Cardiovascular Inflammation Reduction Trial (CIRT)

A randomized, double-blind, placebo-controlled, event-driven trial of weekly low-dose methotrexate (LDM) in the prevention of recurrent cardiovascular events among stable post-myocardial infarction patients with type 2 diabetes or metabolic syndrome

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This document contains the following items:

1. Original protocol (March 6, 2013), final protocol (October 30, 2014), summary of changes
2. Original statistical analysis plan (March 25, 2013), this is the original and final version (no changes were made)

PROTOCOL

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Center for Cardiovascular Disease Prevention
Brigham and Women's Hospital

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Abbreviations

ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
AE	Adverse event
AHA	American Heart Association
ALT	Alanine aminotrasferase
AST	Aspartate aminotransferase
CBC	Complete blood count
CCC	Clinical Coordinating Center
CEC	Clinical Endpoints Committee
CIRT	Cardiovascular Inflammation Reduction Trial
CrCl	Creatinine clearance
CRP	C reactive protein
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
DVT	Deep venous thrombosis
eGFR	Estimate glomerular filtration rate
HbA1c	Hemoglobin A1c
HDL-C	High density lipoprotein cholesterol
HY27	Cholesterol 27-hydroxylase
IL-1	Interleukin-1
IL1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IRB	Institutional review board
GFR	Glomerular filtration rate
LDM	Low dose methotrexate
LDL-C	Low density lipoprotein cholesterol
LLN	Lower limit of normal
NHLBI	National Heart Lung and Blood Institute
PE	Pulmonary embolus
RA	Rheumatoid arthritis
SAE	Serious adverse event
TNF	Tumor necrosis factor
ULN	Upper limit of normal
WBC	White blood cell count

1.0. TRIAL OVERVIEW

While inflammation contributes crucially to atherothrombosis, it is unknown whether inhibition of inflammation per se will lower vascular event rates. The primary aim of the **Cardiovascular Inflammation Reduction Trial** (CIRT) is to directly test the inflammatory hypothesis of atherothrombosis by evaluating whether or not low-dose methotrexate (LDM) will reduce rates of recurrent myocardial infarction, stroke, and cardiovascular death among stable post-myocardial infarction patients with type 2 diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response. CIRT is a randomized, double-blind, placebo-controlled, multi-center, event-driven trial that will randomize 7,000 men and women from the United States and Canada. Following a five- to six-week open-label run-in (maximum 8 weeks), eligible participants who have suffered documented myocardial infarction in the past five years will be randomly allocated over a three to four year period to usual care plus placebo or usual care plus LDM. The target methotrexate dose among those allocated to active therapy is 15 to 20 mg po per week, a dose within the range of that commonly used for the treatment of rheumatoid arthritis. All study participants will additionally receive 1.0 mg oral folate to be taken daily six days per week. LDM complications will be minimized through education programs for all investigators and coordinators, through enhanced communication with study participants, by limiting enrollment to those with no evidence of malignancy, hepatitis, renal dysfunction, chronic infection, pulmonary disease, or other risk factors for toxicity; by conducting an initial 5- to 6-week active-therapy run-in (maximum 8 weeks) designed to eliminate individuals who are either intolerant of or unable to adhere to treatment before randomization; and through regular monitoring of liver function and hematologic indices using a centralized methodology designed to ensure participant safety, allow for dose adjustments while maintaining the study blind, and provide an efficient method to address issues of compliance and follow-up on a cost-effective centralized basis. The primary trial endpoint is the rate of recurrent myocardial infarction, stroke, or cardiovascular death. Secondary and tertiary endpoints include all-cause mortality, coronary revascularization, incident congestive heart failure, incident peripheral artery disease, incident venous thrombosis, clinically significant aortic stenosis, incident atrial fibrillation, incident diabetes among those with metabolic syndrome but not diabetes at study entry, and hemoglobin A1c (HbA1c) control among those with diabetes at study entry. The trial is event driven such that in the absence of extreme effects, the trial will conclude after accrual of at least 530 primary endpoints, an effect estimated to provide 90 percent power to detect a 25 percent relative risk reduction. The potential clinical impact of CIRT is broad as it has sufficient power to directly address core issues in the inflammatory hypothesis of atherothrombosis, and thus, if successful, will open major new directions for cardiovascular treatment.

2.0. SPECIFIC AIMS

Abundant laboratory and translational data demonstrate that inflammation plays a major role in all stages of the atherothrombotic process[1,2]. These observations have generated the hypothesis that targeted anti-inflammatory therapy can lower vascular event rates. To date, however, no clinical trial has directly addressed this critical biologic hypothesis[3,4].

The primary scientific aim of CIRT is to directly test the inflammatory hypothesis of atherothrombosis. Specifically, CIRT will evaluate whether LDM will reduce rates of recurrent myocardial infarction, stroke, or cardiovascular death among patients with a recent history of

myocardial infarction and either type 2 diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response. LDM is an effective anti-inflammatory therapy widely used to treat rheumatoid arthritis that lowers plasma levels of interleukin-6 (IL-6), tumor necrosis factor (TNF), and C-reactive protein (CRP) but does not otherwise have beneficial effects on lipids or biomarkers of hemostasis and thrombosis. Thus, a randomized trial of LDM provides an innovative approach to target inflammation while minimizing confounding effects that might accrue from activation or inhibition of alternative vascular pathways. The wide use of LDM as a mainstay in current therapy for rheumatoid arthritis provides both guidelines for safety monitoring and strong evidence that off-target toxicity is unlikely to be uncovered during the course of this trial.

2.1. Primary Aim

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM given at a target dose of 15 to 20 mg po weekly will reduce rates of *recurrent myocardial infarction, stroke, or cardiovascular death* among patients with a prior history of myocardial infarction and either type 2 diabetes or metabolic syndrome.

2.2. Secondary Aims

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of *all-cause mortality* among patients with a prior history of myocardial infarction and either type 2 diabetes or metabolic syndrome.
b. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus coronary revascularization*.
c. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rates of *hospitalization for congestive heart failure*.
d. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus all-cause mortality plus coronary revascularization plus congestive heart failure*.
e. To determine in a randomized, double-blind, placebo-controlled setting the side effect profile of LDM in a non-rheumatologic population at risk for recurrent vascular events. By so doing, CIRT will evaluate the *net clinical benefit or harm* that might accrue from the hypothesized use of LDM as a novel method for the secondary prevention of myocardial infarction, stroke, and cardiovascular death.
f. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rate of *new onset type 2 diabetes* among those with metabolic syndrome but not diabetes at study entry.

2.3. Tertiary Aims

In addition, we will determine in a randomized, double-blind, placebo-controlled setting whether LDM will

a. reduce rates of the individual components of the primary endpoint
b. reduce rates of the primary endpoint plus unstable angina requiring unplanned coronary revascularization
c. reduce rates of coronary revascularization
d. reduce rates of peripheral artery disease

e. reduce rates of symptomatic deep vein thrombosis or pulmonary embolism, including those considered to be provoked and those considered to be idiopathic
f. reduce rates of clinically significant aortic stenosis
g. reduce rates of atrial fibrillation
h. have positive or negative effects on standardized measures of quality of life and global health status

2.4. Proposed Exploratory and Mechanistic Studies

CIRT will include 7,000 patients with myocardial infarction within the past five years who have either type 2 diabetes or metabolic syndrome. Clinical endpoints of interest that will be prospectively evaluated include incident age-related macular degeneration, sleep apnea, and nephropathy and retinopathy. In addition, a plasma and DNA bank will be established as part of the trial protocol. Thus, cohort accrual and biobanking also allows for the evaluation of several tertiary aims that relate to mechanisms of effect using measured plasma biomarkers of inflammation and glucose metabolism, as well as potential genetic determinants of LDM activity.

a. To evaluate the effect of LDM as compared to placebo on a series of inflammatory biomarkers such as IL-6, TNF, CRP, interleukin-1 (IL-1), and interleukin-1 receptor antagonist (IL1ra), and to ascertain whether any effects on these biomarkers mediate observed benefits or risks of LDM on clinical outcomes in the trial.
b. To evaluate whether genetic polymorphisms associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits or risks of LDM on clinical outcomes observed in the trial.
c. Among subjects with baseline diabetes, to evaluate the effect of LDM as compared to placebo on indices of diabetic progression and glycemic control such as need for diabetes treatment intensification, proportion of subjects achieving optimal glycemic control (HbA1c<7.0%), and change in HbA1c overall and by study visit.

3.0. BACKGROUND AND SIGNIFICANCE

3.1. The Inflammatory Hypothesis of Atherothrombosis

Abundant laboratory evidence indicates that inflammation plays a major role in all stages of atherothrombosis[1,2]. With regard to the translation of inflammation biology to practice, as demonstrated in a comprehensive 2010 meta-analysis, clinical evidence from 54 prospective cohort studies demonstrates that inflammatory biomarkers independently predict vascular risk with a magnitude of effect at least as large as that of blood pressure or cholesterol[5]. However, while the attributable vascular risk associated with inflammation is large and while animal models using targeted anti-inflammatory therapies have shown promise, it remains unknown whether inhibition of inflammation per se will lower vascular event rates.

Despite the importance of this question, no endpoint trial addressing these issues has been initiated. However, the recent JUPITER investigation comparing rosuvastatin to placebo among 17,802 individuals with low levels of low-density lipoprotein cholesterol (LDL-C) but who were at elevated vascular risk on the basis of a pro-inflammatory response reinforces the need for such a trial[6]. In brief, JUPITER demonstrated a 44 percent reduction in major vascular events, which included a 54 percent reduction in myocardial infarction, a 48 percent reduction in stroke, a 46 percent reduction in arterial revascularization, a 43 percent reduction in deep venous thrombosis/pulmonary embolism (DVT/PE), and a 20 percent reduction in mortality.

Underscoring the importance of inflammation as a pathophysiologic factor in selecting this study population, the placebo event rate in JUPITER was higher than that of prior prevention trials limited to those with overt hyperlipidemia[7,8]. Further, within JUPITER, the greatest absolute risk and the greatest absolute risk reduction was observed among those with the highest levels of persistent pro-inflammatory response[9].

From an inflammatory biology perspective, prospective analyses from JUPITER also suggest that achieving low levels of inflammation may be an important treatment goal in a manner analogous to achieving low levels of LDL-C. In pre-specified analyses designed to evaluate the relative effects of inflammation reduction as compared to LDL-C reduction, the JUPITER data suggest that the benefits of statin therapy are magnified among those who not only reduce LDL-C, but who also substantially reduce inflammatory biomarkers[10]. As such, JUPITER prospectively confirms prior data from the CARE[11,12], AFCAPS/TexCAPS[13], PROVE IT – TIMI 22[14], A to Z[15], and REVERSAL[16] trials that best clinical outcomes accrue in statin treated patients who achieve low levels of inflammation as well as cholesterol. All of these data corroborate laboratory evidence of anti-inflammatory properties of statins including reduced cell adhesion and monocyte recruitment to the arterial wall; reduced prenylation of small G proteins and augmented expression of the transcription factor KLF2 with consequent mitigation of inflammatory and thrombotic mediators; altered smooth muscle migration in developing plaques; favorable effects on metalloproteinase expression; and in human hepatocytes, reductions in IL-6 and other cytokines[17,18]. However, statins markedly lower LDL-C as well as reduce inflammation. Thus, although suggestive, these data cannot address whether lowering inflammation alone will lower vascular risk.

3.2. Low-Dose Methotrexate (LDM) and Cardiovascular Disease

A direct test of the inflammatory hypothesis of atherothrombosis requires an agent that (a) inhibits inflammation without having major impact on other components of the atherothrombotic process, and (b) has an acceptable safety profile for evaluation in a large-scale randomized trial[3,4]. LDM has multiple attributes that make it an appropriate agent to test directly the inflammatory hypothesis of atherothrombosis.

First, LDM (range 10 to 30 mg per week) is widely used, has an enviable safety profile among patients with rheumatoid arthritis and psoriasis, and comprehensive guidelines from the American College of Rheumatology exist regarding dosing regimens, drug monitoring, and the identification of high-risk patient subgroups[19-21]. This experience greatly reduces the potential for unanticipated off-target toxicity. The target methotrexate dose in CIRT is 15 to 20 mg po per week, a dose within the range commonly used for the treatment of rheumatoid arthritis.

Second, LDM reduces several inflammatory biomarkers including CRP, IL-6, and TNF-alpha in populations of patients with rheumatoid arthritis (RA) and psoriasis, patient groups at elevated vascular risk on an inflammatory basis. Further, LDM does not have substantive effects on lipid levels, hemostasis, or platelet function. Thus, LDM provides a mechanism to test the inflammatory hypothesis of atherothrombosis without confounding effects on other vascular pathways.

Third, among both rheumatoid arthritis and psoriasis patients assessed in seven cohort and case-control settings[22-28], available observational epidemiologic data suggest that exposure to LDM is associated with reductions in cardiovascular morbidity and mortality, even though those receiving LDM have worse vascular risk factor profiles, data strongly mitigating against indication bias (Table 1). These data have been verified in a recent systematic overview[29]. Of interest, the cardiovascular benefit of LDM was observed despite the fact that patients initiating treatment (mean dose = 13 mg/week) had significantly worse prognostic factors for mortality and significantly worse RA symptoms than did patients not being treated with LDM. Other observational studies of RA patients taking LDM have shown improvement in heart failure[30] and reduction in carotid intima media thickness[28]. The consistent observation of excess vascular risk unexplained by traditional risk factors among those with rheumatoid arthritis or psoriasis also supports the conceptual basis for a trial of anti-inflammatory therapy among those with a persistent enhancement of the innate immune response[31-33].

Table 1. Low Dose Methotrexate and CVD: Observational Evidence

Cohort	Group	HR* (95 % CI)	Endpoint	Exposure
Wichita	**RA**	**0.4 (0.2 - 0.8)**	**Total Mortality**	**LDM**
Choi 2002		**0.3 (0.2 - 0.7)**	**CV Mortality**	**LDM**
		0.4 (0.3 – 0.8)	**CV Mortality**	**LDM < 15 mg/wk**
Netherlands	**RA**	**0.3 (0.1 – 0.7)**	**CVD**	**LDM**
van Helm 2006				
Miami VA	**Psoriasis**	**0.7 (0.6 – 0.9)**	**CVD**	**LDM**
Pradanovich 2005		**0.5 (0.3 – 0.8)**	**CVD**	**LDM < 15 mg/wk**
	RA	**0.8 (0.7 – 1.0)**	**CVD**	**LDM**
		0.6 (0.5 – 0.8)	**CVD**	**LDM < 15 mg/wk**
CORRONA	**RA**	**0.6 (0.3 – 1.2)**	**CVD**	**LDM**
Solomon 2006				
QUEST-RA	**RA**	**0.85 (0.8 – 0.9)**	**CVD**	**LDM**
Narango 2008		**0.82 (0.7 – 0.9)**	**MI**	**LDM**
		0.89 (0.8 - 1.0)	**Stroke**	**LDM**
Insurance				
Hochberg 2008	**RA**	**0.65 (0.59-0.72)**	**CVD**	**LDM**
UK Norfolk	**RA, PSA**	**0.6 (0.4 – 1.0)**	**Total Mortality**	**LDM**
2008		**0.5 (0.3 – 1.1)**	**CV Mortality**	**LDM**

Fourth, mechanistic studies suggest that atheroprotective effects of methotrexate may accrue from enhanced release of adenosine which in turn leads to facilitation of cholesterol efflux and reverse cholesterol transport from arterial wall foam cells[34] via upregulated expression of cholesterol 27-hydroxylase (HY27) and the ATP-binding cassette transporter (ABCA1)[35,36]. Recent data indicating enhanced gene expression of HY27 and ABCA1 with clinical use of methotrexate also supports this emerging hypothesis[37]. Other work suggests that methotrexate has direct effects on apoptosis and on the suppression of adhesion molecule function, both of which play relevant roles in atherothrombosis[38,39].

Finally, LDM is a generic, inexpensive therapy given orally as a once-weekly agent allowing for the efficient and safe conduct of a large simple trial. This simplicity has been incorporated into CIRT in such a way that ongoing safety evaluations can use a centralized methodology that improves participant safety, maintains the study blind while allowing for in-trial dose adjustments, and provides an efficient method to address issues of compliance and follow-up on a cost-effective centralized basis.

3.3. Diabetes, Metabolic Syndrome, and Inflammation

Post-myocardial infarction patients with diabetes or metabolic syndrome have an enhanced pro-inflammatory response and are at high vascular risk, thus providing an excellent target population for CIRT. Further, as is the case for atherothrombosis, the core

pathophysiologic basis underlying insulin resistance and diabetes is hypothesized to entail fundamental abnormalities of the innate immune response[40]. From a clinical perspective, plasma levels of several inflammatory biomarkers increase with increasing numbers of components of metabolic syndrome and several inflammatory biomarkers including IL-6 predict incident type 2 diabetes[41].

With regard to the ability of metabolic syndrome to define a secondary prevention population at increased risk of recurrent vascular events, Table 2 presents data from the 4S, MIRACL, WIZARD, and TNT trials[42-45]. As shown, among those with metabolic syndrome as compared to those without, the hazard ratios for recurrent cardiovascular disease all approximate 1.4 (95%CI 1.2-1.7). Similarly, in a recent meta-analysis of 87 studies, metabolic syndrome was associated with a 2-fold increase in cardiovascular events and a 1.5 fold increase in all-cause mortality[46].

Table 2. Summary of the Association between MetS and Subsequent Cardiovascular Events in Secondary Prevention Trials

Study Population	Cohort Inception Year	Sample Size	Average Follow-Up (yr)	Baseline Prevalence	Outcome	Event Rate		RR/HR
						MetS	No MetS	
4S	1988	2223*	5.4	20%	CVD	7.2 per 1000 person-months	5.2 per 1000 person-months	1.41 (1.16-1.71
MIRACL	1997	3038	0.3	38%	CHD	19.2%	14.3%	1.40 (1.16-1.67)
WIZARD	1997	3319	3.1	53.3	CHD	28.1%	21.1%	1.33 (1.15-1.53)
TNT	1998	10001	4.9	56%	CVD	11.3%	8.0%	1.44 (1.26-1.64)

* Estimates provided for placebo arm only

With regard to the ability of diabetes to define a secondary prevention population at high risk of recurrent vascular events, Table 3 presents data from the 4S, CARE, LIPID, and HPS trials[42,47-49]. As shown, among those with diabetes as compared to those without, the hazard ratios for recurrent cardiovascular disease are again in a range 1.4 to 1.6 when compared to that of individuals without diabetes.

Table 3. Summary of Association between Diabetes and Subsequent CVD Events in Secondary Prevention Trials

Study Population	Cohort Inception Year	Sample Size	Average Follow-Up (yr)	Baseline DM Prevalence	Outcome	Event Rate DM	Event Rate No DM	RR/HR
4S	1988	2223*	5.4	10.4	CVD	NA	NA	1.62 (1.29-2.03)
CARE	1989	4159	5.0	14.1	CHD	19.1	10.5	NA
LIPID	1990	9014	6.1	11.9	CVD	48.9	36.5	1.4 (1.3-1.5)
HPS	1994	20536	5.0	29.0	CHD	35.6	22.7	NA

* Estimates provided for placebo arm only; NA - not available

3.4. Pharmacology of Low Dose Methotrexate (LDM)

LDM is taken weekly by tens of thousands of patients with similar age and co-morbidity profiles as those likely to be enrolled in CIRT. Introduced as a treatment for rheumatoid arthritis in 1951, LDM has an enviable safety and efficacy profile that has allowed it to remain the dominant disease modifying therapy for RA. A clinically relevant anti-inflammatory effect is rapidly achieved for the majority of RA patients at weekly oral doses between 10 and 30 mg. Food minimally affects LDM absorption so the drug can be taken in fasting or non-fasting states. Circulating methotrexate is less than 50 percent protein bound and has minimal interaction with most concomitant medications, including statins, aspirin, beta-blockers, and inhibitors of the renin-angiotensin system. However, there is increased risk when methotrexate is used in

combination with folate depleting drugs such as bactrim (trimethoprim-sulfamethoxazole) or drugs that affect tubular secretion (probenecid). Thus, study participants will be alerted to this potential toxicity and those with allergies that make use of alternative agents impossible will be excluded.

All study participants (including those on placebo) will receive supplementary folic acid (1.0 mg 6 days per week), a simple adjunct known to reduce side-effects of LDM and improve long-term compliance that itself has no direct effect on vascular risk.

Methotrexate is primarily cleared by the kidneys, with 80 to 90 percent being excreted in urine. As such, reduction in creatinine clearance (CrCl) is a determinant of serum levels and systemic toxicity. Patients with baseline CrCl less than 40 ml/min will not be entered into CIRT, and follow-up CrCl will be measured on a regular basis so that drug can be discontinued or dose-reduced in the event of incident renal failure; this safety criterion is conservative as the American College of Rheumatology allows LDM use even when CrCl is reduced to 30 ml/min.

3.5. Safety of LDM and Efforts to Reduce In-Trial Toxicity

The wide use of LDM in clinical practice makes it unlikely that any unknown off-target toxicities will appear during the conduct of CIRT. Further, risk factors associated with LDM toxicity are well known and formal guidelines have been issued by the American College of Rheumatology outlining patient groups where therapy is ill-advised[19]. Broadly, patients with hepatitis, renal dysfunction, chronic infections, and certain pulmonary conditions have increased risk, and these groups are excluded from study participation, as are patients with a significant history of alcohol consumption. As methotrexate can sequester in fluid spaces, participants with known chronic pericardial effusion, pleural effusion, or ascites will not be included in the trial. Because of the fetopathic and teratogenic effects of methotrexate, women of childbearing potential or who intend to breastfeed will not be included in the trial.

The vast majority of life-threatening hepatotoxicity, pulmonary damage, and myelosuppression that have been reported with methotrexate occur at the very high doses used during treatment of malignancy (where methotrexate is dosed cyclically by the gram or more). In the dose range to be used in CIRT (target dose 15 to 20 mg per week), such life-threatening complications are rare. Nonetheless, to reduce the chance of such occurrence within CIRT, screening for hepatitis B and C will be conducted before enrollment and patients who are positive for chronic infection will be excluded. In addition, monitoring of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, creatinine clearance (CrCl), and complete blood count (CBC) will be done throughout the study (monthly for the first 6 months after randomization, then at least bi-monthly for the trial duration). Rarely, in the setting of high dose methotrexate (as used in chemotherapy), hypospermia and the potential for chromosomal damage to sperm has been reported. While it is uncertain if these effects occur with low dose methotrexate, to protect the safety of all study participants and their sexual partners, effective contraception will be recommended during the trial and for six months after a participant completes the trial. Men who intend to father children during the trial period will not be enrolled.

