Coronary Flow Reserve to Assess Cardiovascular Inflammation (CIRT-CFR)

A multi-center, double-blind, placebo-controlled, randomized ancillary imaging trial of the Cardiovascular Inflammation Reduction Trial

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Investigators:

Viviany R. Taqueti, MD, MPH, Brendan M. Everett, MD, MPH, Scott D. Solomon, MD, Paul M. Ridker, MD, MPH, Rob S. Beanlands, MD, Fadi Hage, MD, Marcelo F. Di Carli, MD

Institution:

Brigham and Women's Hospital (BWH)
Boston, MA

Sponsor:

National Heart, Lung and Blood Institute,
National Institutes of Health,
Bethesda, MD

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I. BACKGROUND AND SIGNIFICANCE

Diabetes mellitus (DM) and prediabetic states are enormous public health burdens in the US and worldwide. Recent statistics approximate that 25.8 million children and adults in the US—8.3% of the population have DM[1]. Alarmingly, an additional 79 million people are estimated to have prediabetes, and this is likely to increase with the rising prevalence of obesity in the population[1]. Meta-analyses[2,3] of large prospective studies suggest that, after accounting for other risk factors, DM more than doubles the risk of coronary artery disease (CAD) and ischemic stroke, and that this DM-related risk is higher in women than in men. In contrast with the strong associations observed between type 2 DM and related cardiometabolic disorders and cardiovascular outcomes, a recent meta-analysis of 102 prospective studies found a very modest association of fasting glucose level with CAD and stroke risk[3]. Moreover, large intervention trials[4-7] have shown no beneficial effect of intensive glucose control on primary cardiovascular endpoints. While glucose level itself may play some role in modulating cardiovascular risk, the mechanisms mediating increased risk of cardiovascular mortality and morbidity in diabetics are not well understood. Although a greater extent of CAD[8], as well as abnormalities in cardiac structure and function[9] have been implicated in diabetics, these pathophysiologic features provide only a partial explanation to the excess risk of MI, heart failure and death among diabetics and patients with metabolic syndrome. Indeed, the extent of luminal angiographic CAD with or without left ventricular (LV) dysfunction does not fully account for the excess clinical risk among subjects with[10] or without[11] DM. In our work, the presence of impaired coronary flow reserve (CFR) in patients without overt obstructive CAD identifies patients at increased risk of adverse cardiac events, and diabetic patients without known CAD with impaired CFR show a risk of cardiac death comparable to, and possibly higher than, that for nondiabetic patients with known CAD[12]. This suggests that diffuse atherosclerosis and coronary microvascular dysfunction play a more important role than previously appreciated in the pathophysiologic abnormalities leading to increased risk.

Interestingly, this excess risk may be linked to an enhanced pro-inflammatory state, consistently demonstrated in patients with type 2 DM and metabolic syndrome[13], and following acute MI[14]. Exactly how the enhanced pro-inflammatory response in DM and metabolic syndrome is associated with increased risk of adverse outcomes is not well understood. There is ample laboratory evidence that inflammation plays a major role in all stages of atherothrombosis[15,16] including the early functional abnormalities in vascular endothelial and smooth muscle cell function. There is also growing *in vivo* human evidence that increased systemic inflammation is associated with coronary vascular dysfunction[17-22]. Moreover, there is preliminary evidence that anti-inflammatory therapies may lead to improved coronary vasoreactivity[23-25].

To date, however, no trial has yet proven that directly reducing inflammation lowers cardiovascular event rates, and the Cardiovascular Inflammation Reduction Trial (CIRT)[26] has been launched to address this issue. A possible mechanism for doing so may involve systemic improvement in global coronary vascular function. As such, CIRT provides a ***unique*** opportunity to assess whether CFR might provide a simple noninvasive method to predict which patients best respond to a systemic anti-inflammatory intervention. *By testing the fundamental concept of whether reducing systemic inflammation can lead to improved myocardial blood flow, tissue perfusion and LV function, CIRT-CFR, our proposed randomized placebo-controlled ancillary clinical trial, would provide important mechanistic insights of the capabilities of an anti-inflammatory therapy to improve key clinical determinants of clinical risk.* Equally important for

continued research development programs and eventual future clinical decision-making is the validation of a physiologic imaging biomarker that could also be used to evaluate safety and efficacy of novel therapeutic agents during drug development programs, and/or identify patients at particularly high risk in need of aggressive therapeutic intervention[27].

The Cardiovascular Inflammation Reduction Trial (CIRT) is an NHLBI funded multicenter clinical trial (U01 HL 101422) that will randomly allocate 7,000 patients with stable CAD and either type 2 DM or metabolic syndrome to low-dose methotrexate (LDM; target dose 15 to 20 mg per week) plus usual care, or placebo plus usual care over a follow-up period of 2 to 4 years (average 3 years)[26]. CIRT is an event-driven trial such that in the absence of extreme effects, the trial will conclude after accrual of at least 530 primary endpoints, estimated to provide 90% power to detect a 25% relative risk reduction. The CIRT primary endpoint is a composite of nonfatal myocardial infarction (MI), stroke, and cardiovascular death. Secondary endpoints include total mortality, coronary revascularization, unstable angina requiring unplanned revascularization, and hospitalization for heart failure, with additional pre-specified endpoints such as incident venous thrombosis, atrial fibrillation, peripheral artery disease, and severe aortic stenosis. In addition, effects on measures of quality of life will be assessed using the standardized SF-36 Health Survey. Participants will be recruited from roughly 400 clinical sites in the United States and Canada.

