac arrhythmias may occur, and although the risks can be serious, the likelihood of developing them is very low (74). Voice contact between subject and MR system operator will be available at all times, during both coronary endothelial function studies (isometric handgrip exercise) and brachial endothelial function studies, as well as direct observation via a video camera in the scan room. In addition, a cardiologist or cardiology fellow will be present during the isometric handgrip exercise. Isometric handgrip exercise is terminated prematurely if participants develop symptoms that would typically stop their activities or if abnormal heart rate or blood pressure findings develop. We have performed isometric hand-grip exercise testing in conjunction with cardiac spectroscopy studies since 1988 (75) and have not had any significant complications.

Low dose colchicine is fairly well tolerated but has a relatively narrow therapeutic window. There were no serious adverse events reported in the LoDoCo trial (using a similar colchicine dose 0.5 mg QD to the dose proposed here 0.6mg QD) over three years although 11% of individuals within 30 days and another 11% over the next 3 years withdrew from the study. The most common reasons for withdrawal were unrelated intercurrent illnesses (3.9%), patient choice (1.8%), intestinal upset (2.5%), myalgia (0.9%), myositis (<0.5%), rash (<0.5%), alopecia (<0.5%), itch (<0.5%) and peripheral neuritis (<0.5%)(49). In a study of cardiac patients with atrial fibrillation, no serious adverse events were noted but diarrhea was reported in 8.6% (vs 1.3% on placebo) and nausea in 4.9% (vs 3.8% in placebo) and one case of elevated LFTs which reversed after stopping drug(67). In a study of very low dose methotrexate over 2 years in elderly patients with rheumatoid arthritis there were no serious adverse events(76). At high doses methotrexate has been associated with bone marrow suppression and stomatitis but these are less problematic at lower doses and are attenuated with the concomitant administration of folic acid (which is proposed here at 1mg QD). Use of chronic low dose methotrexate in rheumatoid arthritis has been reported to be well tolerated in many reports but also associated with gastrointestinal toxicity, stomatitis, alopecia, marrow suppression, and liver function abnormalities(77). Toxicity was closely associated with impaired renal function (an exclusion criterion in our study) and not with age(78). Interstitial pulmonary inflammation and fibrosis occur in as many as 1% of the patients studied and necessitates termination of the drug but national guidelines by the American Rheumatologic society do not recommend screening chest x-rays. The combination of low dose colchicine and

very low dose methotrexate has been used in practice to treat primary biliary cirrhosis (PBC). In a study of 93 PBC patients followed for 3.4 years, there were four cases of significant methotrexate side effects (4% of population) with 1 case of interstitial fibrosis (reversed with VLDM withdrawal) and 3 cases of oral aphthous ulcers (that responded to treatment with leucovorin)(57). Because patients with PBC and autoimmune inflammatory diseases are probably more likely to develop side effects related to these conditions and their treatments, than are stable CAD patients, we do not anticipate that the side-effect profile would be more unfavorable in our stable CAD study patient population.

b. Steps taken to minimize the risks.

MRI will be performed with exposure to static and time-dependent magnetic fields within FDA guidelines(72). Special precautions will be taken to exclude patients with implanted metallic objects, including pacemakers, cerebral clips, or prior occupational exposure to small metallic projectiles (eg, lathe operators). All participants will be carefully screened by the investigators for the exclusions stated above. A cardiologist or cardiology fellow will be present for isometric handgrip exercise. Heart rate will be monitored continuously during MR studies via EKG, and blood pressure monitored with a remotely activated sphygmomanometer during isometric handgrip stress. Participants will be in direct verbal contact with the MR system operator at all times. Participants who experience a worsening condition or who wish to end the exam at any time may do so upon request without penalty. The scanner area is equipped with standard medical emergency equipment (including a crash cart) for a hospital-based clinical MR center with appropriately trained personnel in attendance.

To protect against and minimize the potential risks of very low dose methotrexate and low dose colchicine, patients with pre-existing liver or renal disease, blood dyscrasia, hepatitis B or C, and other conditions that might increase risk will be excluded from participation (please see Exclusion criteria above). In addition, participants will undergo a careful history and physical (in a private examination room) and screening laboratory examination at baseline to identify the presence of any condition which might increase their risk from the medications but not have been known to the patients or documented in their medical records. In addition, patients will be evaluated closely at 4 week intervals while on study drug for side-effects and surveillance metabolic and hematologic screening studies. The participants will also return 4 weeks after completing the study (28 weeks after randomization and initial dosing) for a follow-up safety surveillance. That evaluation will not affect the study drug dosing of that participant but if safety concerns are identified, it could be used to minimize risk in future participants. Additionally, methotrexate is a folate inhibitor and so to reduce the risk of side effects folate 1mg will be administered daily to all participants. This is commonly done in practice and does not reduce the clinical efficacy of methotrexate. As mentioned earlier, the dose of methotrexate will also be escalated during the first few weeks (5mg weekly for week 1, 10mg weekly for weeks 2-3, 15mg weekly thereafter) to improve tolerance and observe for side effects during initial dosing. This dosing schedule is identical that used in the current CIRT trial (56).

Women of childbearing potential will be excluded and men who have sexual intercourse with women of childbearing potential must agree to use a condom, as the study drug may cause damage to sperm.

c. Plan for reporting unanticipated problems or study deviations.

All unanticipated problems or study deviations will be reported to the Data Safety and Monitoring Committee and according to The Johns Hopkins Medicine Institutional Review Boards published guidelines.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

Once the data are acquired, they will be assigned a code for each subject with identification secured and accessible only to the PI, senior investigators, and the research nurse (Mrs. Steinberg). Patient clinical information such as copies of clinical studies and imaging reports shall be filed in a locked filing cabinet with access under control of Dr. Weiss and Mrs. Steinberg. Patient information summarized and/or converted into electronic form (tables, images, etc) shall be identified by the assigned code. It is a Johns Hopkins Institutional policy that all identifiers in images and data acquired under IRB-approved research protocols must be removed if they leave the institution, for example in presentations.

e. Financial risks to the participants.

All study related costs will be paid from research sources, as stated in the consent form.

9. Benefits

a. Description of the probable benefits for the participant and for society.

Results from this study may benefit patients with coronary heart disease in the future by providing information on the role of anti-inflammatory medications and inflammatory biomarkers in coronary atherosclerosis of CAD patients. This would support the use of anti-inflammatory medications like these to complement existing cardiovascular prevention strategies. However, we do not currently know whether participants will have an immediate benefit. It could be that these agents (methotrexate and/or colchicine) reduce inflammation and improve coronary endothelial function and this would, at least in the short term, benefit the patients receiving those drugs. If the patients' physicians felt the participants benefited, they could make the decision, apart from this study, to continue the medications since they are clinically available. In addition, low dose methotrexate and low dose colchicine are associated with reduced heart disease risk in patients with arthritis. Thus in the CAD patients that we are studying here, the benefit in terms of reduced risk of heart disease may be even greater.

Contraindications to MRI studies as listed above will be strictly observed and should not constitute added risks for patients enrolled in this study. The research primarily involves non-invasive imaging and thus involves minimal risk, therefore the low risk is reasonable in relation to the potential knowledge gained that may guide future therapeutic studies.

10. Payment and Remuneration

a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Participants will receive parking, a meal voucher and compensation of $75 for each completed MRI for a possible total compensation of $225.

11. Costs

a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

There is no cost to the participants related to the study procedures or drugs.

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