All participants must lack significant pulmonary disease at enrollment, and surveillance questionnaires seeking symptoms of pulmonary disease will be given every four months during study follow-up. A chest X-ray in the 12 months prior to enrollment must be free of evidence of interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis. In instances where a chest X-ray is not available in the prior 12 months, a baseline chest X-ray will be obtained as part of the study protocol. With regard to potential nephrotoxicity, enrollment will be limited to those with CrCl $\geq$ 40 ml/min with dose adjustments or discontinuation built into the protocol in the event of renal deterioration. The protocol also incorporates short-term suspension of LDM for patients with intercurrrent infections, those receiving antibiotic therapy, those undergoing surgery, and

those who develop new clinically significant pericardial effusion, pleural effusion, or ascites. All of these steps are consistent with or more conservative than guidelines issued by the American College of Rheumatology for use of LDM[19].

Five aspects of the CIRT protocol are specifically designed to minimize these issues and maximize long-term follow-up and compliance. First, an active therapy 5- to 6-week run-in (maximum 8 weeks) has been incorporated into CIRT so that any individuals with short-term intolerance to LDM will be excluded prior to randomization. Second, the CIRT protocol targets a maximal methotrexate dose of 15 to 20 mg po weekly, a dose well within the range of that commonly used in the treatment of rheumatoid arthritis. Titration to the 20 mg weekly dose will only occur among those trial participants who have tolerated without complication the 15 mg weekly dose for a minimum of three months. Third, the protocol titration algorithms and drug packaging (calendar blister packs) have been designed in such a way that in response to either clinical need, participant report of adverse effects, or to any monitored laboratory evidence of hepatic, renal, acute infection, or hematologic abnormality, dosing for individual participants can be discontinued on a short term basis or reduced to either a 5, 10 or 15 mg dose/wk as tolerated. After clinical resolution and a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated in 5 mg increments toward the maximal target dose of 20 mg po weekly. Fourth, targeted face-to-face education programs will be required for all study investigators at the investigator meetings, followed by mandatory web-based safety seminars; these educational programs will be provided by the rheumatologic members of the CIRT Steering Committee and will ensure a high level of drug safety awareness among all trial physicians and staff. Fifth, an ongoing communication program is incorporated into CIRT which allows the investigative team to stay in regular contact with trial participants, withhold study drug for fevers or inter-current infection, systematically evaluate subjective symptoms, and ensure an almost continuous flow of safety data throughout the trial experience. This regular communication will also improve compliance and a sense of community for individual participants.

4.0. INVESTIGATIONAL PLAN

4.1. Study Design - Overview

CIRT is a randomized, double-blind, placebo-controlled, event-driven trial of LDM (target dose 15 to 20 mg/wk) in the secondary prevention of myocardial infarction, stroke, and cardiovascular death among stable post myocardial infarction patients who have either diagnosed type 2 diabetes or who meet the formal 2004 American Heart Association (AHA) / National Heart Lung and Blood Institute (NHLBI) definition of metabolic syndrome which 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. All trial participants will have had a documented myocardial infarction in the 5 years prior to enrollment and be clinically stable for at least 60 days after the qualifying event. The qualifying myocardial infarction is the most recent myocardial infarction that meets criteria for the diagnosis of myocardial infarction. Any planned coronary revascularization procedures associated with the qualifying event must also be completed at least 60 days prior to enrollment.

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 nuisance side effects that can be associated with LDM but that has been shown in multiple major trials to have no vascular benefit so there will be no confounding due to folate use.

Following American College of Rheumatology Guidelines for use of LDM[19], acceptable levels of white blood cell count, hematocrit, platelets, CrCl, and liver function (cutoffs provided in Exclusion Criteria, section 4.4), as well as negative screens for hepatitis B and C will be required prior to active run-in. Individuals with known hepatic disease, chronic pulmonary disease (specifically: interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis), or HIV related disease will be excluded. A chest X-ray in the 12 months prior to enrollment must be free of evidence of interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis. Individuals with known chronic pericardial effusion, pleural effusion, or ascites will be excluded. 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. During the 5- to 6- week open-label run-in, all potentially eligible study participants will initiate LDM at a dose of 5 mg in week one. In the absence of adverse effects or intolerance, this 5 mg dose will be increased to 10 mg for the second and third week of the run-in phase, and then to 15 mg for the fourth week. At the end of this 5- to 6-week run-in period safety labs will be repeated. Only those participants who demonstrate compliance during this one-month run in and who are free of side effects with no significant changes in hematologic or hepatic indices will be considered randomizable to active therapy or to placebo.

Once randomized to active therapy or placebo, all study participants over the first 6 months of the trial will undergo monthly evaluation for CBC, ALT, AST, albumin, and CrCl using blinded and centralized study procedures that have been designed both to ensure patient safety and allow for drug discontinuation or dose-adjustment, as well as ongoing evaluations of study compliance. At 4 months, those participants randomly allocated to active LDM who have tolerated the 15 mg dose without complication will be titrated up to the target dose of 20 mg LDM weekly. Six months after randomization, the blood-based safety evaluations will occur at least bi-monthly until trial completion. Pre-labeled calendar blister packs similar in design to that used in the run-in will be used throughout the study to improve compliance and reduce complexity. To maintain the blind as much as possible, sham titrations will be conducted in the placebo group proportionate to the number of actual titrations required in the active treatment group.

For any participants developing side effects or in whom laboratory abnormalities develop, the protocol allows for drug discontinuation and/or down-titration. The protocol also builds in procedures for short-term discontinuation of LDM in circumstances such as fever, antibiotic therapy for bacterial infections, during and immediately after surgical procedures, or if new clinically significant pericardial effusion, pleural effusion, or ascites develops. Most of these steps will be managed centrally to protect the study blind, ensure common compliance across study sites, and allow centralized rapid management of any safety issues on an immediate basis. After clinical resolution of any events that lead to drug discontinuation and after a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated over time back toward the maximal target dose of 20 mg po weekly, as tolerated. Procedures for these dose adjustments, temporary drug discontinuation, and re-initiation of study drug are described in detail in the trial algorithms.

An electronic data capture (EDC) system will be developed and used to collect and transmit source data throughout the course of the trial. This system is 21 CFR part 11 compliant and meets all relevant governmental regulations. The system will be maintained at SOCAR Research, an independent clinical research organization. System functionality will be thoroughly tested and validated prior to implementation. Furthermore, to ensure compliance with standards

of use of electronic trial data, standard operating procedures will be maintained for the use of the system, an audit trail of data changes will ensure that there is no modification of entered data without documentation, and security systems will be maintained to protect against unauthorized access. Furthermore, adequate procedures will be used to backup the data and safeguard the blinding of the study. As original observations are entered by clinic staff directly into the computerized system, the electronic record is considered the source document. The EDC system will thus be used in each of the following steps to create, modify, maintain, archive, retrieve and/or transmit source data: 1) creation of case report forms, 2) resolution of data discrepancies through data queries and checks, 3) implementation of the study drug titration, 4) monitoring of drug distribution, 5) reporting of adverse events and endpoints, and 6) endpoint adjudication

4.2. Study Population

CIRT will randomize 7,000 men and women, age 18 years and over, who have suffered a documented myocardial infarction in the past five years, have completed any planned coronary revascularization procedures associated with the qualifying event, have been on a stable secondary prevention regimen for a minimum of 60 days, and have either a clinical diagnosis of type 2 diabetes or metabolic syndrome.

For purposes of this trial, the formal 2004 AHA/NHLBI definition of metabolic syndrome will be used and requires evidence that any 3 of the following 5 diagnostic criteria are present: waist circumference ≥ 102 cm in men or ≥ 88 cm in women; triglycerides ≥ 150 mg/dl (1.7 mmol/L) or on drug treatment for elevated triglycerides (fibrates, nicotinic acid, or omega 3 fatty acids); HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on drug treatment for reduced HDL-C (fibrates or nicotinic acid); 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.

4.3. Inclusion Criteria

a. Age ≥ 18 years at screening;
b. Documented myocardial infarction within the past five years, completed any planned coronary revascularization procedures associated with the qualifying event, and have been clinically stable for at least 60 days prior to screening; the qualifying prior myocardial infarction must be documented either by hospital records or by evidence on current ECG of Q waves in two contiguous leads and/or an imaging test demonstrating wall motion abnormality or scar;
c. History of type 2 diabetes or metabolic syndrome at time of study enrollment;
d. Willingness to participate as evidenced by signing the study informed consent.

4.4. 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 < 4,000/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.

4.5. Open Label Run-In

For all potentially eligible patients who provide informed consent and declare interest in participation, baseline ALT, AST, hepatitis screens, CrCl, albumin, and CBC will be obtained prior to the 5- to 6-week active run-in phase to ensure they meet trial enrollment criteria. A urine sample will be assayed for albumin and creatinine. A blood sample for plasma and buffy coat will also be shipped to the central lab for long-term storage and to provide a bank for future blood-based biomarker and genetic sub-studies. Quality of life questionnaires will also be administered.

A 5 to 6-week (maximum 8 week) course of open-label LDM will then be given to all eligible study participants in a pre-randomization compliance and tolerability run-in. During this run-in, participants will be given an initial 5 mg oral weekly LDM dose along with an adequate supply of 1.0 mg folic acid to be taken 6 days per week using similar drug calendar packs to those used throughout the active trial period. In the absence of adverse effects or intolerance, this 5 mg dose will be increased to 10 mg for the second and third week of the run-in phase, and then to 15 mg for the fourth and subsequent weeks. This run-in will allow subjects who are poorly compliant, who develop any immediate side effects, or who withdraw informed consent to be excluded before randomization. Participants must tolerate LDM 15 mg two weeks in a row in order to continue in the trial. All subjects who successfully tolerate LDM 15 mg two weeks in a row and are still willing to be randomized will have repeat blood evaluation for AST, ALT, albumin, CBC, and CrCl, and any individuals with substantive changes in these parameters will be excluded prior to randomization. Urine for albumin and creatinine will also be collected. The run-in will thus enhance long-term compliance and eliminate risk of exposure for any individuals with immediate intolerance to LDM.

4.6. Randomization Procedures

All participants who successfully complete the run-in will be eligible for randomization to the study drug calendar blister packages that contain either active LDM or placebo as well as active tablets containing 1.0 mg folic acid to be taken daily 6 days per week. The randomization visit will involve collection of key medical, social, and anthropometric information from the participant, as well as a brief physical examination including assessment of the participant's level of physical function. The responsible site investigator will use an interactive computer system for randomization. The randomization process will involve stratifying participants by time since the qualifying myocardial infarction (< 6 months, ≥ 6 months), by presence of either diabetes or metabolic syndrome, and by site. All post-randomization safety monitoring and dose adjustments will be performed using a standardized centrally run system that has been designed to ensure patient safety, maintain double-blinding while allowing for dose titration or discontinuation in both the active and placebo groups, provide a central mechanism for tracking patient compliance, and that will allow for efficient follow-up and eventual study close-out. To reduce inadvertent trial unblinding and further ensure patient safety, procedures have been designed to allow for more frequent blood ascertainment at all clinical sites on an as needed basis such as might occur during a concomitant infection or change in participant status (see Manual of Operations).

4.7. Cohort Follow up and Clinic Visits

All subjects will be seen by the study physician at the time of randomization. From that point forward throughout the trial, all participants will be required to see a member of the randomizing physician's study team at least once every 4 months in addition to the regularly scheduled laboratory and symptom monitoring procedures described below. At those visits, compliance (measured by pill count) as well as evidence of side effects will be ascertained by self-report, and in the absence of a study endpoint or laboratory abnormality, a new 4-month supply of study drug (or blinded placebo) provided. A Patient Contact Information Form will be updated at each of these 4-month visits to facilitate long-term follow-up.

Cohort follow-up will include a visit form filled out at each of these appointments. Information will similarly be sought concerning trial endpoints, issues of compliance will be reviewed, anthropometric measures and brief physical examination including reassessment of physical function will be performed, participant questions answered, the drug supply ensured, and any outstanding study forms completed and/or updated. In addition, signs or symptoms of early drug toxicity will be assessed, as well as the occurrence of any primary or secondary trial endpoints or other clinical events of interest. Nephropathy, a clinical event of interest, will be assessed by collecting urine for albumin and creatinine at 8 months, 12 months, and then every 6 months after randomization. Since these data will be obtained in an identical manner from participants randomly allocated to LDM as well as to placebo, this procedure will also provide a mechanism for monitoring by the Data Coordinating Center (DCC) and reports to the Data and Safety and Monitoring Board (DSMB) of any differences between treatment groups with regard to side effects or efficacy. At each regularly scheduled safety lab visit (once a month for the first 6 months after randomization, then at least bi-monthly), participants will be asked about clinical symptoms and side effects of the study medication so that study drug dosage may be adjusted or temporarily stopped if necessary. In order to better understand the toxicities of methotrexate, at 6 months a blood sample will be collected for the measurement of key metabolites of methotrexate.

For the first 6 months post-randomization, monthly blinded laboratory evaluations for ALT, AST, albumin, CBC, and CrCl will be obtained; after that time, evaluation will be done on at least a bi-monthly basis until trial completion. As described in the study algorithms, laboratory

values that cross a pre-defined safety threshold (for example, a decline in the total WBC count to < 3,000/ul) will trigger a call to the subject to inform the participant not to take the following week's dose of LDM and to ascertain any signs or symptoms of potential toxicity. As described in the study algorithms, temporary suspension of study drug will occur whenever subjects are being treated with antibiotics, have a clinical infection, develop new clinically significant effusion or ascites, or are scheduled for surgery. Similarly, patients will be informed not to take the next scheduled study medication if they develop a serious unexplained cough or shortness of breath suggestive of interstitial lung disease, or if they develop stomatitis, vomiting, or persistent fever. Details of the methotrexate titration algorithm, including up- and down-titration, temporary study drug interruption, and permanent study drug discontinuation are included in the trial algorithms. The responsible physician will be informed of any changes in study drug dosing.

After any temporary discontinuation of study drug, study algorithms will be used to determine if and when re-initiation of study medication can occur. If a participant's study medication is repeatedly stopped because of laboratory abnormalities or clinical symptoms, the medical monitor will review the case to consider a permanent discontinuation of study medication. As described in the study algorithms, permanent drug discontinuation can also be done at the discretion of the responsible physician.

4.8. Monitoring Participant Laboratory Values and Subsequent Dose Adjustments

In addition to the algorithms designed for safety monitoring in this trial, an additional safety feature of CIRT is that when certain laboratory thresholds are crossed, the physician responsible for the subject will be notified.

The specific thresholds for drug discontinuation, re-initiation, down-titration, and up-titration are described in detail in the trial algorithms that will be used to monitor safety and make dose changes across the study on a central and consistent basis. No increase in weekly dose will occur unless all of the following criterion are met: the WBC count is ≥ 4000/ul, the platelet count is ≥ 75,000/ul, the CrCl is ≥ 40ml/min, ALT/AST levels are ≤ 1.5x ULN, hematocrit is ≥ 27 percent, and there are no clinically important symptoms (defined as stomatitis, diarrhea, , vomiting, or cough either productive of sputum or associated with a fever or with severe shortness of breath). As also described in the study algorithms, a reduction in dose will occur if there are changes in some of these parameters that may suggest early toxicity but that are not severe enough to warrant drug discontinuation. For example, for a subject taking 20 mg of the study drug, while a drop in the platelet count below 50,000 will lead to temporary drug discontinuation, a reduction below 75,000 but above 50,000 would lead to a 5 mg decrease in the current weekly dosing. After clinical resolution of any events that lead to drug discontinuation and after a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated over time in 5 mg increments back toward the maximal target dose of 20 mg po weekly, as tolerated. Responsible physicians will be informed of any changes in study drug dosing recommended by the clinical coordinating center. Adverse events, patient symptoms, laboratory abnormalities, or other reasons for discontinuation of or reduction in dose of study drug will be collected through case report forms and monitored centrally by the Data Coordinating Center (DCC).

4.9. Monitoring Participant Symptoms and Subsequent Dose Adjustments

Participants will have regularly scheduled visits with the local investigator during which the study staff will carefully assess the patients for any signs or symptoms of drug toxicity, in addition to the occurrence of any trial endpoints. A questionnaire designed to capture information about drug toxicity (including symptoms of a pulmonary, infectious, gastrointestinal,

hematologic, dermatologic, and other toxicities), intercurrent hospitalizations, and elective or planned surgical procedures will be administered to all study participants at the time of regularly scheduled safety lab draws (once a month for the first 6 months after randomization, then at least bi-monthly).

The questionnaire will be administered both by local study staff via the telephone or in person. Study algorithms will combine data from recent laboratory safety evaluations with patient reported symptoms to determine study drug dosing and/or cessation, including sham placebo dose adjustments. Responsible physicians will be informed of any changes in the study medication dosing. As noted above, adverse events, patient symptoms, laboratory abnormalities, or other reasons for discontinuation of or reduction in dose of study drug will be collected through case report forms and monitored centrally by the Data Coordinating Center (DCC).

Study coordinators are given instructions to contact the Medical Monitor for symptoms or signs that raise the possibility of drug toxicity, including the development of new clinically significant effusions or ascites. As noted above, in order to better understand the toxicities of methotrexate, at 6 months a blood sample will be collected from all participants for the measurement of key metabolites of methotrexate. Furthermore, if requested by the DSMB, aggregate data on adverse events, stratified by blinded treatment group, will be reviewed on a quarterly basis by the Chair of the DSMB, who may then request a more detailed analysis and/or forward the data to the full Board.

4.10. Medical Monitor

A Medical Monitor will be on call 24 hours a day 7 days each week. A senior board-certified rheumatologist with expertise in drug safety (Senior Rheumatologist) will supervise the Monitors. The Monitors will be rheumatologists (fellows or junior faculty) who will be trained by the Senior Rheumatologist and will be accessible via pager. The Monitors will be referred subjects based on the pre-laboratory questionnaire or laboratory abnormalities and will have access to the subjects' laboratory data and clinical information via the EDC system. The Monitors will have the option of unblinding the treatment allocation of a subject, if the symptoms and acuity of illness make doing so warranted.

Monitors will help sites determine whether the symptoms being described by patients are concerning, whether interruptions in study drug are warranted, and whether re-initiating study drug is safe. While all Monitors will have experience with methotrexate prescribing, the Senior Rheumatologist will compile a list of scenarios as they occur that will allow the Monitors to have a consistent approach. The Monitors as a group will meet monthly with the Senior Rheumatologist to discuss these scenarios. As the trial progresses, these meetings are anticipated to occur every quarter.

4.11. Endpoint Definitions and Documentation

The primary endpoint is defined as the time to the first adjudication committee confirmed major adverse cardiovascular event (MACE) occurring during the double-blind treatment period, which is a composite of CV death, non-fatal MI, and stroke.

An independent Clinical Endpoints Committee (CEC) will review and adjudicate all clinical events that constitute the primary composite endpoint (CV deaths, non-fatal MI, and stroke). In addition, the CEC will adjudicate a number of secondary endpoints, including all deaths, all hospitalizations for unstable angina requiring unplanned revascularization, all hospitalizations for congestive heart failure, and all arterial revascularization procedures. The CEC will be blinded to treatment assignment. Formal definitions for the individual components of the primary endpoint, hospitalization for unstable angina requiring unplanned revascularization,

hospitalizations for congestive heart failure, and arterial revascularization are provided in the Clinical Endpoint Committee (CEC) Charter. The secondary and tertiary endpoints listed in sections 2.2 and 2.3 not adjudicated by the CEC will be validated by non-CEC staff. For example, incident diabetes will be confirmed by a combination of patient or physician report, new anti-diabetic medications, or by protocol-based measures of glucose and HbA1c performed at least twice annually. Incident venous thromboembolism will be confirmed using documentation of imaging studies including a venous ultrasound or venogram showing deep venous thrombosis or a pulmonary arteriogram, CT scan, or ventilation-perfusion scan showing pulmonary embolism.

Other non-adjudicated clinical events of interest include microvascular disease (nephropathy and retinopathy), sleep apnea, and age-related macular degeneration.

Full descriptions of the methods for endpoint ascertainment and the specific definitions of each adjudicated trial endpoint are contained in the Clinical Endpoint Charter (CEC).

4.12. Procedures for Emergency Unblinding

As part of the trial monitoring structure, a medical monitor will also be available on a 24 hour basis who has the ability to electronically access individual patient data files should unblinding be needed. All of these instances will be tracked within the EDC system.

4.13. Investigator Education Programs

To ensure that all investigators and study coordinators are comfortable with LDM regardless of specialty area, a face-to-face targeted education program will be provided as part of the trial Investigator Meetings and a series of on-line teleconference/webinars will be provided every 6 months where educational information regarding LDM will be updated and reinforced.

4.14. Drug Compliance Monitoring

The primary measure of compliance will be based on the return of calendar packs to the physician at each of the 4-month office visits (at which time new calendar packs will be issued as long as no trial endpoint or major side-effects have occurred).

As an additional measure of compliance, washed packed red blood cells collected 6 months after randomization will be stored in a central laboratory for measures of methotrexate levels. This will be done using a methotrexate polyglutamate assay that provides a semi-quantitative method of evaluating methotrexate absorption and metabolism. The results of this assay will not be available to blinded study staff until after the study has completed and the database has been locked.

4.15. Central Laboratory and Bio-bank

Blood samples for storage in a bio-bank at Brigham and Women's Hospital will be obtained at the pre-run-in, randomization, and at 8 and 24 months after randomization. Processing and long-term storage of these samples will be done in a liquid nitrogen biobank facility in Boston, which has been used for this purpose in multiple prior NIH-funded trials and large cohort studies. White blood cells from the pre-run-in sample will also be stored in this facility to allow for DNA extraction at a later date. As described in the informed consent documents, participation in the genetic bio-banking portions of CIRT will be on an "opt-out" basis and are not a requirement for participation in the main trial.

4.16. Serious Adverse Events (SAEs)

All subjects will be monitored at the local study sites on a regular basis during the course of study involvement. All SAEs will be reported within 24 hours of local event awareness (but no later than the next business day) to the Data Coordinating Center (DCC). Initial reports will often not be complete. Central study staff will guide the site regarding the completion of a SAE form along with retrieval of the pertinent medical records for determination of a final diagnosis. An updated full written report will be filed as additional information becomes available within 10 working days (14 calendar days). The report will include a complete description of the event, use of all concomitant medications, and the local investigator's assessment of causality of the SAE to study therapy. All serious adverse events will be recorded in the core study database within 1 week of report by the local study site personnel. SAE reporting for all subjects will occur in accordance with the central institutional review board (IRB) requirements. Study staff may contact patients directly to determine the resolution of adverse events.

4.17. Data Coordination and Treatment Masking

The DCC is responsible for the facilitation of prompt evaluation of reported adverse events and symptoms. Upon receipt from a site of a report of specific adverse events, combinations of symptoms, or symptoms persisting over time that meet pre-specified criteria, the data will be combined with recent laboratory assays and the safety monitor will be notified that there is a report requiring evaluation.