In addition to LDM or matching placebo, all study participants (including those on placebo) will receive folic acid 1.0 mg 6 days per week, a therapy known to reduce side effects that can be associated with LDM. To further ensure safety and improve long-term compliance, the trial design incorporates a 5- to 6-week open-label, active-therapy run-in (maximum 8 weeks) for all potentially eligible participants so that those unable to initially tolerate LDM are excluded prior to randomization. Additional details of the CIRT parent trial are included in the appended CIRT IRB application, approved on 6/13/2012 (Appendix).

The proposed ancillary imaging trial, CIRT-CFR, is a randomized placebo-controlled clinical trial that will test directly whether reducing systemic inflammation in stable CAD patients improves myocardial perfusion reserve, as measured by CFR, a novel quantitative imaging marker of clinical risk. Coronary flow reserve (CFR) refers to the ratio of global myocardial blood flow quantified at stress over that at rest. CFR provides a robust measure of the *integrated* hemodynamic effects of epicardial CAD, diffuse atherosclerosis, vessel remodeling, and microvascular dysfunction on myocardial perfusion. CFR quantified using vasodilator PET can identify patients at high risk for serious cardiac adverse events, including cardiac death, even in the absence of overt obstructive disease (i.e. independently of perfusion score)[28-31], and these associations are especially evident among high, but heterogeneous risk cohorts including patients with DM[12]. The demonstration of improved CFR and myocardial perfusion after low-dose methotrexate would provide further validation of this imaging biomarker as a potential indicator of the complex sequelae of active inflammation in the heart. Doing so may provide a direct, quantifiable target that could be useful in drug discovery programs, and perhaps for initiation and monitoring of therapeutic efficacy.

Specifically, this trial aims to investigate a mechanism by which LDM may modulate cardiovascular outcomes in a high-risk population enriched for chronic, low-level inflammation. Increasing data suggest that LDM, a generic therapy that is widely used among patients with rheumatoid arthritis and psoriasis, mediates its beneficial effects not by direct inhibition of cellular proliferation, but by promoting release of the robust anti-inflammatory autacoid, *adenosine*, at sites of inflammation[32]. Increased extracellular concentrations of adenosine modulate recruitment

and function of leukocytes, including neutrophils, macrophages and regulatory T lymphocytes, leading to local and systemic reduction of inflammatory cytokines such as IL-12, IL-6 and TNF, with far-reaching effects that include facilitated cholesterol efflux and reverse cholesterol transport from arterial wall foam cells[33]. Moreover, adenosine mediates these effects while acting as a potent vasodilator**.** As such, treatment with LDM is poised to affect coronary vasoreactivity in diabetics and prediabetics in complex ways that are ripe for study using PET measures of CFR.

Another fundamental physiologic principle of our study is that, by decreasing inflammation and enhancing myocardial tissue perfusion to the diabetic or prediabetic heart, we may improve cardiac mechanics and, in so doing, prevent or slow the progression to clinical heart failure and possibly death. Although some of these pathophysiologic mechanisms have been explored in experimental animal models[34], the gaps between these experiments in animals and applications in humans are just beginning to be addressed[35].

Thus, we believe that the use of physiologic imaging techniques, integrated into the continuum of clinical care to understand the mechanisms linking inflammation to cardiovascular complications, is not only innovative, but will also facilitate needed clinical translation.

II. SPECIFIC AIMS

Coronary flow reserve (CFR, calculated as the ratio of hyperemic over rest myocardial blood flow) is emerging as a powerful quantitative prognostic imaging marker of clinical cardiovascular risk. CFR provides a robust and reproducible clinical measure of the integrated hemodynamic effects of epicardial CAD, diffuse atherosclerosis, and microvascular dysfunction on myocardial tissue perfusion. Inflammation is a key mediator of this constellation of abnormalities, affecting the entire coronary vasculature, but no clinical trial to date has shown that directly reducing inflammation lowers cardiovascular event rates. As such, the recently launched Cardiovascular Inflammation Reduction Trial (CIRT) provides a unique opportunity for mechanistic investigation of the impact of anti-inflammatory therapy on changes in CFR as a reflection of coronary vascular dysfunction, which may precede clinical outcomes, particularly in patients at high-risk of events. We are ideally positioned to examine the impact of inflammation on CFR, having extensive experience in both the quantitation of CFR using clinically-integrated dynamic positron emission tomography (PET) and the ability to assess its association with cardiovascular outcomes.

