Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT)

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Summary. While inflammation is a crucial component of atherothrombosis and patients with elevated inflammatory biomarkers such as high sensitivity C-reactive protein (hsCRP) are at increased vascular risk, it remains unknown whether inhibition of inflammation per se will lower vascular event rates. The recently completed JUPITER (N Engl J Med 2008, 359, 2195) trial demonstrates that statins reduce myocardial infarction, stroke, and all-cause mortality among healthy individuals with low cholesterol and elevated hsCRP. However, a direct test of the inflammatory hypothesis of atherothrombosis requires an agent that inhibits inflammation without impacting other components of the atherothrombotic process, and has an acceptable safety profile for a trial setting. On this basis, the cardiovascular inflammation reduction trial (CIRT) proposes to allocate 7000 stable coronary artery disease patients with persistent elevations of hsCRP to placebo or very-low-dose-methotrexate (VLDM, 10 mg weekly), a proven anti-inflammatory regimen that reduces TNFα, IL-6, and CRP levels and is in wide use among rheumatoid arthritis patients. If successful, CIRT would both confirm the inflammatory hypothesis of atherothrombosis and open novel approaches to the treatment and prevention of cardiovascular disorders.

Keywords: C-reactive protein, inflammation, methotrexate, myocardial infarction, stroke.

Why test the inflammatory hypothesis of atherothrombosis?

Abundant laboratory and translational evidence has accumulated over the past two decades demonstrating that inflammation plays a major role in all stages of the atherothrombotic process, including the sudden rupture of apparently stable plaque that is the underlying proximate cause of most acute myocardial infarction and stroke events [1,2]. As reviewed elsewhere, components of both the innate and acquired immune systems are relevant to this progression, and the interaction of lipid accumulation and immune function appears to both promote premature atherosclerosis and accelerate plaque fissuring, a process that exposes the underlying matrix to circulating thrombogenic factors and ultimately leads to platelet adhesion, vessel occlusion, and downstream hypoxia [3,4].

At the same time, clinical evidence has also accumulated from more than 20 prospective cohort studies demonstrating that inflammatory biomarkers such as high sensitivity C-reactive protein (hsCRP) predict risk of initial as well as recurrent cardiovascular events, including myocardial infarction, stroke, and cardiovascular death [5–9]. Furthermore, multiple lifestyle and pharmacologic therapies that reduce cardiovascular risk including smoking cessation, weight reduction, exercise, and statins lower hsCRP levels, whereas drug therapies that reduce LDL cholesterol but do not reduce hsCRP such as post-menopausal HRT and torcetrapib have failed in randomized trials to reduce vascular risk. Experimental evidence has also strongly suggested that interventions to inhibit inflammation might well improve vasculo-occlusive outcomes. Nonetheless, while the attributable vascular risk associated with inflammation is similar in magnitude to that associated with hyperlipidemia [10] and while animal models using targeted anti-atherosclerotic therapies have been promising [11], it remains unknown as to whether inhibition of inflammation per se will lower vascular event rates, particularly in a population identified as having a persistent inflammatory response despite use of all usual cardiovascular treatments.

To date, no endpoint trial directly addressing these issues has been initiated either by industry or a federal agency. However, results of the recently completed Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) [12] considerably raise interest in pursuing such a trial.

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Addressing the inflammatory hypothesis of atherothrombosis in a post-JUPITER world

JUPITER was designed to address whether rosuvastatin 20 mg daily as compared to placebo would prevent vascular events in a primary prevention population with low levels of LDL cholesterol (< 130 mg dL⁻¹) who were at elevated vascular risk on the basis of an enhanced innate immune response as determined by hsCRP > 2 mg L⁻¹. This is a patient group that is outside current guidelines for prevention with statin therapy, yet has repeatedly been shown to be at high risk despite lacking traditional risk factors. The core hypothesis underlying JUPITER – and its implications for a future trial of direct inflammatory inhibition – reflects several issues.

The JUPITER investigators recognized that inflammation plays a crucial role in atherothrombosis and thus designed a trial that for the first time used inflammatory risk as its primary entry criterion. Part of this recognition comes from the fact that half of all heart attack and stroke events occur among apparently healthy men and women with average or even low cholesterol levels and 20% of all vascular events occur among individuals with no major risk factor at all [13].

Second, although statins are potent lipid lowering agents, they also have anti-inflammatory effects. For example, statins reduce cell adhesion and monocyte recruitment to the endothelial wall, alter smooth muscle migration in developing plaques, favorably affect matrix metalloproteinase expression leading to plaque stabilization, and in human hepatocytes reduce IL-6 induced CRP production [14–17].