The DCC will also provide monthly reports to the Executive Committee. The Progress Report will include: enrollment and randomization rates, overall and by sex and race; reasons for randomization ineligibility; number of completed follow-up visits; number of completed interim safety bloods; number of blood specimens sent to the central repository; number of dose changes; current distribution of doses; number of patients off drug; reasons for discontinuation; number of unblindings and reasons for the unblindings. The Quality Control Report will include tables on inappropriate randomizations; number of missed visits; reasons for missed visits; number of visits taking place outside of the designated time windows; and rates of missing data broken down by form. Both the Progress Report and the Quality Control Report will pool participants across treatment groups to maintain the study blind.

The DCC also assumes responsibility for maintenance of blinded treatment assignments. Data files with these assignments will be kept on a secure, password protected server housed in a locked, climate-controlled room with restricted access or on a password protected encrypted laptop. To preserve the integrity of the trial, unblinded DCC staff will not participate in decisions to modify the trial protocol after participants have been randomized. The Steering Committee will obtain statistical input for such decisions from other academic statisticians, blinded to treatment assignment, who also work at Harvard Medical School.

4.18. Data and Safety Monitoring Board

Due to the potential risks to study subjects, the size and multi-site nature of the study, and the fact that this is a Phase III clinical trial, the study will have a formal, and independent, Data and Safety Monitoring Board (DSMB). The DSMB will be constituted by the NHLBI and will include at a minimum members from the following general areas: 1) physicians with specific expertise in the management of patients' cardiovascular disease 2) a biostatistician with specific expertise in the design, analysis, and safety monitoring of multi-center clinical trials 3) a medical ethicist, and 4) a physician with specific expertise in rheumatologic disease. The DSMB will

report directly to the NHLBI, and would have the responsibility of monitoring outcome measurements/endpoints, adverse events (AE's), and serious adverse events, and recommending termination of the study if it appeared at any point during the trial that subjects (or a subgroup of subjects) were being placed at undue risk as a result of their participation. Aggregate data on adverse events, stratified by blinded treatment group, will be reviewed on a bimonthly basis by the Chair of the DSMB, and can be brought before the entire DSMB for further review if requested by the Chair.

The DSMB will meet (face-to-face or by teleconference) at designated intervals (semi-annually) to review accumulated data on safety and efficacy, and if appropriate, conduct an interim analysis of the data. Data will be prepared by the Data Coordinating Center (DCC) prior to each meeting of the DSMB. Serious Adverse Events (SAEs) will be initially reviewed with DSMB members blinded to treatment group. If, however, aggregate data suggested a trend toward more frequent SAEs among one treatment group, an unblinded interim analysis would be reviewed by the DSMB. Guidelines for the possible early termination of the study will be formulated by the DSMB and a formal charter agreed upon prior to trial enrollment. The proceedings of each meeting of the DSMB will be recorded in minutes. Any patient-specific protected health information reviewed by the DSMB would be kept completely confidential. Access to the unblinded minutes of the DSMB meetings by Executive/Operations Committee Members, Clinical Site Investigators, or members of the DCC, will be prohibited until after the database for the study has been locked and the study has been unblinded. A formal report will be submitted by the DSMB to the NHLBI, with a recommendation that the study be continued, modified in a manner to enhance subject safety, or terminated.

4.19. Trial Conclusion and Close Out Visits

At the close of the trial, all randomized participants will be asked to return to the local study sites to see a member of the physician's study team. Compliance, side effects, and patient contact information will be collected, and study end points will again be assessed by the local study staff. Therapy with study drug will be discontinued, and the patient's remaining calendar packs of study drug will be collected. While no observational registry is currently planned, the Clinical Coordinating Center may request that local study sites approach study participants about enrolling in a post-trial observational registry.

To assess the impact of LDM withdrawal on diabetes incidence and glycemic control, participants will have one final laboratory evaluation for ascertainment of HbA1c 3 months after discontinuing study drug therapy.

5.0. DATA ANALYSIS PLAN

5.1. Statistical Analysis

The randomized design and large sample size of CIRT should provide balanced distributions of baseline characteristics between the two treatment groups. Nonetheless, initial analyses will be conducted to identify any chance imbalances in these distributions. In particular, these analyses will form part of the routine monitoring of the trial and will be regularly reported to the DSMB. For continuous and ordinal variables, including age and baseline levels of risk factors including lipid levels, blood pressure, and body mass index, comparisons will use the Wilcoxon rank-sum test. For categorical variables, including sex, race, current and former cigarette smoking, diabetes, greater than 1 prior myocardial infarction, stroke, congestive heart failure, atrial fibrillation, peripheral vascular disease, hypertension, and concomitant therapy with

statins, aspirin, clopidogrel, warfarin, beta blockers, ACE inhibitors, and angiotensin receptor blockers, comparisons will use Chi-square tests. These hypothesis tests are intended for data monitoring and quality control, and not to determine which baseline covariates to include in efficacy analyses[50].

The primary endpoint of the trial is the time from randomization to the first occurrence of any component of the clinical composite endpoint including myocardial infarction, stroke and cardiovascular death. The primary analysis of the trial will use a likelihood ratio test based on a proportional hazards model stratified on time since index myocardial infarction (≥ 6 months vs. < 6 months) to test the null hypothesis of no association between assignment to active methotrexate and the rate of the primary endpoint. All analyses will classify patients according to their randomized treatment assignment, i.e. according to the intention to treat principle, and will base evaluation of statistical significance on a two-sided test with level 0.05. Secondary analyses will further stratify on study site, although these analyses will likely be less efficient because of sparse strata that will arise because of small numbers of participants from some sites. The estimated relative hazard in the methotrexate group compared to the placebo group with an accompanying 95% confidence interval will quantify the treatment effect[51]. If this relative hazard is less than 1, then 100*(1-estimated relative hazard) will be defined as the percent reduction in hazard associated with methotrexate treatment.

Rates of occurrence of the primary endpoint will be defined as the total number of subjects who have this event in a treatment group per 100 person-years of follow-up, counting all time from randomization until the first of the event, death, end of trial, or withdrawal of consent. Estimates of the probability of the primary endpoint by time after randomization within treatment groups will be based on the method of Kaplan and Meier[52]. We will also use the proportional hazards model to control for baseline factors that might influence the rate of the primary endpoint (e.g. age, race, gender, baseline comorbidities, and concomitant medications), as control for these variables may yield more efficient estimates of relative treatment effects[53]. If Kaplan-Meier plots of event free survival by study time, or related plots of log(-log)(survival), indicate violations of the proportional hazards assumption, or a formal test of trend in the scaled Schoenfeld residuals indicates such a violation, then weighted log-rank tests will be used according to strategies described by Pecková and Fleming[54]. However, even in the presence of an apparent violation of the proportional hazards assumption, the primary analysis described above gives a valid (although perhaps not optimal) test of the main trial hypothesis and will remain the primary analytic strategy, with these weighted log-rank tests serving as sensitivity analyses.

5.2. Sample Size and Power

Sample size and power for CIRT have been estimated under several alternative assumptions about the rate of the primary endpoint in the placebo group and the likely reduction in this rate among those in the methotrexate group. All estimates are based on a two-sided log-rank test comparing the time to recurrence between two treatment groups at the 0.05 significance level. These estimates use the approach of Lachin and Foulkes under the assumption of a uniform hazard and to account for attrition due to drop-out[55]. The following assumptions have been made:

(a) Based on previous trials in individuals with a prior myocardial infarction, and considering the increased rate of major cardiovascular events associated with either diabetes or metabolic syndrome, it is anticipated that annual event rates between 3.25 and 4.0 per 100 person-years in the placebo group.

(b) A clinically meaningful reduction in the rate of the primary endpoint is assumed to be in the range from 25% to 35%.

(c) The recruitment period will be split into a ramp-up period, followed by steady-state randomization. Each randomized patient will be asked to continue blinded treatment until study completion.
(d) As these patients have strong affiliations with their treatment centers and will have been tested in a run-in period, low rates of loss to follow-up are anticipated. Power calculations assume a 5% annual rate of loss to follow-up.

Under these assumptions Lachin and Foulkes show that the power of the trial with N total randomized subjects in the methotrexate and placebo groups combined is

$$Power = \Phi^{-1}\left(\sqrt{N}\left(\lambda_c - \lambda_e - 3.92\sqrt{\Psi(\bar{\lambda})}\right) / \left(2\Psi(\lambda_e) + 2\Psi(\lambda_c)\right)^{1/2}\right)$$

where Φ is the standard normal distribution function,

$\Psi(\lambda) = \lambda(\lambda + .05) / (1 - [\exp(-2(\lambda + .05)) - \exp(-4(\lambda + .05)]/(2(\lambda + .05))$,

λ_e is the incidence rate in the methotrexate group,

λ_c is the incidence rate in the placebo group

$\bar{\lambda} = .5\lambda_e + .5\lambda_c$

follow-up for uncensored subjects without events ranges from 2 to 4 years.

Table 4 shows the power of CIRT to detect alternative relative hazard rates in an active treatment group with 3500 patients compared to 3500 patients who receive placebo. The trial has good power (>90%) to detect hazard reductions of 25% or greater for the range of reasonable event rates in the placebo group, and power of 95% or greater to detect a reduction of 25% or greater if event rates in the placebo group are 4.0 per 100 person-years or greater.

These power calculations are based on intention to treat analyses of observed event rates. As such, they incorporate the effects of non-compliance. We estimate, based on experience observed in other trials, that, in addition to those who drop out, 10% of the methotrexate group will discontinue active therapy but that none of the placebo group will initiate open-label therapy (drop-in). The impact of non-compliance on power can be evaluated from interpolation using Table 4. For example, if the true rate of major cardiovascular events in persons meeting eligibility criteria but not on methotrexate is 3.5 per 100-person years, and fully compliant methotrexate reduces this rate by 30%, we estimate a rate of the primary endpoint of 2.555 per 100 person-years in the methotrexate group and 3.5 per 100 person-years in the placebo group. This would correspond to an observed 27% reduction in the active treatment group relative to placebo with the above non-compliance and drop-in rates. The proposed trial would thus have power above 90% to detect such a true effect. However, we base primary evaluation of study power on observed event rates and intention to treat analyses, as summarized in Table 4.

Table 4. Power of CIRT for alternative event rates and effect sizes in this proposal

	Rate of Major Cardiovascular Events in the Placebo Group (per 100 person-years)			
Relative Rate	3.25	3.5	3.75	4.0
.75	91%	93%	94%	95%
.7	98%	99%	99%	99%
.65	>99%	>99%	>99%	>99%

Another perspective on sample size estimation based on the above formulation of enrollment indicates that, regardless of the rate of major cardiovascular events in the placebo group, the trial must accrue 514 total confirmed major cardiovascular events in order to have 90% power to detect a 25% reduction in this rate, based on a two-sided test with alpha=0.05. Given a conservative interim monitoring plan such as that described below, then the approach of Reboussin et al[56] indicates that sample size needs to be increased by 1.9% to maintain 90% power in the presence of monitoring. We therefore stipulate that the trial will require accrual of 530 total primary events. With respect to the above assumptions on accrual and drop out, and the range of event rates in the placebo group shown in the above table, the proposed trial with

7,000 randomized subjects would be expected to accrue between 529 endpoints (placebo event rate=0.0325/100 person-years) and 645 endpoints (placebo event rate=0.040/100 person-years) under the assumption of a 25% reduction in hazard associated with methotrexate.

5.3. Stratification and Randomization

Patients willing and eligible to be randomized will be stratified by time since their confirmed index myocardial infarction (< 6 months vs. ≥ 6 months), the presence of diabetes or metabolic syndrome at entry, and by site.

5.4. Interim Feasibility Analysis

Per NHLBI agreement, an interim feasibility analysis will be performed jointly by the DSMB and the NHLBI after 1000 patients have been randomized and followed for at least 6 months. At this time, trial feasibility and safety will be reviewed, the trial dose range will be evaluated, suitability of the trial algorithms considered, and recommendations will be made to the investigators with regard to any needed protocol changes at that time.

Thus, for purposes of the feasibility analysis, the investigators will submit a report to the NHLBI including the cumulative recruitment experience to date, the adherence among randomized participants, the rates of adverse events both overall and according to the major disease categories and the percent of participants willing to continue. It is expected that this report will pool information across treatment groups (i.e. maintain the treatment blind). However, a parallel report with information by treatment group will be submitted simultaneously to the trial's Independent Data and Safety Monitoring Board.

5.5. Interim DSMB Analyses

Interim analyses of rates of the primary outcome, as well as rates of the individual components of the composite endpoint, and the pre-specified secondary endpoints will be prepared for presentation to the Data and Safety Monitoring Board (DSMB). Reports to the DSMB will also include comparisons of baseline characteristics between treatment groups, displays of cumulative recruitment by study time, comparisons of post-randomization laboratory values by treatment group, and rates of adverse events, both overall and within systems, by treatment group. The frequency of the meetings will be determined by the independent DSMB appointed by the NHLBI.

While the frequency of meetings and the approach to interim monitoring will be the choice of the DSMB, we anticipate at least twice yearly meetings to monitor recruitment and retention, with quarterly safety reports. To preserve alpha and to minimize the likelihood of an inflated effect estimate associated with early stopping, pre-planned efficacy analyses will occur only upon accrual of 50% and 75% of the planned study endpoints, i.e. 265 and 398 confirmed primary endpoints. Additional interim analyses of efficacy data may be carried out by the DSMB. The design of the trial, including evaluation of the implications of interim monitoring on study power, considered that stopping boundaries would be based on an alpha-spending function that approximates an O'Brien-Fleming boundary. Specifically, efficacy monitoring would utilize the Lan-DeMets procedure with spending function $\alpha(t^*)=2-2\Phi(Z\alpha/2/\sqrt{t^*})$, where t^* is the information fraction, Φ is the standard normal distribution function, α is the two-sided type 1 error rate, and $Z\alpha/2$ is its $100(1-\alpha/2)$th percentile. Under this approach, the Z-values for the boundaries at the 50% and 75% information times would be ±2.963 and ±2.359, respectively, corresponding to two-sided P-values of 0.0030 and 0.0183, and observed hazard ratios of 0.695 and 0.789, respectively.

The DSMB has voted to prefer more conservative boundaries, and chose a P-value of 0.0001 at both information times, which corresponds to a Z-value of ±3.891. Approximate hazard ratios associated with this Z-value are 0.620 at 50% information and 0.677 at 75% information.

As a guideline for considering a recommendation to stop the study early because of convincing evidence of inefficacy (futility), pre-planned inefficacy bounds will also be considered upon accrual of 50% and again upon accrual of 75% of the targeted numbers of confirmed primary endpoints, i.e. upon accrual of 265 and 398 confirmed primary endpoints. Based upon the Linear 20% Inefficacy Boundary approach described by Freidlin, Korn, and Gray (Clin Trials 2010; 7: 197-208), the inefficacy boundary will be crossed if the observed relative hazard of the primary endpoint associated with methotrexate assignment is greater than 0.99 at the first interim futility analysis, or is greater than 0.97 at the second interim futility analysis. Simulations performed by Freidlin et al indicate that their Linear 20% Inefficacy Boundary approach is associated with a less than 1% loss of power due to inefficacy monitoring. Further, their approach is more conservative than a 10 or 30% conditional power approach in later follow-up (i.e. after 70% of information is accrued). However, the Linear 20% Inefficacy Boundary approach is more aggressive than a 10% (or even a 20%) conditional power rule at the 50% information accrual point, so a more conservative boundary of an observed relative hazard associated with methotrexate assignment greater than 1.11 at the first interim futility analysis (the cutpoint associated with conditional power below 10%), may be preferred at that time, especially as use of this cutpoint will preserve power.

5.6. Secondary Analysis

In addition to the primary comparisons of methotrexate treatment with placebo, pre-specified secondary endpoints (Section 2.2) will also be compared between treatment groups.

Additional analyses will separately evaluate whether the relative effects of methotrexate versus placebo on primary and secondary endpoints is uniform over the follow-up period. These evaluations will be based on the tests for significant interactions between study time and treatments proposed by Cox[51] as well as consideration of trends in scaled Schoenfeld residuals in the proportional hazards model. Specifically, residuals will be plotted and a significant rank correlation of residuals with time will be indicative of a changing effect[60]. In the presence of significant correlation, separate effects by time period will be reported. However, even with a significant correlation of residuals with time, the best overall estimate of the effect of treatment will be the estimate obtained from the proportional hazards model without the interaction.

Separate proportional hazards models will also be used to compare the effects of methotrexate treatment on time to each of the individual components of the composite endpoint. Analyses will use methods of competing risks survival analysis and compare the relative effects of randomized treatments on the different components of the composite outcome[61,62]. The approach of Lunn and McNeil[61] provides a readily accessible implementation of a classical approach to competing risk analysis developed by Kalbfleisch and Prentice[63].

While all primary analyses are on an intention-to-treat basis, CIRT is also functioning as a proof of concept trial and thus analyses of those compliant with the respective LDM or placebo regimens will also be conducted in secondary analyses.

Longitudinal analyses will quantify the impact of methotrexate on lipid levels, biomarkers of inflammation, and change in HbA1c, with the latter analyses stratified by presence of diabetes at baseline. We will also assess whether any effects on these biomarkers mediate observed benefits or risks of LDM on clinical outcomes in the trial.

5.7. Subgroup Analyses

Additional planned exploratory analyses include evaluation of whether treatment effects vary across categories of baseline covariates including age, gender, race, presence of diabetes or metabolic syndrome at baseline, baseline lipid levels, baseline inflammatory biomarker levels, baseline background treatments including those known to interact with LDM therapy, and nutritional measures related to adenosine function such as estimated daily total caffeine intake. Within each subgroup, we will use proportional hazards models to estimate the relative rate of the primary endpoint associated with active treatment versus placebo. Both crude analyses and models including limited baseline covariates will be fitted. To test for the significance of modification of treatment effects by a baseline characteristic, we will include interaction terms between this characteristic and treatment in the proportional hazards model, with statistical significance determined by a likelihood ratio test comparing models with and without the interaction terms between treatment and the categories of a specific covariate.

We will conduct these subgroup analyses regardless of whether or not the overall analyses of treatment effects are significant. Our approach to interpretation of subgroup analyses has always been very cautious and recognizes that, even in large trials, it is not likely to be possible to identify reliably subgroups of patients in whom treatment is especially effective or ineffective. In the absence of prior evidence for real heterogeneity, the overall trial result may provide the best evidence for the presence of a benefit in a subgroup. Our approach thus corresponds to an informal empirical Bayes procedure in which effects in an outlying subgroup require interpretation in light of the overall treatment effect.

5.8. Ancillary Studies

A number of CIRT ancillary studies are planned. These studies include but are not limited to a proposal to develop an evidence-based approach to monitoring the safety of LDM in a large population; a proposal to examine lipid, inflammatory, metabolic, and myocardial biomarkers as determinants of risk in a secondary prevention population; a proposal to determine the effect of LDM on echocardiographic indices of aortic stenosis; a proposal to determine the effect of LDM on the ankle brachial index and incident peripheral artery disease; a proposal to examine the genetic determinants of LDM safety and efficacy; and a proposal to examine the impact of LDM on non-alcoholic fatty liver disease and hepatic inflammation. Additional planned ancillary studies include proposals focused on anemia, cognitive decline, depression, and vascular function. For any ancillary study that requires participant interactions beyond that described in the main trial, additional IRB approval and informed consent will be obtained.

6.0. REFERENCES

1. Ross R. Atherosclerosis--an inflammatory disease. *The New England Journal of Medicine.* 1999;340(2):115-126.
2. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* 2009;54(23):2129-2138.
3. Ridker PM. The time for cardiovascular inflammation reduction trials has arrived: how low to go for hsCRP? *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2008;28(7):1222-1224.
4. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). *Journal of thrombosis and haemostasis : JTH.* 2009;7 Suppl 1:332-339.
5. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet.*375(9709):132-140.
6. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *The New England journal of medicine.* 2008;359(21):2195-2207.
7. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. 1995. *Atherosclerosis.Supplements.* 2004;5(3):91-97.
8. Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA : the journal of the American Medical Association.* 1998;279(20):1615-1622.
9. Ridker PM, MacFadyen J, Libby P, Glynn RJ. Relation of baseline high-sensitivity C-reactive protein level to cardiovascular outcomes with rosuvastatin in the Justification for Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER). *Am J Cardiol.*106(2):204-209.
10. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet.* 2009;373(9670):1175-1182.
11. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1999;100(3):230-235.
12. Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1998;98(9):839-844.
13. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *The New England journal of medicine.* 2001;344(26):1959-1965.
14. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *The New England Journal of Medicine.* 2005;352(1):20-28.
15. Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation.* 2006;114(4):281-288.
16. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *The New England journal of medicine.* 2005;352(1):29-38.
17. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nature reviews.Drug discovery.* 2005;4(12):977-987.
18. Arnaud C, Braunersreuther V, Mach F. Toward immunomodulatory and anti-inflammatory properties of statins. *Trends in cardiovascular medicine.* 2005;15(6):202-206.