The *central hypothesis* of this ancillary proposal, CIRT-CFR, is that reducing systemic inflammation using low-dose methotrexate (LDM) will, compared to placebo, quantitatively improve myocardial blood flow and coronary flow reserve as measured by PET over 1-12 months, in stable CAD patients with type 2 diabetes or metabolic syndrome enrolled in CIRT. In so doing, improvement in coronary vasoreactivity, endothelial function, and tissue perfusion may have beneficial effects on myocardial mechanics, left ventricular deformation and function and, ultimately, symptoms and prognosis.

AIM 1. To test the hypothesis that systemic inflammation is a critical determinant of coronary flow reserve and myocardial tissue perfusion, as assessed by quantitative PET imaging, in stable CAD patients with type 2 diabetes or metabolic syndrome. Specifically, we propose that treatment with LDM at a target dose of 15 to 20 mg orally weekly for 1-12 months will, compared to placebo, result in:

Hypothesis #1: Improved global coronary flow reserve in response to dipyridamole, reflecting primarily endothelium-independent vasodilation.
Hypothesis #2: Reduced total stress perfusion deficit, a semi-quantitative score that combines scar and ischemia.

AIM 2. To test the hypothesis that LDM at a target dose of 15 to 20 mg orally weekly for 1-12 months will, compared to placebo, result in improved left ventricular (LV) myocardial mechanics, as assessed by transthoracic echocardiography, and that this functional improvement will be correlated with the change in coronary flow reserve after 1-12 months of treatment. In this population of patients without severe heart failure, we propose to test the following hypotheses:
Hypothesis #3: LDM for 1-12 months will result in improved LV systolic function, as assessed by measures of LV peak global longitudinal strain, and diastolic function, as assessed by measures of tissue Doppler mitral annular early diastolic relaxation velocity (E').
Hypothesis #4: Changes in LV systolic and diastolic function after 1-12 months of LDM therapy will be correlated with changes in coronary flow reserve.

III. SUBJECT SELECTION

Inclusion and exclusion criteria were selected to reflect those of the parent CIRT study. Specifically:

Inclusion criteria:

a. Age ≥18 years at screening;
b. Documented past history of MI OR past evidence of multivessel CAD by angiography, completed any planned coronary revascularization associated with a qualifying event at least 60 days prior to enrollment, and clinically stable for ≥60 days prior to enrollment; qualifying prior MI must be documented either by hospital records, evidence on current ECG of Q waves in 2 contiguous leads, and/or an imaging test demonstrating wall motion abnormality or scar; qualifying evidence of multivessel CAD by angiography must be documented by CAD in at least two major epicardial vessels defined either as the presence of a stent, a coronary artery bypass graft, or an angiographic lesion of 60% or greater (left main CAD that has been revascularized with a stent or bypass graft will qualify as multivessel disease, as will the presence of a 50% or greater isolated left main stenosis);
c. History of type 2 DM or metabolic syndrome (meeting 2004 AHA/NHLBI definition[*]) at time of study enrollment; [*]includes any 3 of the following 5 diagnostic criteria: waist circumference ≥ 102 cm in men or 88 cm in women; triglycerides ≥ 150 mg/dl or on drug treatment for elevated triglycerides; high-density lipoprotein cholesterol (HDL-C)< 40 mg/dL in men or < 50 mg/dL in women or on drug treatment for reduced HDL-C; systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or on drug treatment for hypertension; and elevated fasting glucose ≥ 100 mg/dL or on drug treatment for elevated glucose.
d. Willingness to participate as evidenced by signing the CIRT and CIRT-CFR informed consent.

Exclusion criteria:

a. Prior history of chronic infectious disease, tuberculosis, or severe fungal disease; chronic hepatitis B or C infection; renal insufficiency; interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis; known chronic pericardial effusion, pleural effusion, or ascites; chronic liver disease; myeloproliferative disorders in the past 5 years; non-basal cell malignancy or treated lymphoproliferative disease within the past 5 years; known HIV positive; life expectancy of <3 years;
b. Chronic inflammatory condition such as lupus or rheumatoid arthritis, ulcerative colitis or Crohn's disease
c. White blood cell count <3,500/ul, hematocrit < 32 percent, or platelet count < 75,000/ul
d. Liver transaminase levels (AST or ALT) >upper limit of normal (ULN) or albumin < the lower limit of normal (LLN);
e. Creatinine clearance < 40 ml/min as estimated with the Cockroft-Gault equation;
f. History of alcohol abuse or unwillingness to limit alcohol consumption to less than 4 drinks per week
g. Women of child bearing potential, even if they are currently using contraception, and women intending to breastfeed.
h. Men who plan to father children during the study period or who are unwilling to use effective forms of contraception.
i. Requirement for use of drugs that alter folate metabolism (trimethoprim/sulfamethoxazol) or reduce tubular excretion (probenecid) or known allergies to antibiotics making avoidance of trimethoprim impossible;
j. Current indication for methotrexate therapy;
k. Chronic use of oral steroid therapy or other immunosuppressive or biologic response modifiers (see Exclusionary Medication List in Manual of Operations). Eligible study participants will be encouraged to have up to date pneumococcal and influenza vaccinations as recommended based on their age and underlying medical conditions.
l. Chest X-ray evidence in the past 12 months of interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis. For participants who do not have a chest X-ray in the prior 12 months, a chest X-ray will be obtained at baseline as part of the study protocol.
m. New York Heart Association Class IV congestive heart failure.