Finally, prior studies demonstrate that statin therapy lowers hsCRP in a largely LDL cholesterol independent manner [18,19] and that among individuals with acute coronary syndromes, those with stable coronary disease, and even among apparently well individuals [20–23], the observed benefit of statin therapy relates not only to LDL cholesterol reduction, but also to reductions in hsCRP. Most relevant, in the hypothesis-generating AFCAPS/TexCAPS trial [23], no benefit of statin therapy on clinical events accrued among individuals with low baseline levels of both LDL cholesterol and hsCRP, even though LDL cholesterol levels were further reduced with therapy. In contrast, within the same AFCAPS/TexCAPS cohort, statin therapy was highly effective in lowering vascular event rates among those with low levels of LDL cholesterol but elevated levels of hsCRP, again an effect that was largely independent of LDL reduction. This critical observation, that statin therapy might have greater benefit in the presence of inflammation than in its absence is consistent with data published from the CARE trial almost a decade ago [22], but was made on a post hoc basis, and thus a large-scale hypothesis-testing trial was needed.

As reported at the November 2008 meetings of the American Heart Association, JUPITER was stopped early at the recommendation of its Independent Data Monitoring Board following a 44% reduction in the primary endpoint of myocardial infarction, stroke, hospitalization for unstable angina, arterial revascularization, or cardiovascular death (P < 0.00001) [12] (Fig. 1). Demonstrating the importance of inflammation as a vascular risk factor, the placebo event rate in JUPITER was higher than that of prior prevention trials limited to those with overt hyperlipidemia. Furthermore, each individual component of the JUPITER primary endpoint was significantly reduced including a 54% reduction in myocardial infarction (P = 0.0002), a 48% reduction in stroke (P = 0.002), and a 46% reduction in need for arterial revascularization (P < 0.0001). A 20% reduction in all cause mortality was also observed (P = 0.02).

All pre-specified subgroups within JUPITER showed a statistically significant benefit with rosuvastatin, including groups traditionally assumed to be at low risk such as those with Framingham scores < 10% (Fig. 2). From a public health perspective, absolute as well as relative risk reductions were clinically important. The 5-year Number Needed to Treat (NNT) in JUPITER for the endpoint of myocardial infarction, stroke, or death from any cause (5-year NNT = 32) was smaller than 5-year NNT values previously observed for the use of statin therapy among individuals with overt hyperlipidemia (5-year NNT 50) [24] and far smaller than the 5-year NNT associated with treatment of hypertension (5-year NNT 80–160) [25].

From an inflammatory pathology perspective, the most important subgroup within JUPITER is that which included 6375 individuals with elevated hsCRP, but no other major risk factor. In this group with low LDL cholesterol, high HDL cholesterol, and no evidence of diabetes, hypertension, or cigarette consumption, the overall event rate remained high on the basis of an elevated hsCRP alone, and the observed benefit was consistent with that seen in the trial as a whole (HR 0.63, 95% CI 0.44–0.92). Ongoing analyses from the JUPITER investigators also confirm that achieving low levels of hsCRP is an important treatment goal in a manner analogous to achieving low levels of LDL cholesterol. Thus, JUPITER prospectively confirms prior data from the PROVE IT–TIMI 22 [20], A-Z [21], and REVERSAL [26] trials, all of which support the ‘dual target’ concept of reducing inflammation as well as LDL cholesterol in order to maximize statin benefits [27].

Despite the consistency and intriguing nature of these data, rosuvastatin markedly lowers LDL cholesterol as well as hsCRP. Thus, although the JUPITER data are suggestive, the trial cannot directly address whether lowering inflammation alone lowers vascular risk. Nonetheless, given the success of JUPITER, an immediate and substantial clinical need has arisen for evidence demonstrating that adjunctive therapies beyond statins can be effective at lowering vascular risk among high-risk patients with persistently elevated levels of hsCRP.

Why a trial of very low dose methotrexate (VLDM)?

Fundamentally, a direct test of the inflammatory hypothesis of atherothrombosis requires an agent that: (i) inhibits inflammation without having major impact on other components of the
atherothrombotic process, and (ii) is known to have an acceptable safety profile for evaluation in a large-scale randomized trial. Very low dose methotrexate (VLDM, 10 mg per weekly) has multiple attributes that make it a promising agent to directly test the inflammatory hypothesis of atherothrombosis.