19. Saag KG, Teng GG, Patkar NM, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis and Rheumatism.* 2008;59(6):762-784.
20. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacological reviews.* 2005;57(2):163-172.
21. Hirshberg B, Muszkat M, Schlesinger O, Rubinow A. Safety of low dose methotrexate in elderly patients with rheumatoid arthritis. *Postgraduate medical journal.* 2000;76(902):787-789.
22. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet.* 2002;359(9313):1173-1177.
23. van Halm VP, Nurmohamed MT, Twisk JW, Dijkmans BA, Voskuyl AE. Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study. *Arthritis research & therapy.* 2006;8(5):R151.
24. Prodanovich S, Ma F, Taylor JR, Pezon C, Fasihi T, Kirsner RS. Methotrexate reduces incidence of vascular diseases in veterans with psoriasis or rheumatoid arthritis. *Journal of the American Academy of Dermatology.* 2005;52(2):262-267.
25. Solomon DH, Avorn J, Katz JN, et al. Immunosuppressive medications and hospitalization for cardiovascular events in patients with rheumatoid arthritis. *Arthritis and Rheumatism.* 2006;54(12):3790-3798.
26. Suissa S, Bernatsky S, Hudson M. Antirheumatic drug use and the risk of acute myocardial infarction. *Arthritis and Rheumatism.* 2006;55(4):531-536.
27. Naranjo A, Sokka T, Descalzo MA, et al. Cardiovascular disease in patients with rheumatoid arthritis: results from the QUEST-RA study. *Arthritis research & therapy.* 2008;10(2):R30.
28. Hochberg MC, Johnston SS, John AK. The incidence and prevalence of extra-articular and systemic manifestations in a cohort of newly-diagnosed patients with rheumatoid arthritis between 1999 and 2006. *Curr Med Res Opin.* 2008;24(2):469-480.
29. Westlake SL, Colebatch AN, Baird J, et al. The effect of methotrexate on cardiovascular disease in patients with rheumatoid arthritis: a systematic literature review. *Rheumatology (Oxford).*49(2):295-307.
30. Bernatsky S, Hudson M, Suissa S. Anti-rheumatic drug use and risk of hospitalization for congestive heart failure in rheumatoid arthritis. *Rheumatology (Oxford).* 2005;44(5):677-680.
31. Solomon DH, Goodson NJ, Katz JN, et al. Patterns of cardiovascular risk in rheumatoid arthritis. *Annals of the Rheumatic Diseases.* 2006;65(12):1608-1612.
32. Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. *Jama.* 2006;296(14):1735-1741.
33. Roman MJ, Salmon JE. Cardiovascular manifestations of rheumatologic diseases. *Circulation.* 2007;116(20):2346-2355.
34. Reiss AB, Carsons SE, Anwar K, et al. Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages. *Arthritis Rheum.* 2008;58(12):3675-3683.
35. Coomes E, Chan ES, Reiss AB. Methotrexate in atherogenesis and cholesterol metabolism. *Cholesterol.*2011:503028.
36. Chan ES, Cronstein BN. Methotrexate--how does it really work? *Nat Rev Rheumatol.*6(3):175-178.
37. Chen DY, Chih HM, Lan JL, Chang HY, Chen WW, Chiang EP. Blood lipid profiles and peripheral blood mononuclear cell cholesterol metabolism gene expression in patients with and without methotrexate treatment. *BMC Med.*9:4.
38. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. *Clinical immunology (Orlando, Fla.).* 2005;114(2):154-163.
39. Gerards AH, de Lathouder S, de Groot ER, Dijkmans BA, Aarden LA. Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. *Rheumatology (Oxford, England).* 2003;42(10):1189-1196.
40. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol.*11(2):98-107.

41. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA.* 2001;286(3):327-334.

42. Girman CJ, Rhodes T, Mercuri M, et al. The metabolic syndrome and risk of major coronary events in the Scandinavian Simvastatin Survival Study (4S) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Am J Cardiol.* 2004;93(2):136-141.

43. Schwartz GG, Olsson AG, Szarek M, Sasiela WJ. Relation of characteristics of metabolic syndrome to short-term prognosis and effects of intensive statin therapy after acute coronary syndrome: an analysis of the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial. *Diabetes Care.* 2005;28(10):2508-2513.

44. Aguilar D, Fisher MR, O'Connor CM, et al. Metabolic syndrome, C-reactive protein, and prognosis in patients with established coronary artery disease. *Am Heart J.* 2006;152(2):298-304.

45. Deedwania P, Barter P, Carmena R, et al. Reduction of low-density lipoprotein cholesterol in patients with coronary heart disease and metabolic syndrome: analysis of the Treating to New Targets study. *Lancet.* 2006;368(9539):919-928.

46. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol.*56(14):1113-1132.

47. Goldberg RB, Mellies MJ, Sacks FM, et al. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. The Care Investigators. *Circulation.* 1998;98(23):2513-2519.

48. Keech A, Colquhoun D, Best J, et al. Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose: results from the LIPID trial. *Diabetes Care.* 2003;26(10):2713-2721.

49. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet.* 2003;361(9374):2005-2016.

50. Senn S. Baseline adjustment in longitudinal studies. In: Armitage P, Colton T, eds. *Biostatistics*. 2 ed2005:294-297.

51. Cox D. Regression models and life tables (with discussion). *J Roy Statist Soc Ser B.* 1972;34(2):187-220.

52. Kaplan E, Meir P. Nonparametric estimation from incomplete observations. *J Am Statist Assoc.* 1958;53:457-481.

53. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat Med.* 2002;21(19):2917-2930.

54. Peckova M, Fleming TR. Adaptive test for testing the difference in survival distributions. *Lifetime Data Anal.* 2003;9(3):223-238.

55. Lachin JM, Foulkes MA. Evaluation of sample size and power for analyses of survival with allowance for nonuniform patient entry, losses to follow-up, noncompliance, and stratification. *Biometrics.* 1986;42(3):507-519.

56. Reboussin DM, DeMets DL, Kim KM, Lan KK. Computations for group sequential boundaries using the Lan-DeMets spending function method. *Control Clin Trials.* 2000;21(3):190-207.

57. Lan K, DeMets D. Discrete sequential boundaries for clinical trials. *Biometrika.* 1983;76:659-663.

58. Freidlin B, Korn EL, Gray R. A general inefficacy interim monitoring rule for randomized clinical trials. *Clin Trials.* 2010;7(3):197-208.

59. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *The New England Journal of Medicine.* 2011;365(24):2255-2267.

60. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika.* 1994;81(3):515-526.

61. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics.* 1995;51:524-532.

62. Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. *Am J Epidemiol.* 2005;162(10):975-982.

63. Kalbfleisch J, Prentice R. *The Statistical Analysis of Failure Time Data*. New York: John Wiley and Sons; 1980.

PROTOCOL
V1.7

Cardiovascular Inflammation Reduction Trial (CIRT):

A randomized, double-blind, placebo-controlled, event-driven trial of weekly low-dose methotrexate (LDM) in the prevention of cardiovascular events among stable coronary artery disease patients with type 2 diabetes or metabolic syndrome

Center for Cardiovascular Disease Prevention
Brigham and Women's Hospital

Funding: National Heart Lung and Blood Institute

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IND 116831

Abbreviations

ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1
AE	Adverse event
AHA	American Heart Association
ALT	Alanine aminotrasferase
AST	Aspartate aminotransferase
CBC	Complete blood count
CCC	Clinical Coordinating Center
CEC	Clinical Endpoints Committee
CIRT	Cardiovascular Inflammation Reduction Trial
CrCl	Creatinine clearance
CRP	C reactive protein
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
DVT	Deep venous thrombosis
eGFR	Estimate glomerular filtration rate
HbA1c	Hemoglobin A1c
HDL-C	High density lipoprotein cholesterol
HY27	Cholesterol 27-hydroxylase
IL-1	Interleukin-1
IL1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IRB	Institutional review board
GFR	Glomerular filtration rate
LDM	Low dose methotrexate
LDL-C	Low density lipoprotein cholesterol
LLN	Lower limit of normal
NHLBI	National Heart Lung and Blood Institute
PE	Pulmonary embolus
RA	Rheumatoid arthritis
SAE	Serious adverse event
TNF	Tumor necrosis factor
ULN	Upper limit of normal
WBC	White blood cell count

1.0. TRIAL OVERVIEW

While inflammation contributes crucially to atherothrombosis, it is unknown whether inhibition of inflammation per se will lower vascular event rates. The primary aim of the **Cardiovascular Inflammation Reduction Trial** (CIRT) is to directly test the inflammatory hypothesis of atherothrombosis by evaluating whether or not low-dose methotrexate (LDM) will reduce rates of myocardial infarction, stroke, and cardiovascular death among stable coronary artery disease patients with type 2 diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response. CIRT is a randomized, double-blind, placebo-controlled, multi-center, event-driven trial that will randomize 7,000 men and women from the United States and Canada. Following a five- to six-week open-label run-in (maximum 8 weeks), eligible participants who have either suffered documented myocardial infarction in the past or have angiographically demonstrated multivessel coronary artery disease in the past will be randomly allocated over a three to four year period to usual care plus placebo or usual care plus LDM. The target methotrexate dose among those allocated to active therapy is 15 to 20 mg po per week, a dose within the range of that commonly used for the treatment of rheumatoid arthritis. All study participants will additionally receive 1.0 mg oral folate to be taken daily six days per week. LDM complications will be minimized through education programs for all investigators and coordinators, through enhanced communication with study participants, by limiting enrollment to those with no evidence of malignancy, hepatitis, renal dysfunction, chronic infection, pulmonary disease, or other risk factors for toxicity; by conducting an initial 5- to 6-week active-therapy run-in (maximum 8 weeks) designed to eliminate individuals who are either intolerant of or unable to adhere to treatment before randomization; and through regular monitoring of liver function and hematologic indices using a centralized methodology designed to ensure participant safety, allow for dose adjustments while maintaining the study blind, and provide an efficient method to address issues of compliance and follow-up on a cost-effective centralized basis. The primary trial endpoint is the rate of myocardial infarction, stroke, or cardiovascular death. Secondary and tertiary endpoints include all-cause mortality, coronary revascularization, incident congestive heart failure, incident peripheral artery disease, incident venous thrombosis, clinically significant aortic stenosis, incident atrial fibrillation, incident diabetes among those with metabolic syndrome but not diabetes at study entry, and hemoglobin A1c (HbA1c) control among those with diabetes at study entry. The trial is event driven such that in the absence of extreme effects, the trial will conclude after accrual of at least 530 primary endpoints, an effect estimated to provide 90 percent power to detect a 25 percent relative risk reduction. The potential clinical impact of CIRT is broad as it has sufficient power to directly address core issues in the inflammatory hypothesis of atherothrombosis, and thus, if successful, will open major new directions for cardiovascular treatment.

2.0. SPECIFIC AIMS

Abundant laboratory and translational data demonstrate that inflammation plays a major role in all stages of the atherothrombotic process[1,2]. These observations have generated the hypothesis that targeted anti-inflammatory therapy can lower vascular event rates. To date, however, no clinical trial has directly addressed this critical biologic hypothesis[3,4].

The primary scientific aim of CIRT is to directly test the inflammatory hypothesis of atherothrombosis. Specifically, CIRT will evaluate whether LDM will reduce rates of myocardial infarction, stroke, or cardiovascular death among patients with a recent history of coronary artery disease and either type 2 diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response. LDM is an effective anti-inflammatory therapy widely

used to treat rheumatoid arthritis that lowers plasma levels of interleukin-6 (IL-6), tumor necrosis factor (TNF), and C-reactive protein (CRP) but does not otherwise have beneficial effects on lipids or biomarkers of hemostasis and thrombosis. Thus, a randomized trial of LDM provides an innovative approach to target inflammation while minimizing confounding effects that might accrue from activation or inhibition of alternative vascular pathways. The wide use of LDM as a mainstay in current therapy for rheumatoid arthritis provides both guidelines for safety monitoring and strong evidence that off-target toxicity is unlikely to be uncovered during the course of this trial.

2.1.Primary Aim

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM given at a target dose of 15 to 20 mg po weekly will reduce rates of *myocardial infarction, stroke, or cardiovascular death* among patients with a prior history of coronary artery disease and either type 2 diabetes or metabolic syndrome.

2.2. Secondary Aims

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of *all-cause mortality* among patients with a prior history of coronary artery disease and either type 2 diabetes or metabolic syndrome.
b. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus coronary revascularization*.
c. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rates of *hospitalization for congestive heart failure*.
d. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus all-cause mortality plus coronary revascularization plus congestive heart failure*.
e. To determine in a randomized, double-blind, placebo-controlled setting the side effect profile of LDM in a non-rheumatologic population at risk for vascular events. By so doing, CIRT will evaluate the *net clinical benefit or harm* that might accrue from the hypothesized use of LDM as a novel method for the secondary prevention of myocardial infarction, stroke, and cardiovascular death.
f. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rate of *new onset type 2 diabetes* among those with metabolic syndrome but not diabetes at study entry.

2.3. Tertiary Aims

In addition, we will determine in a randomized, double-blind, placebo-controlled setting whether LDM will

a. reduce rates of the individual components of the primary endpoint
b. reduce rates of the primary endpoint plus unstable angina requiring unplanned coronary revascularization
c. reduce rates of coronary revascularization
d. reduce rates of peripheral artery disease
e. reduce rates of symptomatic deep vein thrombosis or pulmonary embolism, including those considered to be provoked and those considered to be idiopathic
f. reduce rates of clinically significant aortic stenosis

g. reduce rates of atrial fibrillation
h. have positive or negative effects on standardized measures of quality of life and global health status

2.4.Proposed Exploratory and Mechanistic Studies

CIRT will include 7,000 patients with either myocardial infarction within the past or angiographically demonstrated multivessel coronary artery disease within the past who have either type 2 diabetes or metabolic syndrome. Clinical endpoints of interest that will be prospectively evaluated include incident age-related macular degeneration, sleep apnea, and nephropathy and retinopathy. In addition, a plasma and DNA bank will be established as part of the trial protocol. Thus, cohort accrual and biobanking also allows for the evaluation of several tertiary aims that relate to mechanisms of effect using measured plasma biomarkers of inflammation and glucose metabolism, as well as potential genetic determinants of LDM activity.

a. To evaluate the effect of LDM as compared to placebo on a series of inflammatory biomarkers such as IL-6, TNF, CRP, interleukin-1 (IL-1), and interleukin-1 receptor antagonist (IL1ra), and to ascertain whether any effects on these biomarkers mediate observed benefits or risks of LDM on clinical outcomes in the trial.
b. To evaluate whether genetic polymorphisms associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits or risks of LDM on clinical outcomes observed in the trial.
c. Among subjects with baseline diabetes, to evaluate the effect of LDM as compared to placebo on indices of diabetic progression and glycemic control such as need for diabetes treatment intensification, proportion of subjects achieving optimal glycemic control (HbA1c<7.0%), and change in HbA1c overall and by study visit.

3.0. BACKGROUND AND SIGNIFICANCE

3.1. The Inflammatory Hypothesis of Atherothrombosis

Abundant laboratory evidence indicates that inflammation plays a major role in all stages of atherothrombosis[1,2]. With regard to the translation of inflammation biology to practice, as demonstrated in a comprehensive 2010 meta-analysis, clinical evidence from 54 prospective cohort studies demonstrates that inflammatory biomarkers independently predict vascular risk with a magnitude of effect at least as large as that of blood pressure or cholesterol[5]. However, while the attributable vascular risk associated with inflammation is large and while animal models using targeted anti-inflammatory therapies have shown promise, it remains unknown whether inhibition of inflammation per se will lower vascular event rates.

Despite the importance of this question, no endpoint trial addressing these issues has been initiated. However, the recent JUPITER investigation comparing rosuvastatin to placebo among 17,802 individuals with low levels of low-density lipoprotein cholesterol (LDL-C) but who were at elevated vascular risk on the basis of a pro-inflammatory response reinforces the need for such a trial[6]. In brief, JUPITER demonstrated a 44 percent reduction in major vascular events, which included a 54 percent reduction in myocardial infarction, a 48 percent reduction in stroke, a 46 percent reduction in arterial revascularization, a 43 percent reduction in deep venous thrombosis/pulmonary embolism (DVT/PE), and a 20 percent reduction in mortality. Underscoring the importance of inflammation as a pathophysiologic factor in selecting this study population, the placebo event rate in JUPITER was higher than that of prior prevention trials

limited to those with overt hyperlipidemia[7,8]. Further, within JUPITER, the greatest absolute risk and the greatest absolute risk reduction was observed among those with the highest levels of persistent pro-inflammatory response[9].

From an inflammatory biology perspective, prospective analyses from JUPITER also suggest that achieving low levels of inflammation may be an important treatment goal in a manner analogous to achieving low levels of LDL-C. In pre-specified analyses designed to evaluate the relative effects of inflammation reduction as compared to LDL-C reduction, the JUPITER data suggest that the benefits of statin therapy are magnified among those who not only reduce LDL-C, but who also substantially reduce inflammatory biomarkers[10]. As such, JUPITER prospectively confirms prior data from the CARE[11,12], AFCAPS/TexCAPS[13], PROVE IT – TIMI 22[14], A to Z[15], and REVERSAL[16] trials that best clinical outcomes accrue in statin treated patients who achieve low levels of inflammation as well as cholesterol. All of these data corroborate laboratory evidence of anti-inflammatory properties of statins including reduced cell adhesion and monocyte recruitment to the arterial wall; reduced prenylation of small G proteins and augmented expression of the transcription factor KLF2 with consequent mitigation of inflammatory and thrombotic mediators; altered smooth muscle migration in developing plaques; favorable effects on metalloproteinase expression; and in human hepatocytes, reductions in IL-6 and other cytokines[17,18]. However, statins markedly lower LDL-C as well as reduce inflammation. Thus, although suggestive, these data cannot address whether lowering inflammation alone will lower vascular risk.

3.2. Low-Dose Methotrexate (LDM) and Cardiovascular Disease

A direct test of the inflammatory hypothesis of atherothrombosis requires an agent that (a) inhibits inflammation without having major impact on other components of the atherothrombotic process, and (b) has an acceptable safety profile for evaluation in a large-scale randomized trial[3,4]. LDM has multiple attributes that make it an appropriate agent to test directly the inflammatory hypothesis of atherothrombosis.

First, LDM (range 10 to 30 mg per week) is widely used, has an enviable safety profile among patients with rheumatoid arthritis and psoriasis, and comprehensive guidelines from the American College of Rheumatology exist regarding dosing regimens, drug monitoring, and the identification of high-risk patient subgroups[19-21]. This experience greatly reduces the potential for unanticipated off-target toxicity. The target methotrexate dose in CIRT is 15 to 20 mg po per week, a dose within the range commonly used for the treatment of rheumatoid arthritis.

Second, LDM reduces several inflammatory biomarkers including CRP, IL-6, and TNF-alpha in populations of patients with rheumatoid arthritis (RA) and psoriasis, patient groups at elevated vascular risk on an inflammatory basis. Further, LDM does not have substantive effects on lipid levels, hemostasis, or platelet function. Thus, LDM provides a mechanism to test the inflammatory hypothesis of atherothrombosis without confounding effects on other vascular pathways.

Third, among both rheumatoid arthritis and psoriasis patients assessed in seven cohort and case-control settings[22-28], available observational epidemiologic data suggest that exposure to LDM is associated with reductions in cardiovascular morbidity and mortality, even though those receiving LDM have worse vascular risk factor profiles, data strongly mitigating against indication bias (Table 1). These data have been verified in a recent systematic overview[29]. Of interest, the cardiovascular benefit of LDM was observed despite the fact that patients initiating treatment (mean dose = 13 mg/week) had significantly worse prognostic factors for mortality and significantly worse RA symptoms than did patients not being treated with LDM. Other observational studies of RA patients taking LDM have shown improvement in heart failure[30] and reduction in carotid intima media thickness[28]. The consistent observation of excess vascular risk unexplained by traditional risk factors among those with rheumatoid arthritis or psoriasis also supports the conceptual basis for a trial of anti-inflammatory therapy among those with a persistent enhancement of the innate immune response[31-33].

Table 1. Low Dose Methotrexate and CVD: Observational Evidence

Cohort	Group	HR* (95 % CI)	Endpoint	Exposure
Wichita	**RA**	**0.4 (0.2 - 0.8)**	**Total Mortality**	**LDM**
Choi 2002		**0.3 (0.2 - 0.7)**	**CV Mortality**	**LDM**
		0.4 (0.3 – 0.8)	**CV Mortality**	**LDM < 15 mg/wk**
Netherlands	**RA**	**0.3 (0.1 – 0.7)**	**CVD**	**LDM**
van Helm 2006				
Miami VA	**Psoriasis**	**0.7 (0.6 – 0.9)**	**CVD**	**LDM**
Pradanovich 2005		**0.5 (0.3 – 0.8)**	**CVD**	**LDM < 15 mg/wk**
	RA	**0.8 (0.7 – 1.0)**	**CVD**	**LDM**
		0.6 (0.5 – 0.8)	**CVD**	**LDM < 15 mg/wk**
CORRONA	**RA**	**0.6 (0.3 – 1.2)**	**CVD**	**LDM**
Solomon 2006				
QUEST-RA	**RA**	**0.85 (0.8 – 0.9)**	**CVD**	**LDM**
Narango 2008		**0.82 (0.7 – 0.9)**	**MI**	**LDM**
		0.89 (0.8 - 1.0)	**Stroke**	**LDM**
Insurance				
Hochberg 2008	**RA**	**0.65 (0.59-0.72)**	**CVD**	**LDM**
UK Norfolk	**RA, PSA**	**0.6 (0.4 – 1.0)**	**Total Mortality**	**LDM**
2008		**0.5 (0.3 – 1.1)**	**CV Mortality**	**LDM**

Fourth, mechanistic studies suggest that atheroprotective effects of methotrexate may accrue from enhanced release of adenosine which in turn leads to facilitation of cholesterol efflux and reverse cholesterol transport from arterial wall foam cells[34] via upregulated expression of cholesterol 27-hydroxylase (HY27) and the ATP-binding cassette transporter (ABCA1)[35,36]. Recent data indicating enhanced gene expression of HY27 and ABCA1 with clinical use of methotrexate also supports this emerging hypothesis[37]. Other work suggests that methotrexate has direct effects on apoptosis and on the suppression of adhesion molecule function, both of which play relevant roles in atherothrombosis[38,39].

Finally, LDM is a generic, inexpensive therapy given orally as a once-weekly agent allowing for the efficient and safe conduct of a large simple trial. This simplicity has been incorporated into CIRT in such a way that ongoing safety evaluations can use a centralized methodology that improves participant safety, maintains the study blind while allowing for in-trial dose adjustments, and provides an efficient method to address issues of compliance and follow-up on a cost-effective centralized basis.

3.3. Diabetes, Metabolic Syndrome, and Inflammation

Coronary artery disease patients with diabetes or metabolic syndrome have an enhanced pro-inflammatory response and are at high vascular risk, thus providing an excellent target population for CIRT. Further, as is the case for atherothrombosis, the core

pathophysiologic basis underlying insulin resistance and diabetes is hypothesized to entail fundamental abnormalities of the innate immune response[40]. From a clinical perspective, plasma levels of several inflammatory biomarkers increase with increasing numbers of components of metabolic syndrome and several inflammatory biomarkers including IL-6 predict incident type 2 diabetes[41].

With regard to the ability of metabolic syndrome to define a secondary prevention population at increased risk of vascular events, Table 2 presents data from the 4S, MIRACL, WIZARD, and TNT trials[42-45]. As shown, among those with metabolic syndrome as compared to those without, the hazard ratios for recurrent cardiovascular disease all approximate 1.4 (95%CI 1.2-1.7). Similarly, in a recent meta-analysis of 87 studies, metabolic syndrome was associated with a 2-fold increase in cardiovascular events and a 1.5 fold increase in all-cause mortality[46].