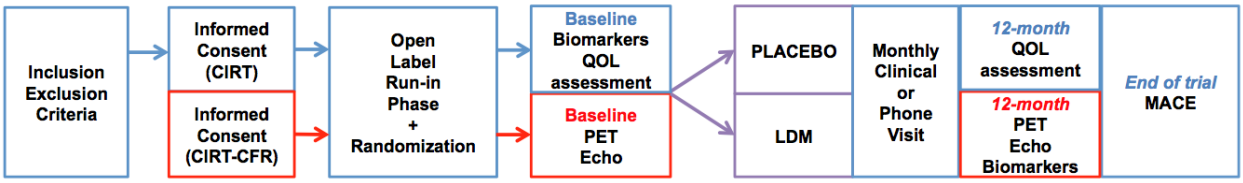
IV. SUBJECT ENROLLMENT

We propose to recruit 150 patients for CIRT-CFR. This will be a collaborative effort between 6 clinical sites, with 4 sites in the Boston area: (1) *Brigham and Women's Hospital, BWH* (PI: Samuel Goldhaber, MD), (2) *Joslin Diabetes Center* (PI: Allison Goldfine, MD), (3) *Massachusetts General Hospital* (PIs: Linda C. Hemphill, MD, and Laura Benzaquen, MD), (4) *North Shore Medical Center* (PI: Lawrence S. Block, MD), and two additional sites in the Ottawa area: (5) *Ottawa Heart Institute, OHI* (PI: Rob Beanlands, MD), and in Birmingham, Alabama: (6) University of Alabama, UAB (PI: Fadi Hage, MD). We will have 3 imaging sites (BWH, PI: Marcelo Di Carli, MD; OHI, PI: Robert Beanlands, MD; and UAB, PI: Fadi Hage, MD), with **BWH serving as the Core Imaging Lab**, and all 4 Boston area sites referring patients for imaging to BWH. The subjects of the trial will be patients with CAD and type 2 DM or metabolic syndrome enrolled in the parent study (CIRT) and recruited from participating

CIRT sites local to BWH, OHI, and UAB. All patients identified at the 6 participating clinical sites as being potentially eligible for CIRT are automatically eligible for participation in this ancillary study.

V. STUDY PROCEDURES

Randomization and double-blind study treatment period to either placebo or LDM (1:1) of willing and eligible patients will occur at the end of the open label run-in phase per the parent CIRT protocol, and will be stratified by time since the qualifying event (< 6 or ≥ 6 months from the date of MI or most recent angiogram), type of event (MI or multivessel CAD), presence of either type 2 DM or metabolic syndrome, and site, which will ensure balance in the proposed study. Patients willing to participate in CIRT will be asked to enroll into the sub-study and may sign the CIRT-CFR informed consent at any point between signing the parent CIRT informed consent and completing the parent CIRT randomization visit (Visit 4). After giving informed consent for the ancillary CIRT-CFR, patients will undergo the baseline PET scan along with echocardiography at any point between the parent CIRT post run-in visit (Visit 3) and up to 4 weeks after randomization (Visit 4). Per the parent CIRT protocol: i) blood will be collected and stored for biomarkers at baseline (Visit 3), ii) patients will complete quality of life (SF-36) questionnaires at enrollment (Visit 2) and 1-12 months (Visit 7) after randomization, and iii) patients will be called at 3, 6, and 10 months after randomization to assure compliance. Final PET scan and echocardiogram will occur at approximately 1-12 months after randomization or within 4 weeks (+/- 2 weeks) of Visit 7. At that time, blood will be collected and stored for biomarkers (using the same protocol as in the parent CIRT) to coincide with follow-up imaging in CIRT-CFR. The data collection schedule for CIRT and CIRT-CFR is summarized in the Appendix. Trial schema is as follows:



Imaging will be performed at the 3 imaging centers (BWH, OHI, and UAB). To minimize participant and site burden, only a baseline and single follow-up imaging time point will be pursued. The CIRT-CFR Screening Visit has been incorporated into the Consent Visit in an attempt to further reduce participant and site burden. Imaging tests (PET and echo) will be scheduled on the same day for patient convenience if possible, and no more than one week apart. "Baseline" study visit imaging will follow the open label run-in period of the parent trial to enhance long-term compliance and eliminate risk of radiation exposure for any individuals with immediate intolerance to the LDM study protocol. The imaging tests proposed are non-invasive, routinely performed, and historically well tolerated by patients. The radiation incurred through PET scanning is small (~2.8 mSv per study), and comparable to that incurred by an average American in a given year from background radiation. Clinical coordinators will be encouraged to enroll all CIRT-eligible patients at the 5 clinical sites participating in this ancillary study, so as to remove the possibility of selection bias. To minimize and understand potential selection bias, we will maintain an evaluation log of all patients who were screened and enrolled at the 6 sites.