First, low doses of methotrexate (range 10–25 mg per week) have been in use for almost two decades among patients with rheumatoid arthritis (RA), and guidelines from the American College of Rheumatology provide extensive information regarding dosing regimens, drug monitoring, and the identification of high-risk patient subgroups that should not receive therapy [28]. The long-term safety of VLDM has already been established among RA patients, a cohort with similar age distribution and co-morbidities as those likely to be enrolled in a trial of patients with stable cardiovascular disease [29,30]. Circulating MTX is < 50% protein bound and as a result has minimal interaction with most concomitant medications, including statins, aspirin, beta-blockers, and inhibitors of the renin-angiotensin system that are in common use among post-infarction patients. The wide use of VLDM in clinical practice further reduces the potential for unanticipated off-target toxicity. Interestingly, as use of VLDM for the treatment of RA has increased, significant declines in mortality from acute myocardial infarction have been reported in the RA community [31].

VLDM has also previously been shown to reduce several inflammatory biomarkers including CRP, IL-6, and TNF-α in populations of patients with rheumatoid arthritis and psoriasis, patient groups at elevated vascular risk on an inflammatory basis [32–34]. In these and other studies of VLDM, no major effects on platelet function were observed. VLDM has minimal effect on LDL-C and at most leads to a marginal increase in HDL-C [35]. This is a markedly different lipid response from that observed for other potential anti-inflammatory agents including TNF-inhibitors (infliximab, entercept) and IL-6 inhibitors (tocilizumab) that are more potent inhibitors of CRP than LDM, but also lead to substantive lipid abnormalities [36–38]. Thus, VLDM would test the inflammatory hypothesis of atherothrombosis without major confounding effects on lipid levels, platelet function, or other indices of hemostasis and thrombosis.

Fig. 1. Cumulative incidence of cardiovascular events in the JUPITER trial, according to rosuvastatin or placebo allocation. Adopted from Ridker et al. [12].
Prior observational evidence for VLDM

Among both RA and psoriasis patients assessed in prospective cohort and nested case-control settings, available epidemiologic data consistently suggest that exposure to VLDM is associated with reductions in cardiovascular morbidity and mortality, despite the fact that those receiving VLDM have worse vascular risk factor profiles, data strongly mitigating against indication bias (Fig. 3).

For example, in a major prospective cohort study of patients with RA, a net survival benefit was observed in association with the use of VLDM, and this effect was due almost entirely to a reduction in cardiovascular mortality, even after adjusting for multiple other covariates for disease risk. Specifically, among 1240 patients with RA followed prospectively in the Wichita Arthritis Center, 588 patients (mean age 57 years) were treated with VLDM, whereas a variety of other disease modifying anti-rheumatic drugs (DMARDs) were used among the remaining cohort participants [39]. During follow-up, total mortality was reduced 60% (HR 0.4, 95% CI 0.2–0.8) and cardiovascular mortality was reduced 70% among those treated with VLDM (HR 0.3, 95% CI 0.2–0.7) compared to those receiving alternative therapies. This cardiovascular benefit was observed despite the fact that patients initiating treatment with VLDM (mean dose = 13 mg per week) had significantly worse prognostic factors for mortality and significantly worse RA symptoms than did patients not being treated with VLDM. Particularly relevant given the JUPITER results, risk reductions in the Wichita Arthritis Study associated with VLDM were similar among those already taking statin agents.

Similar data have been presented in a prospective nested case-control analysis of 613 RA patients of whom 72 were found on follow-up to have developed incident CVD [40]. Compared to RA patients who never received any DMARD,
those taking VLDM (dose range 7.5–30 mg per week) had an age, gender, and smoking adjusted odds ratio for CVD of 0.16 (95% CI 0.04–0.66) and similar effects were observed among those who were taking LDM and a second DMARD agent (OR 0.20, 95% CI 0.08–0.54). While these effects were attenuated in analyses after further adjustment for hypertension, diabetes, and hyperlipidemia, the best estimates of effect in all subgroup analyses were still consistent with 30–60% risk reductions. As in the Wichita Arthritis Study, indication bias is an unlikely alternative explanation for these results as patients receiving VLDM had worse cardiovascular risk profiles at study entry. Comparable data linking non-randomized use of VLDM to lowered vascular risk have been observed in other registries and prospective cohorts [41,42] (Fig. 3).

Patients with psoriasis are often treated with VLDM, and in this setting observational data is also promising; in a cohort of 7615 Veterans Administration outpatients with psoriasis, those treated with VLDM had significantly reduced risk of vascular disease compared to those who were not prescribed VLDM (fully adjusted hazard ratio 0.73, 95% CI 0.55–0.98) [43]. This effect was present even among those taking below median doses of VLDM (adjusted HR 0.50, 95% CI 0.31–0.79).