Table 2. Summary of the Association between MetS and Subsequent Cardiovascular Events in Secondary Prevention Trials

Study Population	Cohort Inception Year	Sample Size	Average Follow-Up (yr)	Baseline Prevalence	Outcome	Event Rate		RR/HR
						MetS	No MetS	
4S	1988	2223*	5.4	20%	CVD	7.2 per 1000 person-months	5.2 per 1000 person-months	1.41 (1.16-1.71
MIRACL	1997	3038	0.3	38%	CHD	19.2%	14.3%	1.40 (1.16-1.67)
WIZARD	1997	3319	3.1	53.3	CHD	28.1%	21.1%	1.33 (1.15-1.53)
TNT	1998	10001	4.9	56%	CVD	11.3%	8.0%	1.44 (1.26-1.64)

* Estimates provided for placebo arm only

With regard to the ability of diabetes to define a secondary prevention population at high risk of vascular events, Table 3 presents data from the 4S, CARE, LIPID, and HPS trials[42,47-49]. As shown, among those with diabetes as compared to those without, the hazard ratios for cardiovascular events are again in a range 1.4 to 1.6 when compared to that of individuals without diabetes.

Table 3. Summary of Association between Diabetes and Subsequent CVD Events in Secondary Prevention Trials

Study Population	Cohort Inception Year	Sample Size	Average Follow-Up (yr)	Baseline DM Prevalence	Outcome	Event Rate DM	Event Rate No DM	RR/HR
4S	1988	2223*	5.4	10.4	CVD	NA	NA	1.62 (1.29-2.03)
CARE	1989	4159	5.0	14.1	CHD	19.1	10.5	NA
LIPID	1990	9014	6.1	11.9	CVD	48.9	36.5	1.4 (1.3-1.5)
HPS	1994	20536	5.0	29.0	CHD	35.6	22.7	NA

* Estimates provided for placebo arm only; NA - not available

3.4. Pharmacology of Low Dose Methotrexate (LDM)

LDM is taken weekly by tens of thousands of patients with similar age and co-morbidity profiles as those likely to be enrolled in CIRT. Introduced as a treatment for rheumatoid arthritis in 1951, LDM has an enviable safety and efficacy profile that has allowed it to remain the dominant disease modifying therapy for RA. A clinically relevant anti-inflammatory effect is rapidly achieved for the majority of RA patients at weekly oral doses between 10 and 30 mg. Food minimally affects LDM absorption so the drug can be taken in fasting or non-fasting states. Circulating methotrexate is less than 50 percent protein bound and has minimal interaction with most concomitant medications, including statins, aspirin, beta-blockers, and inhibitors of the renin-angiotensin system. However, there is increased risk when methotrexate is used in combination with folate depleting drugs such as bactrim (trimethoprim-sulfamethoxazole) or

drugs that affect tubular secretion (probenecid). Thus, study participants will be alerted to this potential toxicity and those with allergies that make use of alternative agents impossible will be excluded.

All study participants (including those on placebo) will receive supplementary folic acid (1.0 mg 6 days per week), a simple adjunct known to reduce side-effects of LDM and improve long-term compliance that itself has no direct effect on vascular risk.

Methotrexate is primarily cleared by the kidneys, with 80 to 90 percent being excreted in urine. As such, reduction in creatinine clearance (CrCl) is a determinant of serum levels and systemic toxicity. Patients with baseline CrCl less than 40 ml/min will not be entered into CIRT, and follow-up CrCl will be measured on a regular basis so that drug can be discontinued or dose-reduced in the event of incident renal failure; this safety criterion is conservative as the American College of Rheumatology allows LDM use even when CrCl is reduced to 30 ml/min.

3.5. Safety of LDM and Efforts to Reduce In-Trial Toxicity

The wide use of LDM in clinical practice makes it unlikely that any unknown off-target toxicities will appear during the conduct of CIRT. Further, risk factors associated with LDM toxicity are well known and formal guidelines have been issued by the American College of Rheumatology outlining patient groups where therapy is ill-advised[19]. Broadly, patients with hepatitis, renal dysfunction, chronic infections, and certain pulmonary conditions have increased risk, and these groups are excluded from study participation, as are patients with a significant history of alcohol consumption. As methotrexate can sequester in fluid spaces, participants with known chronic pericardial effusion, pleural effusion, or ascites will not be included in the trial. Because of the fetopathic and teratogenic effects of methotrexate, women of childbearing potential or who intend to breastfeed will not be included in the trial.

The vast majority of life-threatening hepatotoxicity, pulmonary damage, and myelosuppression that have been reported with methotrexate occur at the very high doses used during treatment of malignancy (where methotrexate is dosed cyclically by the gram or more). In the dose range to be used in CIRT (target dose 15 to 20 mg per week), such life-threatening complications are rare. Nonetheless, to reduce the chance of such occurrence within CIRT, screening for hepatitis B and C will be conducted before enrollment and patients who are positive for chronic infection will be excluded. In addition, monitoring of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, creatinine clearance (CrCl), and complete blood count (CBC) will be done throughout the study (monthly for the first 6 months after randomization, then at least bi-monthly for the trial duration). Rarely, in the setting of high dose methotrexate (as used in chemotherapy), hypospermia and the potential for chromosomal damage to sperm has been reported. While it is uncertain if these effects occur with low dose methotrexate, to protect the safety of all study participants and their sexual partners, effective contraception will be recommended during the trial and for six months after a participant completes the trial. Men who intend to father children during the trial period will not be enrolled.

All participants must lack significant pulmonary disease at enrollment, and surveillance questionnaires seeking symptoms of pulmonary disease will be given every four months during study follow-up. A chest X-ray in the 12 months prior to enrollment must be free of evidence of interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis. In instances where a chest X-ray is not available in the prior 12 months, a baseline chest X-ray will be obtained as part of the study protocol. With regard to potential nephrotoxicity, enrollment will be limited to those with CrCl≥ 40 ml/min with dose adjustments or discontinuation built into the protocol in the event of renal deterioration. The protocol also incorporates short-term suspension of LDM for patients with intercurrrent infections, those receiving antibiotic therapy, those undergoing surgery, and those who develop new clinically significant pericardial effusion, pleural effusion, or ascites. All

of these steps are consistent with or more conservative than guidelines issued by the American College of Rheumatology for use of LDM[19].

Five aspects of the CIRT protocol are specifically designed to minimize these issues and maximize long-term follow-up and compliance. First, an active therapy 5- to 6-week run-in (maximum 8 weeks) has been incorporated into CIRT so that any individuals with short-term intolerance to LDM will be excluded prior to randomization. Second, the CIRT protocol targets a maximal methotrexate dose of 15 to 20 mg po weekly, a dose well within the range of that commonly used in the treatment of rheumatoid arthritis. Titration to the 20 mg weekly dose will only occur among those trial participants who have tolerated without complication the 15 mg weekly dose for a minimum of three months. Third, the protocol titration algorithms and drug packaging (calendar blister packs) have been designed in such a way that in response to either clinical need, participant report of adverse effects, or to any monitored laboratory evidence of hepatic, renal, acute infection, or hematologic abnormality, dosing for individual participants can be discontinued on a short term basis or reduced to either a 5, 10 or 15 mg dose/wk as tolerated. After clinical resolution and a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated in 5 mg increments toward the maximal target dose of 20 mg po weekly. Fourth, targeted face-to-face education programs will be required for all study investigators at the investigator meetings, followed by mandatory web-based safety seminars; these educational programs will be provided by the rheumatologic members of the CIRT Steering Committee and will ensure a high level of drug safety awareness among all trial physicians and staff. Fifth, an ongoing communication program is incorporated into CIRT which allows the investigative team to stay in regular contact with trial participants, withhold study drug for fevers or inter-current infection, systematically evaluate subjective symptoms, and ensure an almost continuous flow of safety data throughout the trial experience. This regular communication will also improve compliance and a sense of community for individual participants.

4.0. INVESTIGATIONAL PLAN

4.1. Study Design - Overview

CIRT is a randomized, double-blind, placebo-controlled, event-driven trial of LDM (target dose 15 to 20 mg/wk) in the secondary prevention of myocardial infarction, stroke, and cardiovascular death among stable coronary artery disease patients who have either diagnosed type 2 diabetes or who meet the formal 2004 American Heart Association (AHA) / National Heart Lung and Blood Institute (NHLBI) definition of metabolic syndrome which 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. All trial participants will have had either a documented myocardial infarction within the past or angiographically demonstrated multivessel coronary artery disease **in the past** and be clinically stable for at least 60 days after the qualifying event. The qualifying myocardial infarction is the most recent myocardial infarction that meets criteria for the diagnosis of myocardial infarction. The qualifying coronary angiogram is the most recent angiogram that meets the criteria for the diagnosis of multivessel coronary artery disease, defined as the presence of a stent, bypass graft, or ≥ 60 percent stenosis in at least two major epicardial vessels. Left main coronary artery disease 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. Any planned coronary revascularization procedures associated with the qualifying myocardial infarction or angiogram must also be completed at least 60 days prior to enrollment.

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 nuisance side effects that can be associated with LDM but that has been shown in multiple major trials to have no vascular benefit so there will be no confounding due to folate use.

Following American College of Rheumatology Guidelines for use of LDM[19], acceptable levels of white blood cell count, hematocrit, platelets, CrCl, and liver function (cutoffs provided in Exclusion Criteria, section 4.4), as well as negative screens for hepatitis B and C will be required prior to active run-in. Individuals with known hepatic disease, chronic pulmonary disease (specifically: interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis), or HIV related disease will be excluded. A chest X-ray in the 12 months prior to enrollment must be free of evidence of interstitial pneumonitis, bronchiectasis, or pulmonary fibrosis. Individuals with known chronic pericardial effusion, pleural effusion, or ascites will be excluded. 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. During the 5- to 6- week (maximum 8-week) open-label run-in, all potentially eligible study participants will initiate LDM at a dose of 5 mg in week one. In the absence of adverse effects or intolerance, this 5 mg dose will be increased to 10 mg for the second and third week of the run-in phase, and then to 15 mg for the fourth week. At the end of this 5- to 6-week run-in period safety labs will be repeated. Only those participants who demonstrate compliance during this one-month run in and who are free of side effects with no significant changes in hematologic or hepatic indices will be considered randomizable to active therapy or to placebo.

Once randomized to active therapy or placebo, all study participants over the first 6 months of the trial will undergo monthly evaluation for CBC, ALT, AST, albumin, and CrCl using blinded and centralized study procedures that have been designed both to ensure patient safety and allow for drug discontinuation or dose-adjustment, as well as ongoing evaluations of study compliance. At 4 months, those participants randomly allocated to active LDM who have tolerated the 15 mg dose without complication will be titrated up to the target dose of 20 mg LDM weekly. Six months after randomization, the blood-based safety evaluations will occur at least bi-monthly until trial completion. Pre-labeled calendar blister packs similar in design to that used in the run-in will be used throughout the study to improve compliance and reduce complexity. To maintain the blind as much as possible, sham titrations will be conducted in the placebo group proportionate to the number of actual titrations required in the active treatment group.

For any participants developing side effects or in whom laboratory abnormalities develop, the protocol allows for drug discontinuation and/or down-titration. The protocol also builds in procedures for short-term discontinuation of LDM in circumstances such as fever, antibiotic therapy for bacterial infections, during and immediately after surgical procedures, or if new clinically significant pericardial effusion, pleural effusion, or ascites develops. Most of these steps will be managed centrally to protect the study blind, ensure common compliance across study sites, and allow centralized rapid management of any safety issues on an immediate basis. After clinical resolution of any events that lead to drug discontinuation and after a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated over time back toward the maximal target dose of 20 mg po weekly, as tolerated. Procedures for these dose adjustments, temporary drug discontinuation, and re-initiation of study drug are described in detail in the trial algorithms.

An electronic data capture (EDC) system will be developed and used to collect and

transmit source data throughout the course of the trial. This system is 21 CFR part 11 compliant and meets all relevant governmental regulations. The system will be maintained at SOCAR Research, an independent clinical research organization. System functionality will be thoroughly tested and validated prior to implementation. Furthermore, to ensure compliance with standards of use of electronic trial data, standard operating procedures will be maintained for the use of the system, an audit trail of data changes will ensure that there is no modification of entered data without documentation, and security systems will be maintained to protect against unauthorized access. Furthermore, adequate procedures will be used to backup the data and safeguard the blinding of the study. As original observations are entered by clinic staff directly into the computerized system, the electronic record is considered the source document.The EDC system will thus be used in each of the following steps to create, modify, maintain, archive, retrieve and/or transmit source data: 1) creation of case report forms, 2) resolution of data discrepancies through data queries and checks, 3) implementation of the study drug titration, 4) monitoring of drug distribution, 5) reporting of adverse events and endpoints, and 6) endpoint adjudication

4.2. Study Population

CIRT will randomize 7,000 men and women, age 18 years and over, who have either suffered a documented myocardial infarction within the past or have multivessel coronary disease by angiography in the past, have completed any planned coronary revascularization procedures associated with the qualifying event, have been on a stable secondary prevention regimen for a minimum of 60 days, and have either a clinical diagnosis of type 2 diabetes or metabolic syndrome.

For purposes of this trial, the formal 2004 AHA/NHLBI definition of metabolic syndrome will be used and requires evidence that any 3 of the following 5 diagnostic criteria are present: waist circumference ≥102 cm in men or≥ 88 cm in women; triglycerides ≥ 150 mg/dl (1.7 mmol/L) or on drug treatment for elevated triglycerides (fibrates, nicotinic acid, or omega 3 fatty acids); HDL-C < 40 mg/dL in men or < 50 mg/dL in women or on drug treatment for reduced HDL-C (fibrates or nicotinic acid); 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.

4.3. Inclusion Criteria

a. Age ≥ 18 years at screening;
b. Documented **past history of** myocardial infarction OR **past** evidence of multivessel coronary artery disease by angiography.
    i. To qualify on the basis of a **past history** myocardial infarction**,** the event must be documented either by hospital records or by evidence on current ECG of Q waves in two contiguous leads and/or an imaging test demonstrating wall motion abnormality or scar. The patient must also have completed any planned coronary revascularization procedures associated with the qualifying event, and be clinically stable for at least 60 days prior to screening.
    ii. To qualify on the basis of multivessel coronary disease, there must be **past** angiographic evidence of atherosclerosis in at least 2 major epicardial vessels defined either as the presence of a stent, a coronary bypass graft, or an angiographic lesion of 60% or greater. Left main coronary artery disease 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. The patient must also have completed any planned coronary revascularization procedures associated with the qualifying event, and be clinically stable for at least 60 days prior to screening.

c. History of type 2 diabetes or metabolic syndrome at time of study enrollment;
d. Willingness to participate as evidenced by signing the study informed consent.

4.4. 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.

4.5. Open Label Run-In

For all potentially eligible patients who provide informed consent and declare interest in participation, baseline ALT, AST, hepatitis screens, CrCl, albumin, and CBC will be obtained prior to the 5- to 6-week (maximum 8-week) active run-in phase to ensure they meet trial enrollment criteria. A urine sample will be assayed for albumin and creatinine. A blood sample for plasma and buffy coat will also be shipped to the central lab for long-term storage and to

provide a bank for future blood-based biomarker and genetic sub-studies. Quality of life questionnaires will also be administered.

A 5- to 6-week (maximum 8-week) course of open-label LDM will then be given to all eligible study participants in a pre-randomization compliance and tolerability run-in. During this run-in, participants will be given an initial 5 mg oral weekly LDM dose along with an adequate supply of 1.0 mg folic acid to be taken 6 days per week using similar drug calendar packs to those used throughout the active trial period. In the absence of adverse effects or intolerance, this 5 mg dose will be increased to 10 mg for the second and third week of the run-in phase, and then to 15 mg for the fourth and subsequent weeks. This run-in will allow subjects who are poorly compliant, who develop any immediate side effects, or who withdraw informed consent to be excluded before randomization. Participants must tolerate LDM 15 mg two weeks in a row in order to continue in the trial. All subjects who successfully tolerate LDM 15 mg two weeks in a row and are still willing to be randomized will have repeat blood evaluation for AST, ALT, albumin, CBC, and CrCl, and any individuals with substantive changes in these parameters will be excluded prior to randomization. Urine for albumin and creatinine will also be collected. The run-in will thus enhance long-term compliance and eliminate risk of exposure for any individuals with immediate intolerance to LDM.

4.6. Randomization Procedures

All participants who successfully complete the run-in will be eligible for randomization to the study drug calendar blister packages that contain either active LDM or placebo as well as active tablets containing 1.0 mg folic acid to be taken daily 6 days per week. The randomization visit will involve collection of key medical, social, and anthropometric information from the participant, as well as a brief physical examination including assessment of the participant's level of physical function. The responsible site investigator will use an interactive computer system for randomization. The randomization process will involve stratifying participants by time since the qualifying event (< 6 months or ≥ 6 months from either the date of the MI or most recent angiogram), type of event (myocardial infarction or multivessel coronary disease), presence of either diabetes or metabolic syndrome, and by site. All post-randomization safety monitoring and dose adjustments will be performed using a standardized centrally run system that has been designed to ensure patient safety, maintain double-blinding while allowing for dose titration or discontinuation in both the active and placebo groups, provide a central mechanism for tracking patient compliance, and that will allow for efficient follow-up and eventual study close-out. To reduce inadvertent trial unblinding and further ensure patient safety, procedures have been designed to allow for more frequent blood ascertainment at all clinical sites on an as needed basis such as might occur during a concomitant infection or change in participant status (see Manual of Operations).

4.7. Cohort Follow up and Clinic Visits

All subjects will be seen by the study physician at the time of randomization. From that point forward throughout the trial, all participants will be required to see a member of the randomizing physician's study team at least once every 4 months in addition to the regularly scheduled laboratory and symptom monitoring procedures described below. At those visits, compliance (measured by pill count) as well as evidence of side effects will be ascertained by self-report, and in the absence of a study endpoint or laboratory abnormality, a new 4-month supply of study drug (or blinded placebo) provided. A Patient Contact Information Form will be updated at each of these 4-month visits to facilitate long-term follow-up.

Cohort follow-up will include a visit form filled out at each of these appointments. Information will similarly be sought concerning trial endpoints, issues of compliance will be

reviewed, anthropometric measures and brief physical examination including reassessment of physical function will be performed, participant questions answered, the drug supply ensured, and any outstanding study forms completed and/or updated. In addition, signs or symptoms of early drug toxicity will be assessed, as well as the occurrence of any primary or secondary trial endpoints or other clinical events of interest. Nephropathy, a clinical event of interest, will be assessed by collecting urine for albumin and creatinine at 8 months, 12 months, and then every 6 months after randomization. Since these data will be obtained in an identical manner from participants randomly allocated to LDM as well as to placebo, this procedure will also provide a mechanism for monitoring by the Data Coordinating Center (DCC) and reports to the Data and Safety and Monitoring Board (DSMB) of any differences between treatment groups with regard to side effects or efficacy. At each regularly scheduled safety lab visit (once a month for the first 6 months after randomization, then at least bi-monthly), participants will be asked about clinical symptoms and side effects of the study medication so that study drug dosage may be adjusted or temporarily stopped if necessary. In order to better understand the toxicities of methotrexate, at 6 months a blood sample will be collected for the measurement of key metabolites of methotrexate.

For the first 6 months post-randomization, monthly blinded laboratory evaluations for ALT, AST, albumin, CBC, and CrCl will be obtained; after that time, evaluation will be done on at least a bi-monthly basis until trial completion. As described in the study algorithms, laboratory values that cross a pre-defined safety threshold (for example, a decline in the total WBC count to < 3,000/ul) will trigger a call to the subject to inform the participant not to take the following week's dose of LDM and to ascertain any signs or symptoms of potential toxicity. As described in the study algorithms, temporary suspension of study drug will occur whenever subjects are being treated with antibiotics, have a clinical infection, develop new clinically significant effusion or ascites, or are scheduled for surgery. Similarly, patients will be informed not to take the next scheduled study medication if they develop a serious unexplained cough or shortness of breath suggestive of interstitial lung disease, or if they develop stomatitis, vomiting, or persistent fever. Details of the methotrexate titration algorithm, including up- and down-titration, temporary study drug interruption, and permanent study drug discontinuation are included in the trial algorithms. The responsible physician will be informed of any changes in study drug dosing.

After any temporary discontinuation of study drug, study algorithms will be used to determine if and when re-initiation of study medication can occur. If a participant's study medication is repeatedly stopped because of laboratory abnormalities or clinical symptoms, the medical monitor will review the case to consider a permanent discontinuation of study medication. As described in the study algorithms, permanent drug discontinuation can also be done at the discretion of the responsible physician.

4.8. Monitoring Participant Laboratory Values and Subsequent Dose Adjustments

In addition to the algorithms designed for safety monitoring in this trial, an additional safety feature of CIRT is that when certain laboratory thresholds are crossed, the physician responsible for the subject will be notified.

The specific thresholds for drug discontinuation, re-initiation, down-titration, and up-titration are described in detail in the trial algorithms that will be used to monitor safety and make dose changes across the study on a central and consistent basis. No increase in weekly dose will occur unless all of the following criterion are met: the WBC count is ≥ 3,500/ul, the platelet count is ≥ 75,000/ul, the CrCl is ≥40ml/min, ALT/AST levels are ≤ 1.5x ULN, hematocrit is ≥27 percent, and there are no clinically important symptoms (defined as stomatitis, diarrhea, vomiting, or cough either productive of sputum or associated with a fever or with severe shortness of breath). As also described in the study algorithms, a reduction in dose will occur if

there are changes in some of these parameters that may suggest early toxicity but that are not severe enough to warrant drug discontinuation. For example, for a subject taking 20 mg of the study drug, while a drop in the platelet count below 50,000 will lead to temporary drug discontinuation, a reduction below 75,000 but above 50,000 would lead to a 5 mg decrease in the current weekly dosing. After clinical resolution of any events that lead to drug discontinuation and after a return of all laboratory abnormalities to a safe range, study drug will be re-initiated and up-titrated over time in 5 mg increments back toward the maximal target dose of 20 mg po weekly, as tolerated. Responsible physicians will be informed of any changes in study drug dosing recommended by the clinical coordinating center. Adverse events, patient symptoms, laboratory abnormalities, or other reasons for discontinuation of or reduction in dose of study drug will be collected through case report forms and monitored centrally by the Data Coordinating Center (DCC).

4.9. Monitoring Participant Symptoms and Subsequent Dose Adjustments

Participants will have regularly scheduled visits with the local investigator during which the study staff will carefully assess the patients for any signs or symptoms of drug toxicity, in addition to the occurrence of any trial endpoints. A questionnaire designed to capture information about drug toxicity (including symptoms of a pulmonary, infectious, gastrointestinal, hematologic, dermatologic, and other toxicities), intercurrent hospitalizations, and elective or planned surgical procedures will be administered to all study participants at the time of regularly scheduled safety lab draws (once a month for the first 6 months after randomization, then at least bi-monthly).

The questionnaire will be administered both by local study staff via the telephone or in person. Study algorithms will combine data from recent laboratory safety evaluations with patient reported symptoms to determine study drug dosing and/or cessation, including sham placebo dose adjustments. Responsible physicians will be informed of any changes in the study medication dosing. As noted above, adverse events, patient symptoms, laboratory abnormalities, or other reasons for discontinuation of or reduction in dose of study drug will be collected through case report forms and monitored centrally by the Data Coordinating Center (DCC).