Timing of baseline imaging assessments. Baseline PET scanning and echocardiography will follow CIRT's post run-in (open label, 6-8 week run-in phase) visit. The justification for this decision is based on the pharmacodynamics of LDM in rheumatoid arthritis, as well as additional biologic and trial logistic reasons. There are no data on the timing of changes in myocardial blood flow following LDM. Methotrexate is a prodrug that becomes active only when polyglutamated within cells, a slow iterative process that can take as long as ~27 weeks to reach steady state[36]. In patients with rheumatoid arthritis, a clinical response to methotrexate usually begins within the first 6–12 weeks after initiation of therapy, with a maximal response observed at 6 months[37,38]. This suggests that the longer-chain polyglutamates, which take longer to become detectable and reach steady state, have a more important role in the clinical response to methotrexate[36].

Timing of follow-up imaging assessments. Follow-up PET scanning and echocardiography will occur between 1 and 12 months post randomization.

Positron Emission Tomography (PET): Regional and global myocardial blood flow (MBF) will be assessed using PET imaging in accordance with the study-specific acquisition protocol. The investigative teams at BWH, OHI, and UAB have extensive experience in performing these procedures, and quantifying myocardial blood flow. PET scans will be performed using a whole body PET/CT scanner. Anti-hypertensives and beta-blockers will be withheld on the morning of the study. Patients will be allowed to continue using sublingual nitroglycerin as needed. Studies will be performed after 4 hours of fasting and 24 hours of abstinence from caffeine. The visit will take approximately 5 hours, including patient preparation. MBF will be measured at rest and during dipyridamole, adenosine, or regadenoson infusion, using ^{13}N-ammonia or ^{82}Rubidium, as the flow tracer. Importantly, the same radiopharmaceutical should always be used for the baseline and follow-up scans. After transmission imaging and beginning with the intravenous (IV) bolus administration of ^{13}N-ammonia (~10-20 mCi, adjusted based on whether 2D- or 3D-imaging is used) or ^{82}Rubidium (~10-20 mCi for 3D imaging), list mode images will be acquired for 20 or 7 minutes, respectively. Thirty (^{13}N-ammonia) or 10 (^{82}Rubidium) minutes later, patients will undergo a standard infusion of dipyridamole (0.56 mg/kg over 4 minutes), adenosine (0.14 mg/kg/min for 4 minutes), or regadenoson (0.4 mg bolus over 10 seconds). At peak hyperemia, a second dose of ^{13}N-ammonia (~10-20 mCi, adjusted based on whether 2D- or 3D-imaging is used) or ^{82}Rubidium (~10-20 mCi for 3D-, and 30-50 mCi for 2D imaging) will be injected IV, and images recorded in the same manner. The heart rate, blood pressure, and 12-lead ECG will be recorded at baseline and throughout the infusion of dipyridamole, and at recovery. All studies will be reviewed at the sites by site investigators for clinically important findings. All PET scans will be done for research (non-clinical) purposes only. For safety reasons, all studies will be reviewed at the sites by site investigators for clinically important findings. No reports or analyses will be provided to sites from the PET core laboratory and studies will not be assessed in real-time.

Two-dimensional echocardiography: Echocardiograms will be performed by site sonographers trained and certified by the echo core laboratory in the study-specific acquisition protocol[39]. The noninvasive core laboratory at BWH has extensive experience in quantitative echocardiography in the context of therapeutic clinical trials[40,41]. Dr. Solomon also directs the core laboratory in the analysis of echocardiographic images in the NIH-funded ARIC study[42]. Images will be acquired as recommended by the American Society of Echocardiography (ASE) and in a manner consistent

with standard practices for patient comfort and position. In addition, the echo protocol includes specific views to ensure optimal imaging for specific views to: (1) assess LV diastolic function, and (2) ensure optimal imaging for off-line LV deformation analysis with speckle-tracking software (i.e., images of the LV will be captured at high frame rates of 50-70 fps). The protocol will take approximately 35 minutes, including patient preparation. A superset of a standard echo exam will be performed, emphasizing endocardial border optimization and on-axis imaging. All studies will be acquired and stored in digital DICOM format, with CD/DVD copy for site backup. All studies will be reviewed at the sites by site investigators for clinically important findings. All echos will be done for research (non-clinical) purposes only. No reports or analyses will be provided to the sites from the echo core laboratory and studies will not be assessed in real-time.

VI. BIOSTATISTICAL ANALYSIS

CIRT-CFR sites participating in the proposed ancillary study. Based on our power analysis and sample size calculations, we estimate that each of the 6 clinical sites will be required to enroll approximately 25 patients over approximately 3 years. This is equivalent to 1 patient every 6 weeks per site, which we believe is reasonable and attainable. All sites have received IRB approval for the parent study and are actively enrolling patients in the parent CIRT trial. The proposed imaging studies (PET scans and echos) are routinely performed at the 3 imaging sites (BWH, OHI, and UAB). Drs. Di Carli (PI) and Solomon (both at BWH), Beanlands (OHI), and Hage (UAB) are recognized experts in quantitative PET imaging and echocardiography, have published widely on these topics, and have extensive experience in conducting clinical trials using imaging endpoints[40, 43, 44-48].