Why patients with stable cardiovascular disease and persistently elevated hsCRP?

Given the role of inflammation in both early atherogenesis and in plaque disruption, a direct anti-inflammatory agent could in theory be tested at any stage of the atherothrombotic process. However, the most appropriate population to test this hypothesis is one in which: (i) patients are known to be at increased risk despite current therapy, and (ii) there is biochemical evidence of a persistent heightened inflammatory response despite usual care.

Given these constraints, a primary prevention population would be infeasible due to the exceptionally large sample size required and because an extremely low side effect profile is typically required in that setting. Furthermore, given the success of JUPITER, primary prevention patients with elevated hsCRP levels are likely to be treated with statin therapy, further reducing event rates and increasing sample size in this setting. Similarly, while direct anti-inflammatory therapies might in theory be effective as an initial adjunctive therapy in the immediate acute coronary syndrome setting, use of an agent with the potential to alter wound healing (and thus potentially increase myocardial rupture) will raise additional safety concern regarding overall benefits and risks if this setting is chosen for initial evaluation.

In contrast, patients who have survived a prior myocardial infarction or stroke, are clinically stable, and who have persistently elevated hsCRP levels appear to be an optimal patient population in which to undertake an initial test of the inflammatory hypothesis of atherothrombosis. This population is no longer at risk for infarct rupture due to altered wound healing, yet remains at high risk for recurrent cardiovascular events despite use of all current aggressive therapies. Moreover, as demonstrated in a pre-specified analysis within the PROVE IT – TIMI 22 trial [20] and subsequently confirmed in the fully independent A-to-Z trial [21], patients with known vascular disease who fail to reduce hsCRP despite aggressive therapy are at increased vascular risk even if LDL-C levels are reduced below 70 mg dL−1 (Fig. 4). In these same study populations, those with persistently elevated levels of hsCRP are at markedly increased risk of stroke, an intriguing observation as achieved LDL cholesterol levels did not predict recurrent stroke. In post-hoc analyses of both trials, even greater reductions in risk were observed for those who further reduced hsCRP levels to < 1 mg L−1.

Fig. 3. Observational studies of very low dose methotrexate (VLDM) and vascular events conducted among populations with either rheumatoid arthritis, psoriasis, or psoriatic arthritis.
Proposed overview of CIRT: moving the hypothesis forward

The proposed cardiovascular inflammation reduction trial (CIRT) is a randomized, double-blind, placebo-controlled trial of 3–4 year therapy with very low dose methotrexate (VLDM, 10 mg per week) in the secondary prevention of myocardial infarction, stroke, and cardiovascular death among patients with known prior cardiovascular disease who have evidence of a persistent inflammatory response on the basis of an elevated CRP (>2 mg L\(^{-1}\)) despite usual therapy. All study participants will additionally receive folic acid (1 mg po qd) (Fig. 5).

Following the American College of Rheumatology Guidelines for use of VLDM [28], baseline evaluation of blood counts, renal and hepatic function, as well as screening for hepatitis B and C would be required prior to randomization, with safety monitoring performed thereafter. The CIRT primary endpoint will consist of the first event of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death occurring after randomization. All-cause mortality (and cause-specific mortality) as well as specific safety endpoints relating to possible renal, infectious, hepatic, hematologic, or pulmonary disease will also be ascertained. Although observational data cited above suggests a 40–70% relative risk reduction in cardiovascular mortality associated with VLDM, a more conservative estimate (25–30% relative risk reductions) is consistent with the need for 7000 study participants to be randomized in a 1:1 ratio with a mean follow-up period of approximately 3.5 years.

The potential clinical impact of CIRT is broad since a positive finding would strongly support the inflammatory hypothesis of atherothrombosis, provide a fully novel treatment for patients with chronic cardiovascular disease, and spur a new era for the development of second-generation targeted anti-inflammatory drugs with potential for even greater vasculoprotective benefits [44].

Disclosure of Conflict of Interests

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Fig. 4. Clinical benefits of statin therapy are maximized when LDL-C levels are reduced below 70 mg dL\(^{-1}\) and hsCRP levels are reduced below 2 mg L\(^{-1}\). Left, data from the PROVE IT – TIMI 22 trial (Ridker et al., [20]) and right, data from the A to Z trial (Morrow et al., [21]).

Fig. 5. Schematic diagram for the cardiovascular inflammation reduction trial (CIRT).
cardiovascular disease. These patents comply with guidelines established by the Harvard Medical School and have been licensed to Seimens and Astra-Zeneca.

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