Study coordinators are given instructions to contact the Medical Monitor for symptoms or signs that raise the possibility of drug toxicity, including the development of new clinically significant effusions or ascites. As noted above, in order to better understand the toxicities of methotrexate, at 6 months a blood sample will be collected from all participants for the measurement of key metabolites of methotrexate. Furthermore, if requested by the DSMB, aggregate data on adverse events, stratified by blinded treatment group, will be reviewed on a quarterly basis by the Chair of the DSMB, who may then request a more detailed analysis and/or forward the data to the full Board.

4.10. Medical Monitor

A Medical Monitor will be on call 24 hours a day 7 days each week. A senior board-certified rheumatologist with expertise in drug safety (Senior Rheumatologist) will supervise the Monitors. The Monitors will be rheumatologists (fellows or junior faculty) who will be trained by the Senior Rheumatologist and will be accessible via pager. The Monitors will be referred subjects based on the pre-laboratory questionnaire or laboratory abnormalities and will have access to the subjects' laboratory data and clinical information via the EDC system. The Monitors will have the option of unblinding the treatment allocation of a subject, if the symptoms and acuity of illness make doing so warranted.

Monitors will help sites determine whether the symptoms being described by patients are concerning, whether interruptions in study drug are warranted, and whether re-initiating study drug is safe. While all Monitors will have experience with methotrexate prescribing, the Senior Rheumatologist will compile a list of scenarios as they occur that will allow the Monitors to have a consistent approach. The Monitors as a group will meet monthly with the Senior Rheumatologist to discuss these scenarios. As the trial progresses, these meetings are anticipated to occur every quarter.

4.11. Endpoint Definitions and Documentation

The primary endpoint is defined as the time to the first adjudication committee confirmed major adverse cardiovascular event (MACE) occurring during the double-blind treatment period, which is a composite of CV death, non-fatal MI, and stroke.

An independent Clinical Endpoints Committee (CEC) will review and adjudicate all clinical events that constitute the primary composite endpoint (CV deaths, non-fatal MI, and stroke). In addition, the CEC will adjudicate a number of secondary endpoints, including all deaths, all hospitalizations for unstable angina requiring unplanned revascularization, all hospitalizations for congestive heart failure, and all arterial revascularization procedures. The CEC will be blinded to treatment assignment. Formal definitions for the individual components of the primary endpoint, hospitalization for unstable angina requiring unplanned revascularization, hospitalizations for congestive heart failure, and arterial revascularization are provided in the Clinical Endpoint Committee (CEC) Charter. The secondary and tertiary endpoints listed in sections 2.2 and 2.3 not adjudicated by the CEC will be validated by non-CEC staff. For example, incident diabetes will be confirmed by a combination of patient or physician report, new anti-diabetic medications, or by protocol-based measures of glucose and HbA1c performed at least twice annually. Incident venous thromboembolism will be confirmed using documentation of imaging studies including a venous ultrasound or venogram showing deep venous thrombosis or a pulmonary arteriogram, CT scan, or ventilation-perfusion scan showing pulmonary embolism.

Other non-adjudicated clinical events of interest include microvascular disease (nephropathy and retinopathy), sleep apnea, and age-related macular degeneration.

Full descriptions of the methods for endpoint ascertainment and the specific definitions of each adjudicated trial endpoint are contained in the Clinical Endpoint Charter (CEC).

4.12. Procedures for Emergency Unblinding

As part of the trial monitoring structure, a medical monitor will also be available on a 24 hour basis who has the ability to electronically access individual patient data files should unblinding be needed. All of these instances will be tracked within the EDC system.

4.13. Investigator Education Programs

To ensure that all investigators and study coordinators are comfortable with LDM regardless of specialty area, a face-to-face targeted education program will be provided as part of the trial Investigator Meetings and a series of on-line teleconference/webinars will be provided every 6 months where educational information regarding LDM will be updated and reinforced.

4.14. Drug Compliance Monitoring

The primary measure of compliance will be based on the return of calendar packs to the physician at each of the 4-month office visits (at which time new calendar packs will be issued as long as no trial endpoint or major side-effects have occurred).

As an additional measure of compliance, washed packed red blood cells collected 6 months after randomization will be stored in a central laboratory for measures of methotrexate levels. This will be done using a methotrexate polyglutamate assay that provides a semi-quantitative method of evaluating methotrexate absorption and metabolism. The results of this assay will not be available to blinded study staff until after the study has completed and the database has been locked.

4.15. Central Laboratory and Bio-bank

Blood samples for storage in a bio-bank at Brigham and Women's Hospital will be obtained at the pre-run-in, randomization, and at 8 and 24 months after randomization. Processing and long-term storage of these samples will be done in a liquid nitrogen biobank facility in Boston, which has been used for this purpose in multiple prior NIH-funded trials and large cohort studies. White blood cells from the pre-run-in sample will also be stored in this facility to allow for DNA extraction at a later date. As described in the informed consent documents, participation in the genetic bio-banking portions of CIRT will be on an "opt-out" basis and are not a requirement for participation in the main trial.

4.16. Serious Adverse Events (SAEs)

All subjects will be monitored at the local study sites on a regular basis during the course of study involvement. All SAEs will be reported within 24 hours of local event awareness (but no later than the next business day) to the Data Coordinating Center (DCC). Initial reports will often not be complete. Central study staff will guide the site regarding the completion of a SAE form along with retrieval of the pertinent medical records for determination of a final diagnosis. An updated full written report will be filed as additional information becomes available within 10 working days (14 calendar days). The report will include a complete description of the event, use of all concomitant medications, and the local investigator's assessment of causality of the SAE to study therapy. All serious adverse events will be recorded in the core study database within 1 week of report by the local study site personnel. SAE reporting for all subjects will occur in accordance with the central institutional review board (IRB) requirements. Study staff may contact patients directly to determine the resolution of adverse events.

4.17. Data Coordination and Treatment Masking

The DCC is responsible for the facilitation of prompt evaluation of reported adverse events and symptoms. Upon receipt from a site of a report of specific adverse events, combinations of symptoms, or symptoms persisting over time that meet pre-specified criteria, the data will be combined with recent laboratory assays and the safety monitor will be notified that there is a report requiring evaluation.

The DCC will also provide monthly reports to the Executive Committee. The Progress Report will include: enrollment and randomization rates, overall and by sex and race; reasons for randomization ineligibility; number of completed follow-up visits; number of completed interim safety bloods; number of blood specimens sent to the central repository; number of dose changes; current distribution of doses; number of patients off drug; reasons for discontinuation; number of unblindings and reasons for the unblindings. The Quality Control Report will include tables on inappropriate randomizations; number of missed visits; reasons for missed visits;

number of visits taking place outside of the designated time windows; and rates of missing data broken down by form. Both the Progress Report and the Quality Control Report will pool participants across treatment groups to maintain the study blind.

The DCC also assumes responsibility for maintenance of blinded treatment assignments. Data files with these assignments will be kept on a secure, password protected server housed in a locked, climate-controlled room with restricted access or on a password protected encrypted laptop. To preserve the integrity of the trial, unblinded DCC staff will not participate in decisions to modify the trial protocol after participants have been randomized. The Steering Committee will obtain statistical input for such decisions from other academic statisticians, blinded to treatment assignment, who also work at Harvard Medical School.

4.18. Data and Safety Monitoring Board

Due to the potential risks to study subjects, the size and multi-site nature of the study, and the fact that this is a Phase III clinical trial, the study will have a formal, and independent, Data and Safety Monitoring Board (DSMB). The DSMB will be constituted by the NHLBI and will include at a minimum members from the following general areas: 1) physicians with specific expertise in the management of patients' cardiovascular disease 2) a biostatistician with specific expertise in the design, analysis, and safety monitoring of multi-center clinical trials 3) a medical ethicist, and 4) a physician with specific expertise in rheumatologic disease. The DSMB will report directly to the NHLBI, and would have the responsibility of monitoring outcome measurements/endpoints, adverse events (AE's), and serious adverse events, and recommending termination of the study if it appeared at any point during the trial that subjects (or a subgroup of subjects) were being placed at undue risk as a result of their participation. Aggregate data on adverse events, stratified by blinded treatment group, will be reviewed on a bimonthly basis by the Chair of the DSMB, and can be brought before the entire DSMB for further review if requested by the Chair.

The DSMB will meet (face-to-face or by teleconference) at designated intervals (semi-annually) to review accumulated data on safety and efficacy, and if appropriate, conduct an interim analysis of the data. Data will be prepared by the Data Coordinating Center (DCC) prior to each meeting of the DSMB. Serious Adverse Events (SAEs) will be initially reviewed with DSMB members blinded to treatment group. If, however, aggregate data suggested a trend toward more frequent SAEs among one treatment group, an unblinded interim analysis would be reviewed by the DSMB. Guidelines for the possible early termination of the study will be formulated by the DSMB and a formal charter agreed upon prior to trial enrollment. The proceedings of each meeting of the DSMB will be recorded in minutes. Any patient-specific protected health information reviewed by the DSMB would be kept completely confidential. Access to the unblinded minutes of the DSMB meetings by Executive/Operations Committee Members, Clinical Site Investigators, or members of the DCC, will be prohibited until after the database for the study has been locked and the study has been unblinded. A formal report will be submitted by the DSMB to the NHLBI, with a recommendation that the study be continued, modified in a manner to enhance subject safety, or terminated.

4.19. Trial Conclusion and Close Out Visits

At the close of the trial, all randomized participants will be asked to return to the local study sites to see a member of the physician's study team. Compliance, side effects, and patient contact information will be collected, and study end points will again be assessed by the local study staff. Therapy with study drug will be discontinued, and the patient's remaining

calendar packs of study drug will be collected. While no observational registry is currently planned, the Clinical Coordinating Center may request that local study sites approach study participants about enrolling in a post-trial observational registry.

To assess the impact of LDM withdrawal on diabetes incidence and glycemic control, participants will have one final laboratory evaluation for ascertainment of HbA1c 3 months after discontinuing study drug therapy.

5.0. DATA ANALYSIS PLAN

5.1. Statistical Analysis

The randomized design and large sample size of CIRT should provide balanced distributions of baseline characteristics between the two treatment groups. Nonetheless, initial analyses will be conducted to identify any chance imbalances in these distributions. In particular, these analyses will form part of the routine monitoring of the trial and will be regularly reported to the DSMB. For continuous and ordinal variables, including age and baseline levels of risk factors including lipid levels, blood pressure, and body mass index, comparisons will use the Wilcoxon rank-sum test. For categorical variables, including sex, race, current and former cigarette smoking, diabetes, greater than 1 prior myocardial infarction, stroke, congestive heart failure, atrial fibrillation, peripheral vascular disease, hypertension, and concomitant therapy with statins, aspirin, clopidogrel, warfarin, beta blockers, ACE inhibitors, and angiotensin receptor blockers, comparisons will use Chi-square tests. These hypothesis tests are intended for data monitoring and quality control, and not to determine which baseline covariates to include in efficacy analyses[50].

The primary endpoint of the trial is the time from randomization to the first occurrence of any component of the clinical composite endpoint including myocardial infarction, stroke and cardiovascular death. The primary analysis of the trial will use a likelihood ratio test based on a proportional hazards model stratified on time since qualifying event (≥ 6 months vs. < 6 months), type of event (myocardial infarction vs. multivessel coronary disease), and presence of either diabetes or metabolic syndrome to test the null hypothesis of no association between assignment to active methotrexate and the rate of the primary endpoint. All analyses will classify patients according to their randomized treatment assignment, i.e. according to the intention to treat principle, and will base evaluation of statistical significance on a two-sided test with level 0.05. Secondary analyses will further stratify on study site, although these analyses will likely be less efficient because of sparse strata that will arise because of small numbers of participants from some sites. The estimated relative hazard in the methotrexate group compared to the placebo group with an accompanying 95% confidence interval will quantify the treatment effect[51]. If this relative hazard is less than 1, then 100*(1-estimated relative hazard) will be defined as the percent reduction in hazard associated with methotrexate treatment.

Rates of occurrence of the primary endpoint will be defined as the total number of subjects who have this event in a treatment group per 100 person-years of follow-up, counting all time from randomization until the first of the event, death, end of trial, or withdrawal of consent. Estimates of the probability of the primary endpoint by time after randomization within treatment groups will be based on the method of Kaplan and Meier[52]. We will also use the proportional hazards model to control for baseline factors that might influence the rate of the primary endpoint (e.g. age, race, gender, baseline comorbidities, and concomitant medications), as control for these variables may yield more efficient estimates of relative treatment effects[53]. If Kaplan-Meier plots of event free survival by study time, or related plots of log(-log)(survival), indicate violations of the proportional hazards assumption, or a formal test of trend in the scaled Schoenfeld residuals indicates such a violation, then weighted log-rank tests will be used

according to strategies described by Pecková and Fleming[54]. However, even in the presence of an apparent violation of the proportional hazards assumption, the primary analysis described above gives a valid (although perhaps not optimal) test of the main trial hypothesis and will remain the primary analytic strategy, with these weighted log-rank tests serving as sensitivity analyses.

5.2. Sample Size and Power

Sample size and power for CIRT have been estimated under several alternative assumptions about the rate of the primary endpoint in the placebo group and the likely reduction in this rate among those in the methotrexate group. All estimates are based on a two-sided log-rank test comparing the time to recurrence between two treatment groups at the 0.05 significance level. These estimates use the approach of Lachin and Foulkes under the assumption of a uniform hazard and to account for attrition due to drop-out[55]. The following assumptions have been made:

(a) Based on previous trials in individuals with a prior myocardial infarction or multivessel coronary disease, and considering the increased rate of major cardiovascular events associated with either diabetes or metabolic syndrome, it is anticipated that annual event rates between 3.25 and 4.0 per 100 person-years in the placebo group.

(b) A clinically meaningful reduction in the rate of the primary endpoint is assumed to be in the range from 25% to 35%.

(c) The recruitment period will be split into a ramp-up period, followed by steady-state randomization. Each randomized patient will be asked to continue blinded treatment until study completion.

(d) As these patients have strong affiliations with their treatment centers and will have been tested in a run-in period, low rates of loss to follow-up are anticipated. Power calculations assume a 5% annual rate of loss to follow-up.

Under these assumptions Lachin and Foulkes show that the power of the trial with N total randomized subjects in the methotrexate and placebo groups combined is

$$Power = \Phi^{-1}\left(\sqrt{N}\left(\lambda_c - \lambda_e - 3.92\sqrt{\Psi(\bar{\lambda})}\right) / \left(2\Psi(\lambda_e) + 2\Psi(\lambda_c)\right)^{1/2}\right)$$

where Φ is the standard normal distribution function,

$\Psi(\lambda) = \lambda(\lambda + .05) / (1 - [\exp(-2(\lambda + .05)) - \exp(-4(\lambda + .05)]/(2(\lambda + .05))$,

λ_c is the incidence rate in the methotrexate group,

λ_e is the incidence rate in the placebo group

$\bar{\lambda} = .5\lambda_e + .5\lambda_c$

follow-up for uncensored subjects without events ranges from 2 to 4 years.

Table 4 shows the power of CIRT to detect alternative relative hazard rates in an active treatment group with 3500 patients compared to 3500 patients who receive placebo. The trial has good power (>90%) to detect hazard reductions of 25% or greater for the range of reasonable event rates in the placebo group, and power of 95% or greater to detect a reduction of 25% or greater if event rates in the placebo group are 4.0 per 100 person-years or greater.

These power calculations are based on intention to treat analyses of observed event rates. As such, they incorporate the effects of non-compliance. We estimate, based on experience observed in other trials, that, in addition to those who drop out, 10% of the methotrexate group will discontinue active therapy but that none of the placebo group will initiate open-label therapy (drop-in). The impact of non-compliance on power can be evaluated from interpolation using Table 4. For example, if the true rate of major cardiovascular events in persons meeting eligibility criteria but not on methotrexate is 3.5 per 100-person years, and fully

compliant methotrexate reduces this rate by 30%, we estimate a rate of the primary endpoint of 2.555 per 100 person-years in the methotrexate group and 3.5 per 100 person-years in the placebo group. This would correspond to an observed 27% reduction in the active treatment group relative to placebo with the above non-compliance and drop-in rates. The proposed trial would thus have power above 90% to detect such a true effect. However, we base primary evaluation of study power on observed event rates and intention to treat analyses, as summarized in Table 4.

Table 4. Power of CIRT for alternative event rates and effect sizes in this proposal

	Rate of Major Cardiovascular Events in the Placebo Group (per 100 person-years)			
Relative Rate	3.25	3.5	3.75	4.0
.75	91%	93%	94%	95%
.7	98%	99%	99%	99%
.65	>99%	>99%	>99%	>99%

Another perspective on sample size estimation based on the above formulation of enrollment indicates that, regardless of the rate of major cardiovascular events in the placebo group, the trial must accrue 514 total confirmed major cardiovascular events in order to have 90% power to detect a 25% reduction in this rate, based on a two-sided test with alpha=0.05. Given a conservative interim monitoring plan such as that described below, then the approach of Reboussin et al[56] indicates that sample size needs to be increased by 1.9% to maintain 90% power in the presence of monitoring. We therefore stipulate that the trial will require accrual of 530 total primary events. With respect to the above assumptions on accrual and drop out, and the range of event rates in the placebo group shown in the above table, the proposed trial with 7,000 randomized subjects would be expected to accrue between 529 endpoints (placebo event rate=0.0325/100 person-years) and 645 endpoints (placebo event rate=0.040/100 person-years) under the assumption of a 25% reduction in hazard associated with methotrexate.

5.3. Stratification and Randomization

Patients willing and eligible to be randomized will be stratified by time since their confirmed index event (< 6 months vs. $\geq$ 6 months), the presence of diabetes or metabolic syndrome at entry, type of index event (myocardial infarction or multivessel coronary disease), and by site.

5.4. Interim Feasibility Analysis

Per NHLBI agreement, an interim feasibility analysis will be performed jointly by the DSMB and the NHLBI after 1000 patients have been randomized and followed for at least 6 months. At this time, trial feasibility and safety will be reviewed, the trial dose range will be evaluated, suitability of the trial algorithms considered, and recommendations will be made to the investigators with regard to any needed protocol changes at that time.

Thus, for purposes of the feasibility analysis, the investigators will submit a report to the NHLBI including the cumulative recruitment experience to date, the adherence among randomized participants, the rates of adverse events both overall and according to the major disease categories and the percent of participants willing to continue. It is expected that this report will pool information across treatment groups (i.e. maintain the treatment blind). However, a parallel report with information by treatment group will be submitted simultaneously to the trial's Independent Data and Safety Monitoring Board.

5.5. Interim DSMB Analyses

Interim analyses of rates of the primary outcome, as well as rates of the individual components of the composite endpoint, and the pre-specified secondary endpoints will be prepared for presentation to the Data and Safety Monitoring Board (DSMB). Reports to the DSMB will also include comparisons of baseline characteristics between treatment groups, displays of cumulative recruitment by study time, comparisons of post-randomization laboratory values by treatment group, and rates of adverse events, both overall and within systems, by treatment group. The frequency of the meetings will be determined by the independent DSMB appointed by the NHLBI.

While the frequency of meetings and the approach to interim monitoring will be the choice of the DSMB, we anticipate at least twice yearly meetings to monitor recruitment and retention, with quarterly safety reports. To preserve alpha and to minimize the likelihood of an inflated effect estimate associated with early stopping, pre-planned efficacy analyses will occur only upon accrual of 50% and 75% of the planned study endpoints, i.e. 265 and 398 confirmed primary endpoints. Additional interim analyses of efficacy data may be carried out by the DSMB. The design of the trial, including evaluation of the implications of interim monitoring on study power, considered that stopping boundaries would be based on an alpha-spending function that approximates an O'Brien-Fleming boundary. Specifically, efficacy monitoring would utilize the Lan-DeMets procedure with spending function $\alpha(t^*)=2-2\Phi(Z_{\alpha}/2/\sqrt{t^*})$, where t^* is the information fraction, Φ is the standard normal distribution function, α is the two-sided type 1 error rate, and $Z_{\alpha/2}$ is its 100(1-α/2)th percentile. Under this approach, the Z-values for the boundaries at the 50% and 75% information times would be ±2.963 and ±2.359, respectively, corresponding to two-sided P-values of 0.0030 and 0.0183, and observed hazard ratios of 0.695 and 0.789, respectively.

The DSMB has voted to prefer more conservative boundaries, and chose a P-value of 0.0001 at both information times, which corresponds to a Z-value of ±3.891. Approximate hazard ratios associated with this Z-value are 0.620 at 50% information and 0.677 at 75% information.

As a guideline for considering a recommendation to stop the study early because of convincing evidence of inefficacy (futility), pre-planned inefficacy bounds will also be considered upon accrual of 50% and again upon accrual of 75% of the targeted numbers of confirmed primary endpoints, i.e. upon accrual of 265 and 398 confirmed primary endpoints. Based upon the Linear 20% Inefficacy Boundary approach described by Freidlin, Korn, and Gray (Clin Trials 2010; 7: 197-208), the inefficacy boundary will be crossed if the observed relative hazard of the primary endpoint associated with methotrexate assignment is greater than 0.99 at the first interim futility analysis, or is greater than 0.97 at the second interim futility analysis. Simulations performed by Freidlin et al indicate that their Linear 20% Inefficacy Boundary approach is associated with a less than 1% loss of power due to inefficacy monitoring. Further, their approach is more conservative than a 10 or 30% conditional power approach in later follow-up (i.e. after 70% of information is accrued). However, the Linear 20% Inefficacy Boundary approach is more aggressive than a 10% (or even a 20%) conditional power rule at the 50% information accrual point, so a more conservative boundary of an observed relative hazard associated with methotrexate assignment greater than 1.11 at the first interim futility analysis (the cutpoint associated with conditional power below 10%), may be preferred at that time, especially as use of this cutpoint will preserve power.

5.6. Secondary Analysis

In addition to the primary comparisons of methotrexate treatment with placebo, pre-specified secondary endpoints (Section 2.2) will also be compared between treatment groups.

Additional analyses will separately evaluate whether the relative effects of methotrexate versus placebo on primary and secondary endpoints is uniform over the follow-up period. These evaluations will be based on the tests for significant interactions between study time and treatments proposed by Cox[51] as well as consideration of trends in scaled Schoenfeld residuals in the proportional hazards model. Specifically, residuals will be plotted and a significant rank correlation of residuals with time will be indicative of a changing effect[60]. In the presence of significant correlation, separate effects by time period will be reported. However, even with a significant correlation of residuals with time, the best overall estimate of the effect of treatment will be the estimate obtained from the proportional hazards model without the interaction.