Aim 1. To test the hypothesis that systemic inflammation is a critical determinant of coronary flow reserve (CFR) and myocardial tissue perfusion, as assessed by quantitative PET imaging, in stable CAD patients with *type 2* ***DM or metabolic syndrome.*** Specifically, we propose that treatment with LDM at a target dose of 15 to 20 mg orally weekly for 1-12 months will, compared to placebo, result in:
Hypothesis #1: Improved global CFR in response to dipyridamole, reflecting primarily endotheliumindependent vasodilation.
Hypothesis #2: Reduced total stress perfusion deficit, a semi-quantitative score combining scar and ischemia.

Methods: PET scans will be performed at the 3 imaging sites at randomization and at 1-12-month follow-up using a standardized study protocol. As described in section V, we have previously demonstrated feasibility of measuring myocardial blood flow with high reproducibility[49,50]. In addition to CFR and coronary hemodynamics, we will also measure the total perfusion deficit (TPD) during stress and at rest, and calculate the difference TPD between stress and rest (reflecting total ischemic burden) using previously validated methods[51,52]. The primary endpoint for the efficacy portion of *Aim 1* is the change in global CFR response to dipyridamole, reflecting primarily endothelium-independent coronary vasodilatation, over 1-12 months. A key secondary endpoint is the change in dipyridamole-stimulated TPD_{stress}, reflecting the combined extent and severity of ischemia and scar, over 1-12 months. Tertiary endpoints include the change in hyperemic global and regional (i.e., non-infarcted myocardium)

myocardial blood flow (MBF), and the change in global and regional $TPD_{stress\text{-}rest}$ (reflecting the ischemia score) over 1-12 months.

Statistical analyses: For the analyses of both primary and secondary endpoints included in *Hypotheses 1 and 2*, we will use separate linear regression models with the respective change in global CFR (in response to dipyridamole) and TPD_{stress} over 1-12 months as the outcome variables. Each of these regression models will control for baseline variables of stratum (DM or metabolic syndrome), time since index MI (< 6 months or ≥6 months), imaging site (BWH or OHI), the corresponding baseline global CFR score, as well as treatment assignment. Although we expect the distribution of change scores to be approximately normal, preliminary analyses will evaluate whether a transformation from the Box-Cox family will improve that assumption. From our experience in prior clinical trials using a similar design to CIRT-CFR, we expect a 10-15% attrition rate of patients due to death or loss to follow-up. Because it is not expected that site randomization assignment will influence these events, and because the available information is unlikely to allow for accurate imputation of missing values for CFR, subjects who do not have a repeat 1-12-month PET scan will not be included in the primary or secondary analyses.

Power calculations: For *Aim 1*, we used prior publications from our group to characterize the reproducibility of the global, dipyridamole mediated CFR measurements in patients with DM or metabolic syndrome[43, 53]. We used data from the patients enrolled in the placebo arm of these trials to estimate the standard deviation in the change in global CFR (Time 2 CFR – Time 1 CFR), and found a mean standard deviation of 0.45. **Table 1** shows the sample size needed to reach 80-90% power for an expected effect size of 20% relative improvement in CFR in the active treatment group compared to placebo. Using a total sample size of 130 evaluable patients and a two-tailed alpha of 0.05, power will be 85% for this analysis. Estimated power by this approach should be considered conservative since the control for baseline CFR is expected to enhance power. ***We expect to randomize approximately 150 patients*** to have at least 130 evaluable subjects completing the study (estimating a 10-15% attrition rate between time of randomization and final PET).

Table 1. Power and sample size calculations for Aim 1

AIM 1 Measurement	*Difference (LDM vs. placebo)*	*Standard deviation (placebo)*	*Two-sided α level*	*Total sample size for power*		
				80%	*85%*	*90%*
Primary outcome: $\Delta CFR_{\text{to dipyridamole}}$	0.24	0.45	0.05	114	130	150
Secondary outcome: $\Delta TPD_{\text{stress}}$	2.5%	4.9%	0.05	124	140	164

Aim 2. To test the hypothesis that LDM at a target dose of 15 to 20 mg orally weekly for* 1*-12 months will, compared to placebo, result in improved LV myocardial mechanics, as assessed by transthoracic echocardiography, and that this functional improvement will correlate with the change in CFR after* 1*-12 months of treatment. Specifically:
Hypothesis #3: LDM for 1-12 months will result in improved LV systolic function, as assessed by measures of LV peak global longitudinal strain, and diastolic function, as assessed by measures of tissue Doppler mitral annular early diastolic relaxation velocity (E').