Separate proportional hazards models will also be used to compare the effects of methotrexate treatment on time to each of the individual components of the composite endpoint. Analyses will use methods of competing risks survival analysis and compare the relative effects of randomized treatments on the different components of the composite outcome[61,62]. The approach of Lunn and McNeil[61] provides a readily accessible implementation of a classical approach to competing risk analysis developed by Kalbfleisch and Prentice[63].

While all primary analyses are on an intention-to-treat basis, CIRT is also functioning as a proof of concept trial and thus analyses of those compliant with the respective LDM or placebo regimens will also be conducted in secondary analyses.

Longitudinal analyses will quantify the impact of methotrexate on lipid levels, biomarkers of inflammation, and change in HbA1c, with the latter analyses stratified by presence of diabetes at baseline. We will also assess whether any effects on these biomarkers mediate observed benefits or risks of LDM on clinical outcomes in the trial.

5.7. Subgroup Analyses

Additional planned exploratory analyses include evaluation of whether treatment effects vary across categories of baseline covariates including age, gender, race, presence of diabetes or metabolic syndrome at baseline, index event ≤6 vs. >6 months prior, type of index event (myocardial infarction or multivessel coronary disease), baseline lipid levels, baseline inflammatory biomarker levels, baseline background treatments including those known to interact with LDM therapy, and nutritional measures related to adenosine function such as estimated daily total caffeine intake. Within each subgroup, we will use proportional hazards models to estimate the relative rate of the primary endpoint associated with active treatment versus placebo. Both crude analyses and models including limited baseline covariates will be fitted. To test for the significance of modification of treatment effects by a baseline characteristic, we will include interaction terms between this characteristic and treatment in the proportional hazards model, with statistical significance determined by a likelihood ratio test comparing models with and without the interaction terms between treatment and the categories of a specific covariate.

We will conduct these subgroup analyses regardless of whether or not the overall analyses of treatment effects are significant. Our approach to interpretation of subgroup analyses has always been very cautious and recognizes that, even in large trials, it is not likely to be possible to identify reliably subgroups of patients in whom treatment is especially effective or ineffective. In the absence of prior evidence for real heterogeneity, the overall trial result may provide the best evidence for the presence of a benefit in a subgroup. Our approach thus corresponds to an informal empirical Bayes procedure in which effects in an outlying subgroup require interpretation in light of the overall treatment effect.

5.8. Ancillary Studies

A number of CIRT ancillary studies are planned. These studies include but are not limited to a proposal to develop an evidence-based approach to monitoring the safety of LDM in a large population; a proposal to examine lipid, inflammatory, metabolic, and myocardial biomarkers as determinants of risk in a secondary prevention population; a proposal to determine the effect of LDM on echocardiographic indices of aortic stenosis; a proposal to determine the effect of LDM on the ankle brachial index and incident peripheral artery disease; a proposal to examine the genetic determinants of LDM safety and efficacy; and a proposal to examine the impact of LDM on non-alcoholic fatty liver disease and hepatic inflammation. Additional planned ancillary studies include proposals focused on anemia, cognitive decline, depression, and vascular function. For any ancillary study that requires participant interactions beyond that described in the main trial, additional IRB approval and informed consent will be obtained.

6.0. REFERENCES

1. Ross R. Atherosclerosis--an inflammatory disease. *The New England Journal of Medicine.* 1999;340(2):115-126.
2. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol.* 2009;54(23):2129-2138.
3. Ridker PM. The time for cardiovascular inflammation reduction trials has arrived: how low to go for hsCRP? *Arteriosclerosis, Thrombosis, and Vascular Biology.* 2008;28(7):1222-1224.
4. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). *Journal of thrombosis and haemostasis : JTH.* 2009;7 Suppl 1:332-339.
5. Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet.*375(9709):132-140.
6. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *The New England journal of medicine.* 2008;359(21):2195-2207.
7. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. 1995. *Atherosclerosis.Supplements.* 2004;5(3):91-97.
8. Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA : the journal of the American Medical Association.* 1998;279(20):1615-1622.
9. Ridker PM, MacFadyen J, Libby P, Glynn RJ. Relation of baseline high-sensitivity C-reactive protein level to cardiovascular outcomes with rosuvastatin in the Justification for Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER). *Am J Cardiol.*106(2):204-209.
10. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet.* 2009;373(9670):1175-1182.
11. Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E. Long-term effects of pravastatin on plasma concentration of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1999;100(3):230-235.
12. Ridker PM, Rifai N, Pfeffer MA, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. *Circulation.* 1998;98(9):839-844.
13. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *The New England journal of medicine.* 2001;344(26):1959-1965.
14. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. *The New England Journal of Medicine.* 2005;352(1):20-28.
15. Morrow DA, de Lemos JA, Sabatine MS, et al. Clinical relevance of C-reactive protein during follow-up of patients with acute coronary syndromes in the Aggrastat-to-Zocor Trial. *Circulation.* 2006;114(4):281-288.
16. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. *The New England journal of medicine.* 2005;352(1):29-38.
17. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nature reviews.Drug discovery.* 2005;4(12):977-987.
18. Arnaud C, Braunersreuther V, Mach F. Toward immunomodulatory and anti-inflammatory properties of statins. *Trends in cardiovascular medicine.* 2005;15(6):202-206.
19. Saag KG, Teng GG, Patkar NM, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis and Rheumatism.* 2008;59(6):762-784.
20. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacological reviews.* 2005;57(2):163-172.

21. Hirshberg B, Muszkat M, Schlesinger O, Rubinow A. Safety of low dose methotrexate in elderly patients with rheumatoid arthritis. *Postgraduate medical journal.* 2000;76(902):787-789.

22. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet.* 2002;359(9313):1173-1177.

23. van Halm VP, Nurmohamed MT, Twisk JW, Dijkmans BA, Voskuyl AE. Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study. *Arthritis research & therapy.* 2006;8(5):R151.

24. Prodanovich S, Ma F, Taylor JR, Pezon C, Fasihi T, Kirsner RS. Methotrexate reduces incidence of vascular diseases in veterans with psoriasis or rheumatoid arthritis. *Journal of the American Academy of Dermatology.* 2005;52(2):262-267.

25. Solomon DH, Avorn J, Katz JN, et al. Immunosuppressive medications and hospitalization for cardiovascular events in patients with rheumatoid arthritis. *Arthritis and Rheumatism.* 2006;54(12):3790-3798.

26. Suissa S, Bernatsky S, Hudson M. Antirheumatic drug use and the risk of acute myocardial infarction. *Arthritis and Rheumatism.* 2006;55(4):531-536.

27. Naranjo A, Sokka T, Descalzo MA, et al. Cardiovascular disease in patients with rheumatoid arthritis: results from the QUEST-RA study. *Arthritis research & therapy.* 2008;10(2):R30.

28. Hochberg MC, Johnston SS, John AK. The incidence and prevalence of extra-articular and systemic manifestations in a cohort of newly-diagnosed patients with rheumatoid arthritis between 1999 and 2006. *Curr Med Res Opin.* 2008;24(2):469-480.

29. Westlake SL, Colebatch AN, Baird J, et al. The effect of methotrexate on cardiovascular disease in patients with rheumatoid arthritis: a systematic literature review. *Rheumatology (Oxford).*49(2):295-307.

30. Bernatsky S, Hudson M, Suissa S. Anti-rheumatic drug use and risk of hospitalization for congestive heart failure in rheumatoid arthritis. *Rheumatology (Oxford).* 2005;44(5):677-680.

31. Solomon DH, Goodson NJ, Katz JN, et al. Patterns of cardiovascular risk in rheumatoid arthritis. *Annals of the Rheumatic Diseases.* 2006;65(12):1608-1612.

32. Gelfand JM, Neimann AL, Shin DB, Wang X, Margolis DJ, Troxel AB. Risk of myocardial infarction in patients with psoriasis. *Jama.* 2006;296(14):1735-1741.

33. Roman MJ, Salmon JE. Cardiovascular manifestations of rheumatologic diseases. *Circulation.* 2007;116(20):2346-2355.

34. Reiss AB, Carsons SE, Anwar K, et al. Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages. *Arthritis Rheum.* 2008;58(12):3675-3683.

35. Coomes E, Chan ES, Reiss AB. Methotrexate in atherogenesis and cholesterol metabolism. *Cholesterol.*2011:503028.

36. Chan ES, Cronstein BN. Methotrexate--how does it really work? *Nat Rev Rheumatol.*6(3):175-178.

37. Chen DY, Chih HM, Lan JL, Chang HY, Chen WW, Chiang EP. Blood lipid profiles and peripheral blood mononuclear cell cholesterol metabolism gene expression in patients with and without methotrexate treatment. *BMC Med.*9:4.

38. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action of methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. *Clinical immunology (Orlando, Fla.).* 2005;114(2):154-163.

39. Gerards AH, de Lathouder S, de Groot ER, Dijkmans BA, Aarden LA. Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis. *Rheumatology (Oxford, England).* 2003;42(10):1189-1196.

40. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol.*11(2):98-107.

41. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA.* 2001;286(3):327-334.

42. Girman CJ, Rhodes T, Mercuri M, et al. The metabolic syndrome and risk of major coronary events in the Scandinavian Simvastatin Survival Study (4S) and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Am J Cardiol.* 2004;93(2):136-141.

43. Schwartz GG, Olsson AG, Szarek M, Sasiela WJ. Relation of characteristics of metabolic syndrome to short-term prognosis and effects of intensive statin therapy after acute coronary syndrome: an analysis of the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial. *Diabetes Care.* 2005;28(10):2508-2513.

44. Aguilar D, Fisher MR, O'Connor CM, et al. Metabolic syndrome, C-reactive protein, and prognosis in patients with established coronary artery disease. *Am Heart J.* 2006;152(2):298-304.

45. Deedwania P, Barter P, Carmena R, et al. Reduction of low-density lipoprotein cholesterol in patients with coronary heart disease and metabolic syndrome: analysis of the Treating to New Targets study. *Lancet.* 2006;368(9539):919-928.

46. Mottillo S, Filion KB, Genest J, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol.*56(14):1113-1132.

47. Goldberg RB, Mellies MJ, Sacks FM, et al. Cardiovascular events and their reduction with pravastatin in diabetic and glucose-intolerant myocardial infarction survivors with average cholesterol levels: subgroup analyses in the cholesterol and recurrent events (CARE) trial. The Care Investigators. *Circulation.* 1998;98(23):2513-2519.

48. Keech A, Colquhoun D, Best J, et al. Secondary prevention of cardiovascular events with long-term pravastatin in patients with diabetes or impaired fasting glucose: results from the LIPID trial. *Diabetes Care.* 2003;26(10):2713-2721.

49. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet.* 2003;361(9374):2005-2016.

50. Senn S. Baseline adjustment in longitudinal studies. In: Armitage P, Colton T, eds. *Biostatistics*. 2 ed2005:294-297.

51. Cox D. Regression models and life tables (with discussion). *J Roy Statist Soc Ser B.* 1972;34(2):187-220.

52. Kaplan E, Meir P. Nonparametric estimation from incomplete observations. *J Am Statist Assoc.* 1958;53:457-481.

53. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. *Stat Med.* 2002;21(19):2917-2930.

54. Peckova M, Fleming TR. Adaptive test for testing the difference in survival distributions. *Lifetime Data Anal.* 2003;9(3):223-238.

55. Lachin JM, Foulkes MA. Evaluation of sample size and power for analyses of survival with allowance for nonuniform patient entry, losses to follow-up, noncompliance, and stratification. *Biometrics.* 1986;42(3):507-519.

56. Reboussin DM, DeMets DL, Kim KM, Lan KK. Computations for group sequential boundaries using the Lan-DeMets spending function method. *Control Clin Trials.* 2000;21(3):190-207.

57. Lan K, DeMets D. Discrete sequential boundaries for clinical trials. *Biometrika.* 1983;76:659-663.

58. Freidlin B, Korn EL, Gray R. A general inefficacy interim monitoring rule for randomized clinical trials. *Clin Trials.* 2010;7(3):197-208.

59. Boden WE, Probstfield JL, Anderson T, et al. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *The New England Journal of Medicine.* 2011;365(24):2255-2267.

60. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika.* 1994;81(3):515-526.

61. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics.* 1995;51:524-532.

62. Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. *Am J Epidemiol.* 2005;162(10):975-982.

63. Kalbfleisch J, Prentice R. *The Statistical Analysis of Failure Time Data*. New York: John Wiley and Sons; 1980.

Summary of Changes

Protocol Amendment 1

- To extend CIRT enrollment criterion to include those with multi-vessel coronary artery disease
- To reduce the screening inclusion criterion and threshold for up-titration of drug for the total white blood cell (WBC) count from >= 4,000 to >= 3500

Protocol Amendment 2

- To extend CIRT enrollment criterion to include those with qualifying evens at any time in the past, rather than simply within past 5 years.

Statistical Analysis Plan

Introduction As described in the CIRT primary report, at trial initiation, the primary cardiovascular endpoint was the first occurrence of major adverse cardiovascular events (MACE, inclusive of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death) in a time-to-event analysis. In July 2016, the investigative team petitioned the NHLBI to consider an expansion of the trial primary endpoint to additionally include hospitalization for unstable angina requiring urgent coronary revascularization (MACE-plus) as this endpoint would have greater power and allow for smaller overall enrollment. It was jointly decided to wait for the outcome of CANTOS to see if such a change had scientific merit; when the CANTOS data became available in August of 2017, the NHLBI convened an External Advisory Panel which approved the expanded endpoint in January 2018. Other than the unblinded members of the Data Coordinating Center, who did not participate in this process, no members of the investigative team or the External Advisory Panel were aware of any unblinded trial data.

As a consequence of these intended changes, the anticipated trial sample size was reduced from 7,000 to 5,500 and the targeted number of primary endpoints (now MACE-plus) was increased from 530 to 634 to have 90% power to detect a 23% reduction in the hazard of MACE-plus. However, while approved by the NHLBI External Advisory panel, the formal amendment to alter the protocol had yet to be submitted for IRB approval at the time of the first formal IDSMB meeting occurred to address efficacy and futility (upon accrual of half of the required MACE and MACE-plus endpoints) on March 13, 2018. At that meeting, the IDSMB recommended early termination of the trial based on crossing a pre-specified boundary for futility and absence of evidence for reduction in hsCRP with LD-MTX treatment. The IDSMB further asked for a formal safety follow-up visit for all participants after an additional 6 months of follow-up. These recommendations were accepted by the NHLBI in April 2018.

For the above reasons, the original Statistical Analysis Plan for the trial did not undergo formal revision (and is presented in full below). However, to be consistent with investigator intent and actions taken by the IDSMB, both the MACE and MACE-plus endpoints are presented in the primary report.

Statistical Analysis Plan

Cardiovascular Inflammation Reduction Trial (CIRT):

A randomized, double-blind, placebo-controlled, event-driven trial of weekly low-dose methotrexate (LDM) in the prevention of recurrent cardiovascular events among stable post-myocardial infarction patients with type 2 diabetes or metabolic syndrome

Center for Cardiovascular Disease Prevention
Brigham and Women's Hospital

Funding: National Heart Lung and Blood Institute

March 25, 2013

Abbreviations

ALT	Alanine aminotrasferase
AST	Aspartate aminotransferase
CIRT	Cardiovascular Inflammation Reduction Trial
CRP	C reactive protein
DSMB	Data Safety and Monitoring Board
HbA1c	Hemoglobin A1c
HDL-C	High density lipoprotein cholesterol
ITT	Intention to Treat
LDM	Low dose methotrexate
LDL-C	Low density lipoprotein cholesterol
MACE	Major Adverse Cardiovascular Event
NHLBI	National Heart Lung and Blood Institute
SAE	Serious adverse event
SD	Standard deviation
WBC	White blood cell count

Table of Contents

1.0. STUDY DETAILS

1.1. Study Objectives

The primary aim of the **Cardiovascular Inflammation Reduction Trial** (CIRT) is to directly test the inflammatory hypothesis of atherothrombosis by evaluating whether or not low-dose methotrexate (LDM) will reduce rates of recurrent myocardial infarction, stroke, and cardiovascular death among stable post-myocardial infarction patients with type 2 diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response.

The primary trial endpoint is the rate of recurrent myocardial infarction, stroke, or cardiovascular death. Secondary endpoints include: all-cause mortality, primary endpoint plus coronary revascularization, hospitalization for congestive heart failure, primary endpoint plus all-cause mortality plus coronary revascularization plus congestive heart failure, net clinical benefit or harm, and new onset type 2 diabetes. Tertiary endpoints include: individual components of the primary endpoint, the primary endpoint plus unstable angina requiring unplanned coronary revascularization, coronary revascularization, peripheral artery disease, symptomatic deep vein thrombosis or pulmonary embolism, including those considered to be provoked and those considered to be idiopathic, clinically significant aortic stenosis, atrial fibrillation, and standardized measures of quality of life and global health status.

Additional planned exploratory and mechanistic analyses include evaluation of whether treatment effects vary across categories of baseline covariates including age, gender, race, presence of diabetes or metabolic syndrome at baseline, baseline lipid levels, baseline inflammatory biomarker levels including high-sensitivity C-reactive protein, revascularization status, baseline background treatments which affect adenosine function, and nutritional measures related to adenosine function such as estimated daily total caffeine intake. Clinical endpoints of interest that will be prospectively evaluated include incident age-related macular degeneration, sleep apnea, and nephropathy and retinopathy. Analyses are planned that will evaluate the effect of LDM as compared to placebo on a series of inflammatory biomarkers (hsCRP, IL-6, and others); evaluate whether common genetic variants associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits of LDM; and evaluate the effect of LDM as compared to placebo on indices of diabetes progression (among those with baseline diabetes).

Mediation analyses exploring treatment effect differences by plasma measures of LDM metabolism, by reported LDM adherence, and by achieved reduction in inflammation, are also planned. Longitudinal analyses will quantify the impact of LDM on lipid levels, biomarkers of inflammation, and change in HbA1c, with the latter analyses stratified by presence of diabetes at baseline. Analyses will also evaluate whether genetic polymorphisms associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits or risks of LDM on clinical outcomes observed in the trial.

1.2. Study Design

CIRT is a randomized, double-blind, placebo-controlled, multi-center, event-driven trial. Following an open-label run-in, eligible participants who have suffered documented myocardial infarction in the past five years will be randomly allocated, 1:1, to usual care plus placebo or usual care plus LDM (target dose 15 to 20 mg per week). All study participants will additionally receive 1.0 mg of folic acid 6 days per week. Subjects will be followed over a three to four year period

1.3. Number of Subjects

Approximately 7,000 men and women from the United States and Canada will be enrolled. The trial is event driven such that in the absence of extreme effects, the trial will conclude after accrual of at least 530 primary endpoints, the number needed to provide 90 percent power to detect a 25 percent relative risk reduction with a conservative interim monitoring plan.

Sample size and power for CIRT have been estimated under several alternative assumptions about the rate of the primary endpoint in the placebo group and the likely reduction in this rate among those in the methotrexate group. All estimates are based on a two-sided log-rank test comparing the time to recurrence between two treatment groups at the 0.05 significance level. These estimates use the approach of Lachin and Foulkes under the assumption of a uniform hazard and to account for attrition due to drop-out[1]. The following assumptions have been made:

(a) Based on previous trials in individuals with a prior myocardial infarction, and considering the increased rate of major cardiovascular events associated with either diabetes or metabolic syndrome, it is expected that annual event rates will be between 3.25 and 4.0 per 100 person-years in the placebo group.

(b) A clinically meaningful reduction in the rate of the primary endpoint is assumed to be in the range from 25% to 35%.

(c) The recruitment period will be split into a ramp-up period, followed by steady-state randomization. Each randomized patient will be asked to continue blinded treatment until study completion.

(d) As these patients have strong affiliations with their treatment centers and will have been tested in a run-in period, low rates of loss to follow-up are anticipated. Power calculations assume a 5% annual rate of loss to follow-up.

Under these assumptions Lachin and Foulkes show that the power of the trial with N total randomized subjects in the methotrexate and placebo groups combined is

$$Power = \Phi^{-1}\left(\sqrt{N}\left(\lambda_c - \lambda_e - 3.92\sqrt{\Psi(\bar{\lambda})}\right)/\left(2\Psi(\lambda_e)+2\Psi(\lambda_c)\right)^{1/2}\right)$$

where Φ is the standard normal distribution function,

$\Psi(\lambda) = \lambda(\lambda+.05)/(1-[\exp(-2(\lambda+.05)) - \exp(-4(\lambda+.05)]/(2(\lambda+.05))$,

λ_e is the incidence rate in the methotrexate group,

λ_c is the incidence rate in the placebo group

$\bar{\lambda} = .5\lambda_e + .5\lambda_c$

follow-up for uncensored subjects without events ranges from 2 to 4 years.

Table 1 shows the power of CIRT to detect alternative relative hazard rates in an active treatment group with 3500 patients compared to 3500 patients who receive placebo. The trial has good power (>90%) to detect hazard reductions of 25% or greater for the range of reasonable event rates in the placebo group, and power of 95% or greater to detect a reduction of 25% or greater if

Table 1. Power of CIRT for alternative event rates and effect sizes in this proposal

	Rate of Major Cardiovascular Events in the Placebo Group (per 100 person-years)			
Relative Rate	3.25	3.5	3.75	4.0
.75	91%	93%	94%	95%
.7	98%	99%	99%	99%
.65	>99%	>99%	>99%	>99%

event rates in the placebo group are 4.0 per 100 person-years or greater.

These power calculations are based on intention to treat analyses of observed event rates. As such, they incorporate the effects of non-compliance. We estimate, based on experience observed in other trials, that, in addition to those who drop out, 10% of the methotrexate group will discontinue active therapy but that none of the placebo group will initiate open-label therapy (drop-in). The impact of non-compliance on power can be evaluated from interpolation in the above table. For example, if the true rate of major cardiovascular events in persons meeting eligibility criteria but not on methotrexate is 3.5 per 100-person years, and fully compliant methotrexate reduces this rate by 30%, we estimate a rate of the primary endpoint of 2.555 per 100 person-years in the methotrexate group and 3.5 per 100 person-years in the placebo group. This would correspond to an observed 27% reduction in the active treatment group relative to placebo with the above non-compliance and drop-in rates. The proposed trial would thus have power above 90% to detect such a true effect. However, we base primary evaluation of study power on observed event rates and intention to treat analyses, as summarized in the above table.

Another perspective on sample size estimation based on the above formulation of enrollment indicates that, regardless of the rate of major cardiovascular events in the placebo group, the trial must accrue 514 total confirmed major cardiovascular events in order to have 90% power to detect a 25% reduction in this rate, based on a two-sided test with alpha=0.05. Given a conservative interim monitoring plan such as that described below, then the approach of Reboussin et al[2] indicates that sample size needs to be increased by 1.9% to maintain 90% power in the presence of monitoring. We therefore stipulate that the trial will require accrual of 530 total primary events. With respect to the above assumptions on accrual and drop out, and the range of event rates in the placebo group shown in the above table, the proposed trial with 7,000 randomized subjects would be expected to accrue between 529 endpoints (placebo event rate=3.25/100 person-years) and 645 endpoints (placebo event rate=4.0/100 person-years) under the assumption of a 25% reduction in hazard associated with methotrexate.