Hypothesis #4: Changes in LV systolic and diastolic function after 1-12 months of LDM therapy will be correlated with changes in coronary flow reserve.
Methods: 2-D echocardiograms will be performed at the 3 enrolling imaging sites at baseline and 12 months after randomization in accordance with standard clinical practice and utilizing a standardized study protocol as outlined in section V. As described in section V, we have previously demonstrated feasibility of measuring cardiac mechanics with high reproducibility. The primary efficacy endpoints for *Aim 2* are the change in LV peak global longitudinal strain (GLS) (systolic function) and in tissue Doppler mitral annular early diastolic relaxation velocity (E') (diastolic function). Key secondary endpoints are change in other LV systolic function measures (LVEF and tissue Doppler peak systolic mitral annular velocity (S')), as well as change in other LV diastolic function measures (LV early diastolic strain rate by speckle-tracking, and mitral inflow Doppler E velocity to E' ratio (E/E')). Tertiary endpoints include changes in more conventional measures of LV structure (e.g. volumes, wall thickness, mass) over 1-12 months.

Statistical analyses. *Hypothesis 3***:** We will use separate linear regression models with the respective change in LV peak GLS and E' over 1-12 months as the outcome variables. Each of these regression models will control for baseline variables of stratum (DM or metabolic syndrome), time since index MI (< 6 months or ≥ 6 months), imaging site (BWH, OHI, or UAB), the corresponding baseline LV peak GLS and E' scores, as well as treatment assignment. *Hypothesis 4:* Although temporality cannot be established in correlational analysis of concurrent changes, we will also use regression models to clarify the association of changes in CFR and LV function. Controlling for treatment assignment, baseline stratification variables, baseline CFR and LV function, as well as change in LV function, our outcome will be change in CFR. By adding the baseline and change in LV function to our primary model for *Aim 2*, we will explore whether changes in CFR are associated with changes in LV function, or whether independent treatment effects remain.

Power calculations: For *Aim 2*, we used prior publications from our team to characterize the reproducibility of the LV measures of systolic and diastolic function[40, 54.] Power analysis is based upon the following assumptions: the expected effect size is 10% relative improvement in each of the primary and secondary outcome measures in the active treatment group compared to placebo. **Table 2** shows the sample size needed to reach 80-90% power for each of the primary and secondary efficacy measures. As for *Aim 1*, estimated power by this approach should be considered conservative since the control for baseline scores is expected to enhance power. Of the 150 patients we expect to randomize, there will be at least 130 evaluable subjects completing the study (estimating a 10-15% attrition rate between time of randomization and final echo).

Table 2. Power and sample size calculations for Aim 2

AIM 2 Measurement	*Difference (LDM vs. placebo)*	*Standard deviation (placebo)*	*Two-sided α level*	*Total sample size for power*		
				80%	*85%*	*90%*
Primary outcome:						
Systolic: ΔGLS_{peak}	1.4%	2.8	0.05	126	142	166
Diastolic: $\Delta E'_{velocity}$	0.7 cm/s	1.3	0.05	112	126	148
Secondary outcomes:						
Systolic: $\Delta S'$	0.6 cm/s	1.2	0.05	128	146	172
$\Delta LVEF$	1.5%	3.1	0.05	138	156	182
Diastolic: $\Delta E/E'$	1.1	2.0	0.05	128	146	172
$\Delta LAVI$	1.2 ml/m^2	2.3	0.05	118	134	158

Exploratory analyses: The proposed ancillary study will not be powered to detect changes in clinical outcomes, patient symptoms, or changes in circulating biomarkers. However, we recognize the importance of exploring the correlations between the changes in CFR and LV function over the 1-12-month observation period and these meaningful outcomes. We propose the following exploratory tertiary objectives: (1) Explore an interaction between the change in CFR at 1-12 months with composite MACE that includes cardiac and all-cause mortality, recurrent MI, stroke, coronary revascularization, and hospitalization for unstable angina requiring unplanned revascularization or for congestive heart failure, occurring 3-4 years after randomization. We will use a proportional hazards model to explore relationships of CFR and its change with time until development of the first clinical event occurring through the end of randomization. We recognize that this exploratory analysis, in particular, will be limited because we expect only ~25 composite events in total in our study sample. (2) Correlate the change in CFR at 1-12 months with the corresponding change in QOL scores, as assessed by the standardized SF-36 Health Survey to be administered during enrollment, 12-month, and final study visits of the parent CIRT trial. Prior assessments with this tool have shown significant differences in baseline and 6-month follow-up scores in a modest-sized (n=120) patient population post-MI[55]. (3) Correlate the change in CFR and LV function with the corresponding changes in circulating biomarkers of inflammation (hsCRP, IL-1) and myocardial strain (NTproBNP, hsTroponin), as assessed from stored biorepository samples at 8, 12 and 24 months after randomization. Analyses of changes in QOL scores and biomarkers will use linear regression models, after transforming changes to normality as necessary, and with control for baseline trial stratification variables, baseline QOL scores and biomarker levels, and treatment assignment. Main independent variables will be baseline and 1-12-month change in CFR. (4) Assess correlations among the baseline PET, echo, biomarker, and SF-36 data using linear regression models, as described above for continuous variables.