2.0. ANALYSIS SETS

2.1. Definition of Analysis Sets

The efficacy analyses will be based on an Intention to Treat (ITT) population, defined as all randomized subjects with any follow-up data, analyzed according to their randomized treatment group. Safety analyses will be based upon the Safety population, defined as all subjects entering the study, analyzed according to treatment actually received. Safety analyses will be presented separately for the run-in period and the period after randomization.

Compliance analyses will be based on the Compliance population, defined as all randomized subjects with compliance measures, analyzed according to compliance levels. The primary measure of compliance will be based on pill counts, with participants characterized according to the percent of study pills taken (with denominator that accounts for the directed dose changes) in the interval since the last compliance assessment.

2.2. Violations and Deviations

Violations of entry criteria will be reported to the Data Safety and Monitoring Board (DSMB).

3.0. PRIMARY, SECONDARY AND TERTIARY AIMS

Primary Aim

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM given at a target dose of 15 to 20 mg po weekly will reduce rates of *recurrent myocardial infarction, stroke, or cardiovascular death* among patients with a prior history of myocardial infarction and either type 2 diabetes or metabolic syndrome.

Secondary Aims

a. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of *all-cause mortality* among patients with a prior history of myocardial infarction and either type 2 diabetes or metabolic syndrome.
b. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus coronary revascularization*.
c. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rates of *hospitalization for congestive heart failure*.
d. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce rates of the *primary endpoint plus all-cause mortality plus coronary revascularization plus congestive heart failure*.
e. To determine in a randomized, double-blind, placebo-controlled setting the side effect profile of LDM in a non-rheumatologic population at risk for recurrent vascular events. By so doing, CIRT will evaluate the *net clinical benefit or harm* that might accrue from the hypothesized use of LDM as a novel method for the secondary prevention of myocardial infarction, stroke, and cardiovascular death.
f. To determine in a randomized, double-blind, placebo-controlled setting whether LDM will reduce the rate of *new onset type 2 diabetes* among those with metabolic syndrome but not diabetes at study entry.

Tertiary Aims

In addition, we will determine in a randomized, double-blind, placebo-controlled setting whether LDM will

a. reduce rates of the individual components of the primary endpoint
b. reduce rates of the primary endpoint plus unstable angina requiring unplanned coronary revascularization
c. reduce rates of coronary revascularization
d. reduce rates of peripheral artery disease
e. reduce rates of symptomatic deep vein thrombosis or pulmonary embolism, including those considered to be provoked and those considered to be idiopathic
f. reduce rates of clinically significant aortic stenosis
g. reduce rates of atrial fibrillation
h. have positive or negative effects on standardized measures of quality of life and global health status

Proposed Exploratory and Mechanistic Studies

Clinical endpoints of interest that will be prospectively evaluated include incident age-related macular degeneration, sleep apnea, and nephropathy and retinopathy. In addition, a plasma and DNA bank will be established as part of the trial protocol. Thus, cohort accrual and biobanking also allows for the evaluation of several exploratory aims that relate to mechanisms of effect using measured plasma biomarkers of inflammation and glucose metabolism, as well as potential genetic determinants of LDM activity. Analyses are planned that will

a. Evaluate the effect of LDM as compared to placebo on a series of inflammatory biomarkers such as IL-6, TNF, CRP, interleukin-1 (IL-1), and interleukin-1 receptor antagonist (IL1ra), and to ascertain whether any effects on these biomarkers mediate observed benefits or risks of LDM on clinical outcomes in the trial.
b. Evaluate whether genetic polymorphisms associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits or risks of LDM on clinical outcomes observed in the trial.
c. Among subjects with baseline diabetes, evaluate the effect of LDM as compared to placebo on indices of diabetic progression and glycemic control such as need for diabetes treatment intensification, proportion of subjects achieving optimal glycemic control (HbA1c<7.0%), and change in HbA1c overall and by study visit.

Variables for safety assessment will include:

a. adverse events
b. transaminase levels
c. hematology
d. creatinine clearance
e. reported symptoms

4.0. ANALYSIS METHODS

4.1. General Principles

In the analysis of efficacy, subjects will be grouped by the treatment to which they were randomized. In the analyses of safety, subjects will be grouped by the treatment received.

All analyses will be stratified by time since the confirmed index myocardial infarction (≤ 6 months vs > 6 months) as well as presence of diabetes or metabolic syndrome at entry.

4.2. Demography

The following will be reported on all randomized subjects: age, sex, race, weight, height, BMI, waist circumference, medication use, vital signs, CHD risk factors, education, exercise, alcohol consumption, revascularization status, and significant medical history. We will also collect information on other risk factors for recurrent cardiovascular events, such as severity of the index event, functional status, and relevant comorbidities. For continuous variables (e.g. age), the mean, standard deviation (SD), minimum, maximum, and n will be presented. For categorical variables (e.g sex), frequency counts and percentages in each category will be

provided. Data will be given by center, treatment, and subject. The randomized design and large sample size of CIRT should provide balanced distributions of baseline characteristics between the two treatment groups. Nonetheless, initial analyses will be conducted to identify any chance imbalances in these distributions using Wilcoxon rank-sum tests for continuous variables and Chi-square tests for categorical variables. These hypothesis tests are intended for data monitoring and quality control, and not to determine which baseline covariates to include in efficacy analyses[3]. In particular, these analyses will form part of the routine monitoring of the trial and will be regularly reported to the Data and Safety Monitoring Board.

4.3. Efficacy

Efficacy analyses of the primary and secondary endpoints of the trial will focus on time from randomization to the first occurrence of the event. These analyses will use a likelihood ratio test based on a proportional hazards model stratified on time since index myocardial infarction (≥ 6 months vs < 6 months) and diabetes mellitus vs metabolic syndrome alone at baseline, to test the null hypothesis of no association between assignment to active methotrexate and the rate of the endpoint. Estimation will use the exact method to handle ties[4]. Although randomization is also stratified by site, we judge the potential loss of information associated with additional stratification on this variable to be a greater concern than any associated gain, so that stratification by site will not be part of the primary analysis. All primary and pre-specified secondary analyses will classify patients according to their randomized treatment assignment, i.e. according to the intention to treat principle, and will base evaluation of statistical significance on a two-sided test with level 0.05. The estimated relative hazard in the methotrexate group compared to the placebo group with an accompanying 95% confidence interval will quantify the treatment effect[5]. If this relative hazard is less than 1, then 100*(1-estimated relative hazard) will be defined as the percent reduction in hazard associated with methotrexate treatment.

Rates of occurrence of the endpoint will be defined as the total number of subjects who have this event in a treatment group per 100 person-years of follow-up, counting all time from randomization until the first of the event, death, end of trial, or withdrawal of consent. The primary analysis will estimate the probability of the endpoint by time after randomization within treatment groups based on the method of Kaplan and Meier[6]. As an additional analysis, we will also use the proportional hazards model to control for baseline factors that might influence the rate of the endpoint (e.g. age, race, gender, severity of index event, baseline comorbidities, and concomitant medications), as control for these variables may yield more efficient estimates of relative treatment effects[7]. However, these analyses are secondary, and the logrank test, stratified on time since index MI and baseline diabetes without adjustment for covariates, is the pre-specified, primary analytic approach. If Kaplan-Meier plots of event free survival by study time, or related plots of log(-log)(survival), indicate violations of the proportional hazards assumption, or a formal test of trend in the scaled Schoenfeld residuals indicates such a violation[8], then weighted log-rank tests will be used according to strategies described by Pecková and Fleming[9]. However, even in the presence of an apparent violation of the proportional hazards assumption, the analysis described above gives a valid (although perhaps not optimal) test of the main trial hypothesis and will remain the primary analytic strategy, with these weighted log-rank tests serving as sensitivity analyses.

Separate proportional hazards models will also be used to compare the effects of methotrexate treatment on time to each of the individual components of the composite endpoint. The null hypothesis of no effect of methotrexate on an individual component will be evaluated by the logrank test, stratified on time since index MI and baseline diabetes status. Analyses will

use methods of competing risks survival analysis and compare the relative effects of randomized treatments on the different components of the composite outcome[10,11]. The approach of Lunn and McNeil[10] provides a readily accessible implementation of a classical approach to competing risk analysis developed by Kalbfleisch and Prentice[12].

While all primary and secondary endpoint analyses are on an intention-to-treat basis, CIRT is also functioning as a proof of concept trial and thus secondary analyses of those compliant with the respective LDM or placebo regimens will also be conducted. These analyses will use time-varying proportional hazards models, with an individual's level of adherence updated at each study visit. Adherence will be examined on a time-varying basis using a metric based on percent of study drug taken at last visit, such as half or more vs. less than half, and will also be analyzed cumulatively. Similar analyses will also be conducted on the basis of plasma levels of LDM metabolism.

We will explore in subgroup analyses whether treatment effects vary across categories of baseline covariates including age, gender, race, presence of diabetes or metabolic syndrome at baseline, index MI ≤6 vs >6 months prior, baseline lipid levels, CHF at baseline, baseline inflammatory biomarker levels including high-sensitivity C-reactive protein, revascularization status, baseline background treatments including those known to affect adenosine, such as aggrenox, methylxanthines, and fioricet, nutritional measures related to adenosine function such as estimated daily total caffeine intake, and by plasma measures of LDM metabolism. Cutpoints for analyses will be determined after the final distribution of patients has been established.

Within each subgroup, we will use proportional hazards models to estimate the relative rate of the primary endpoint associated with active treatment versus placebo. Both crude analyses and models including limited baseline covariates will be fitted. To test for the significance of modification of treatment effects by a baseline characteristic, we will include interaction terms between this characteristic and treatment in the proportional hazards model, with statistical significance determined by a likelihood ratio test comparing models with and without the interaction terms between treatment and the categories of a specific covariate.

We will conduct these subgroup analyses regardless of whether or not the overall analyses of treatment effects are significant. Our approach to interpretation of subgroup analyses has always been very cautious and recognizes that, even in large trials, it is not likely to be possible to identify reliably subgroups of patients in whom treatment is especially effective or ineffective. In the absence of prior evidence for real heterogeneity, the overall trial result may provide the best evidence for the presence of a benefit in a subgroup[13]. Our approach thus corresponds to an informal empirical Bayes procedure in which effects in an outlying subgroup require interpretation in light of the overall treatment effect[14].

The pre-specified secondary endpoints (all-cause mortality, primary endpoint plus coronary revascularization, hospitalization for congestive heart failure, primary endpoint plus all-cause mortality plus coronary revascularization plus congestive heart failure, net clinical benefit or harm, and new onset type 2 diabetes) will be evaluated using the same procedures described above for the primary endpoints. The net clinical benefit or harm endpoint will be presented as a table of the incidence of adverse events occurring with greater than 1% frequency in either treatment group, as well as a test of proportions for comparing the incidence in each treatment group. The overall rate of any serious adverse event during the randomized treatment period will also be compared between the methotrexate and placebo groups. Pre-specified tertiary endpoints will also be evaluated using the same procedures described for the primary endpoint.

Measures of quality of life and global health status, assessed at baseline, 1-year and closeout visits, will be analyzed in a mixed-effects linear regression model with subject as a random effect. Of particular interest in this model will be a time by treatment interaction. The

model will use two indicators of post-randomization (1-year and final visit) to evaluate a possible non-linear effect of treatment on outcomes by time.

To enhance interpretation for clinicians through consideration of absolute treatment effects, we will also estimate the number needed to treat or the number needed to harm, for both primary and secondary endpoints. Specifically, we will use the approach of Altman and Andersen[15] to obtain these estimates based on the Kaplan-Meier estimates of time-specific treatment effects.

Longitudinal analyses will quantify the impact of methotrexate on biomarkers including lipid levels, inflammatory markers, creatinine, and change in HbA1c, with the latter analyses stratified by presence of diabetes at baseline. Additional longitudinal analyses will evaluate the time course of methotrexate treatment on important biomarkers of safety including serum creatinine and estimated glomerular filtration rate.

Mediation analyses exploring treatment effect differences by achieved reduction in inflammation and level of adherence to assigned treatment are also planned. Following approaches used in previous analyses of the relationship of changes in lipids and hs-CRP with effects of statin therapy on outcomes[16-18], we will group subjects into achieved target levels of biomarkers and relate these achieved levels to treatment outcomes. This will also be combined with an analysis of compliance to explore whether observed treatment effects are compliance driven.

We also plan to evaluate whether genetic polymorphisms associated with vascular risk, inflammation, thrombosis, or LDM metabolism modify any benefits or risks of LDM on clinical outcomes observed in the trial.

4.4. Safety

Data from all subjects who enter the run-in period will be included in the evaluation of safety. Results from the run-in period will be presented separately from the results after randomization. Adverse events will also be reported separately for the two periods.

Treatment emergent adverse effects are defined as adverse events that start after initiation of study drug or adverse events ongoing from screening which worsen in intensity. The definitions of adverse events and serious adverse events are given in the protocol (Sections 4.9, 4.10, and 4.17).

The proportion of subjects experiencing adverse events will be tabulated by treatment received. In the case of adverse events leading to withdrawal or dose changes, the incidence will also be tabulated and reported by treatment group for all events observed during the randomized treatment phase. Summaries of all treatment emergent adverse events, treatment emergent adverse events leading to death, and treatment emergent serious adverse events will also be presented by treatment group.

Laboratory measures and changes from baseline will be summarized by mean, median, SD, minimum, maximum, and number of subject at each visit at which measurements are taken. Pre-specified alterations in laboratory values will be highlighted and summarized by treatment group, including:

1. Increase in transaminase levels (ALT or AST)
    a. >1.5to <2 times the upper limit of normal
    b. more than 2 times the upper limit of normal
2. Platelets
    a. < 75,000 to 50,000/mm^3
    b. < 50,000/mm^3

3. WBC
    a. < 4,000 to 3,000/mm^3
    b. < 3,000/mm^3
4. Reduction in creatinine clearance to
    a. 30 to < 40 ml/min/1.75m^2
    b. < 30 ml/min
5. Hematocrit
    a. < 27%
6. Albumin
    a. < 0.8 times the lower limit of normal

Vital signs and weight will be summarized at each visit. Physical exam abnormalities at baseline and new or aggravated physical exam abnormalities will be listed, including fevers, cough, shortness of breath, nausea, vomiting, and painful and open mouth sores.

Safety results will be summarized both by randomized treatment assignment and by actual treatment at time of occurrence of an adverse event or measurement of a laboratory value. For the latter, treatment groups are LDM, placebo, and Off Randomized Treatment. Off Randomized Treatment is defined as subjects who have withdrawn from randomized treatment but continue to be followed.

4.5. Other Analysis

The following will be summarized by randomized treatment with frequency counts and percentages:

- Completion and withdrawal from the randomized treatment period
- Reason for withdrawal from the randomized treatment period
- Compliance
- Prior and concomitant medication
- Misrandomization

5.0. INTERIM ANALYSES

5.1. Interim Feasibility Analysis

An interim feasibility analysis will be performed jointly by the DSMB and the NHLBI after 1000 patients have been randomized and followed for at least 6 months. At this time, trial feasibility and safety will be reviewed, the trial dose range will be evaluated, suitability of the trial algorithms considered, and recommendations will be made to the investigators with regard to any needed protocol changes at that time.

Thus, for the purposes of the feasibility analysis, the investigators will submit a report to the NHLBI including the cumulative recruitment experience to date, the adherence among randomized participants, the rates of adverse events both overall and according to the major disease categories and the percent of participants willing to continue. It is expected that this report will pool information across treatment groups (i.e. maintain the treatment blind). However, a parallel report with information by treatment group will be submitted simultaneously to the trial's Independent Data and Safety Monitoring Board.

5.2. DSMB Analyses

Interim analyses of rates of the primary outcome, as well as rates of the individual components of the composite endpoint, and the pre-specified secondary endpoints will be prepared for presentation to the Data and Safety Monitoring Board (DSMB). Reports to the DSMB will also include comparisons of baseline characteristics between treatment groups, displays of cumulative recruitment by study time, comparisons of post-randomization laboratory values by treatment group, and rates of adverse events, both overall and within systems, by treatment group. The frequency of the meetings will be determined by the independent DSMB appointed by the NHLBI.

Specifically, the blinded portion of the report will display, by treatment group and visit number, the number of subjects with an expected visit (i.e. those whose window for that visit closed at least 4 weeks before database lock for the particular interim report), the number and percent of those who remained on drug at that visit, and secondary tables which display the numbers of subjects who gave scheduled blood samples. An additional table will display by treatment group the numbers of subjects who remain in study and on drug, and, for those who have discontinued, the reason for discontinuation. Categories for reasons for stopping which we have used in the past are: Adverse Event, Endpoint, Deceased, Medication Use, Investigator Decision, Site Closure, Protocol Noncompliance, Lost to Follow-up, Withdrew Consent, Other, and Unknown. Additional tables will display, by treatment group and visit number, the rates of adherence based on self-reported pill counts. These last tables will be shown two ways: first, restricted to subjects who attended a visit, and then with 0 imputed for adherence for subjects who missed a visit and whose visit window closed more than 12 weeks before database lock for that interim report. Overall, power for the trial was estimated under the assumption of a 5% annual drop-out and loss to follow-up rate. If the rate exceeds this amount at a scheduled interim report, study power will be re-estimated based on the observed rate.

While the frequency of meetings and the approach to interim monitoring will be the choice of the DSMB, we anticipate at least twice yearly meetings to monitor recruitment and retention, with quarterly safety reports. With respect to efficacy monitoring and possible stopping for benefit, we will recommend to the DSMB monitoring via the Lan-DeMets procedure[19] with spending function $\alpha(t^*)=2-2\Phi(Z_{\alpha/2}/\sqrt{t^*})$, where t* is the information fraction, Φ is the standard normal distribution function, α is the two-sided type 1 error rate, and $Z_{\alpha/2}$ is its 100(1-α/2)th percentile. To preserve alpha and to minimize the likelihood of an inflated effect estimate associated with early stopping, we recommend pre-specification of only two formal evaluations of efficacy. With an event-driven trial targeting 530 total confirmed endpoints, these evaluations would occur upon accrual of 199 and 398 confirmed primary endpoints.The Z-values for the boundaries would be ±3.4786 at the first interim analysis and ±2.3431 at the second efficacy analysis. Based on the formula of Schoenfeld[20], the Z-value is related to the expected value of the log-rank statistic by the formula $Z = \log(HR)\sqrt{D/4}$, where HR is the hazard ratio, and D is the overall number of events. Thus, with D=199 and 398, the hazard ratios at the boundary would be 0.611 and 0.791, respectively.

The DSMB has voted to prefer more conservative boundaries, and chose a P-value of 0.0001 at both information times, which corresponds to a Z-value of ±3.891. Approximate hazard ratios associated with this Z-value are 0.620 at 50% information and 0.677 at 75% information.

As a guideline for considering a recommendation to stop the study early because of convincing evidence of inefficacy (futility), pre-planned inefficacy bounds will also be considered upon accrual of 50% and again upon accrual of 75% of the targeted numbers of confirmed primary endpoints, i.e. upon accrual of 265 and 398 confirmed primary endpoints. These time

points agree with the cutpoints used in the AIM-HIGH trial[21], and are chosen to mitigate the possibility of stopping early with an imprecisely estimated treatment effect. Based upon the Linear 20% Inefficacy Boundary approach described by Freidlin et al[22], the inefficacy boundary will be crossed if the observed relative hazard of the primary endpoint associated with methotrexate assignment is greater than 0.99 at the first interim futility analysis, or is greater than 0.97 at the second interim futility analysis. Simulations performed by Freidlin et al indicate that their Linear 20% Inefficacy Boundary approach is associated with a less than 1% loss of power due to inefficacy monitoring. Further, their approach is more conservative than a 10 or 30% conditional power approach in later follow-up (i.e. after 70% of information is accrued). However, the Linear 20% Inefficacy Boundary approach is more aggressive than a 10% (or even a 20%) conditional power rule at the 50% information accrual point, so a more conservative boundary of an observed relative hazard associated with methotrexate assignment greater than 1.11 at the first interim futility analysis (the cutpoint associated with conditional power below 10%), may be preferred at that time, especially as use of this cutpoint will preserve power.

6.0. REFERENCES

1. Lachin JM, Foulkes MA. Evaluation of sample size and power for analyses of survival with allowance for nonuniform patient entry, losses to follow-up, noncompliance, and stratification. Biometrics 1986; 42: 507-519.
2. Reboussin DM, DeMets DL, Kim KM, Lan KK. Computations for group sequential boundaries using the Lan-DeMets spending function method. Control Clin Trials 2000; 21:190-207.
3. Senn S. Baseline adjustment in longitudinal studies. In, P Armitage and T Colton, eds: Encyclopedia of Biostatistics 2nd edition 2005; 294-297.
4. Peto R. Discussion on Professor Cox's paper. J Roy Statist Soc Ser B 1972; 34: 205-207.
5. Cox DR. Regression models and life tables (with discussion). J Roy Statist Soc Ser B 1972;34:187-220.
6. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Statist Assoc 1958;53:457-481.
7. Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems. Stat Med 2002; 21: 2917-2930.
8. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994; 81: 515-526.
9. Pecková M, Fleming TR. Adaptive test for testing the difference in survival distributions. Lifetime Data Anal 2003; 9: 223-38.
10. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995; 51: 524-532.
11. Glynn RJ, Rosner B. Comparison of risk factors for the competing risks of coronary heart disease, stroke, and venous thromboembolism. Am J Epidemiol 2005;162:975-82.
12. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. New York. John Wiley & Sons 1980; pp 163-188.
13. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17187 cases of suspected acute myocardial infarction: ISIS-2. Lancet 1988;ii:349-360.
14. Morris CN. Parametic empirical bayes inference: theory and applications. J Am Statist Assoc 1983;78:47-55.

15. Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. BMJ. 1999; 319: 1492–1495
16. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gotto AM Jr; Air Force/Texas Coronary Atherosclerosis Prevention Study Investigators. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344:1959-1965.
17. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E; Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005; 352:20-28.
18. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, Macfadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ; JUPITER Trial Study Group. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial.Lancet 2009; 373:1175-1182.
19. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trails. Biometrika 1983;76:659-663.
20. D Schoenfeld. The asymptotic properties of nonparametric tests for comparing survival distributions. Biometrika 1981; 68: 316-319.
21. AIM-HIGH Investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011; 365: 2255-2267.
22. Freidlin B, Korn EL, Gray R. A general inefficacy interim monitoring rule for randomized clinical trials. Clin Trials. 2010;7:197-208.