VII. RISKS AND DISCOMFORTS

Blood Sampling and Intravenous Catheter Risks

There are minor risks and discomforts associated with placement of an intravenous line, including the possibility of bleeding, pain, inflammation (redness and swelling), and leaking of contrast agent outside of the vein at the site of the IV. These problems are usually self-limited or require only minor treatments, such as application of an ice-pack or slight pressure for a few minutes.

Vasodilator Risks
Vasodilator-stress with Dipyridamole (Persantine®), as will be performed in this study, has been used routinely for many years for evaluation of known or suspected coronary artery disease in conjunction with myocardial perfusion imaging. During the Dipyridamole infusion, the patient may experience flushing, chest pain/pressure, nausea, or lightheadedness. If significant symptoms are present, patients will be given aminophylline IV to relieve these symptoms. There will be continuous monitoring of heart rate, blood pressure, and 12-lead ECG throughout the infusion and recovery. These procedures are routinely performed in patients with CAD, as those in the CIRT trial, and are considered safe.

For patients with contraindications to Dipyridamole, the PI or Co-Investigator can decide to use Adenosine or Regadenoson for safety purposes.

Regadenoson stress as performed in this study has been used routinely for many years for evaluating patients with known or suspected CAD. The most common side effects associated with the regadenoson bolus include: flushing, chest pain/pressure, shortness of breath, palpitations, headache, mild hypotension and heart block. These side effects are usually mild and self-limiting. If they are severe in intensity, aminophylline IV (1 mg/kg) will be given as per standard protocol.

Subjects with a history of seizures may receive a medicine called adenosine. The side effects associated with this product include: facial flushing, headache, sweating, palpitations, chest pain, hypotension, shortness of breath/dyspnea, chest pressure, hyperventilation, head pressure, lightheadedness, dizziness, tingling in arms, numbness, apprehension, blurred vision, burning sensation, heaviness in arms, neck and back pain, nausea, metallic taste, tightness in throat, and pressure in groin. Adenosine is administered as a continuous infusion over 4 minutes and administered in a dose of 0.14mg/kg/min. The half life of adenosine is less than 10 seconds. If the patient is having symptoms the infusion can be stopped, no further medication is administered, and the symptoms would stop within seconds because the medication would already be cleared from the heart. Therefore, no reversal medication is needed.

Aminophyline Infusion
Rarely, an aminophylline IV injection may be used to reverse side effects of dypiridamole infusion. Aminophylline is generally well tolerated; possible side effects of aminophylline may include nausea, headache, restlessness, convulsions, rapid breathing, a rapid heart rate, and allergic reactions such as rash.

Radiation Risks
The PET scans will involve exposure to radiation for research purposes. The two radioactive drug studies (^{13}N-ammonia) per visit will expose the patient to approximately 2.82 mSv of radiation for each visit, or a total of approximately 5.64 mSv for both the baseline and follow-up studies (a mSv is a unit of radiation dose). For sites using ^{82}Rubidium, the two radioactive drug studies per visit will expose the patient to approximately 2.6 mSv of radiation for each visit, or a total of approximately 5.2 mSv for both the baseline and follow-up studies (a mSv is a unit of radiation dose). The total whole body effective dose from the ancillary study is equivalent to approximately 11% of the 50 mSv annual limit for a person who works with radiation, or 2 times the natural environmental radiation an average person receives in the United States annually. This risk from

medical imaging is considered low. Women of child-bearing potential are excluded from the parent trial.

Potential Risks from 2-D Echocardiograms
There is minimal discomfort from the echocardiograms including slight discomfort from pressure from the transducer while images are collected, or rarely irritation from the gel used to obtain the echocardiographic images. These studies are routinely performed in patients with CAD, as those in the CIRT trial, and well tolerated.

Reproductive Risks
Pregnant or breastfeeding patients will be excluded from this study.

Unknown Risks
There may be other risks and side effects that are not known at this time.

VIII. POTENTIAL BENEFITS

There are no direct benefits for the patient from taking part in this study. The proposed ancillary study will test the fundamental concept of whether reducing systemic inflammation with LDM can lead to improved myocardial blood flow and tissue perfusion and LV function. Thus, this ancillary study will provide important and unique mechanistic insights of the capabilities of anti-inflammatory therapies to improve key clinical determinants of clinical risk (improve coronary blood flow, reduce ischemia, and improved LV function) and, in so doing, improve patient symptoms and quality of life, as well as outcomes.

IX. MONITORING AND QUALITY ASSURANCE

Safety of patients will be monitored through an independent Data and Safety Monitoring Board (DSMB) for the main CIRT trial.

Ancillary study investigators will report to the CIRT leadership (Ancillary Studies Committee and Steering Committee) on an annual basis regarding the status of study funding, initiation and terminations dates, and success of recruitment and data collection.

The PI will monitor the safety of subjects enrolled at BWH.

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APPENDIX:
CIRT Protocol Summary, Data Collection Schedule, Informed Consent Form and IRB Approval Letter