

Clinical update

# Anti-inflammatory therapies for cardiovascular disease

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Atherothrombosis is no longer considered solely a disorder of lipoprotein accumulation in the arterial wall. Rather, the initiation and progression of atherosclerotic lesions is currently understood to have major inflammatory influences that encompass components of both the innate and acquired immune systems. Promising clinical data for 'upstream' biomarkers of inflammation such as interleukin-6 (IL-6) as well as 'downstream' biomarkers such as C-reactive protein, observations regarding cholesterol crystals as an activator of the IL-1 $\beta$  generating inflammasome, and recent Mendelian randomization data for the IL-6 receptor support the hypothesis that inflammatory mediators of atherosclerosis may converge on the central IL-1, tumour necrosis factor (TNF- $\alpha$ ), IL-6 signalling pathway. On this basis, emerging anti-inflammatory approaches to vascular protection can be categorized into two broad groups, those that target the central IL-6 inflammatory signalling pathway and those that do not. Large-scale Phase III trials are now underway with agents that lead to marked reductions in IL-6 and C-reactive protein (such as canakinumab and methotrexate) as well as with agents that impact on diverse non-IL-6-dependent pathways (such as varespladib and darapladib). Both approaches have the potential to benefit patients and reduce vascular events. However, care should be taken when interpreting these trials as outcomes for agents that target IL-6 signalling are unlikely to be informative for therapies that target alternative pathways, and vice versa. As the inflammatory system is redundant, compensatory, and crucial for survival, evaluation of risks as well as benefits must drive the development of agents in this class.

## Keywords

Inflammation • Atherosclerosis • C reactive protein • Interleukin-6 • Inflammasome • Methotrexate • Canakinumab • Salsalate • Darapladib • Colchicine

## Introduction

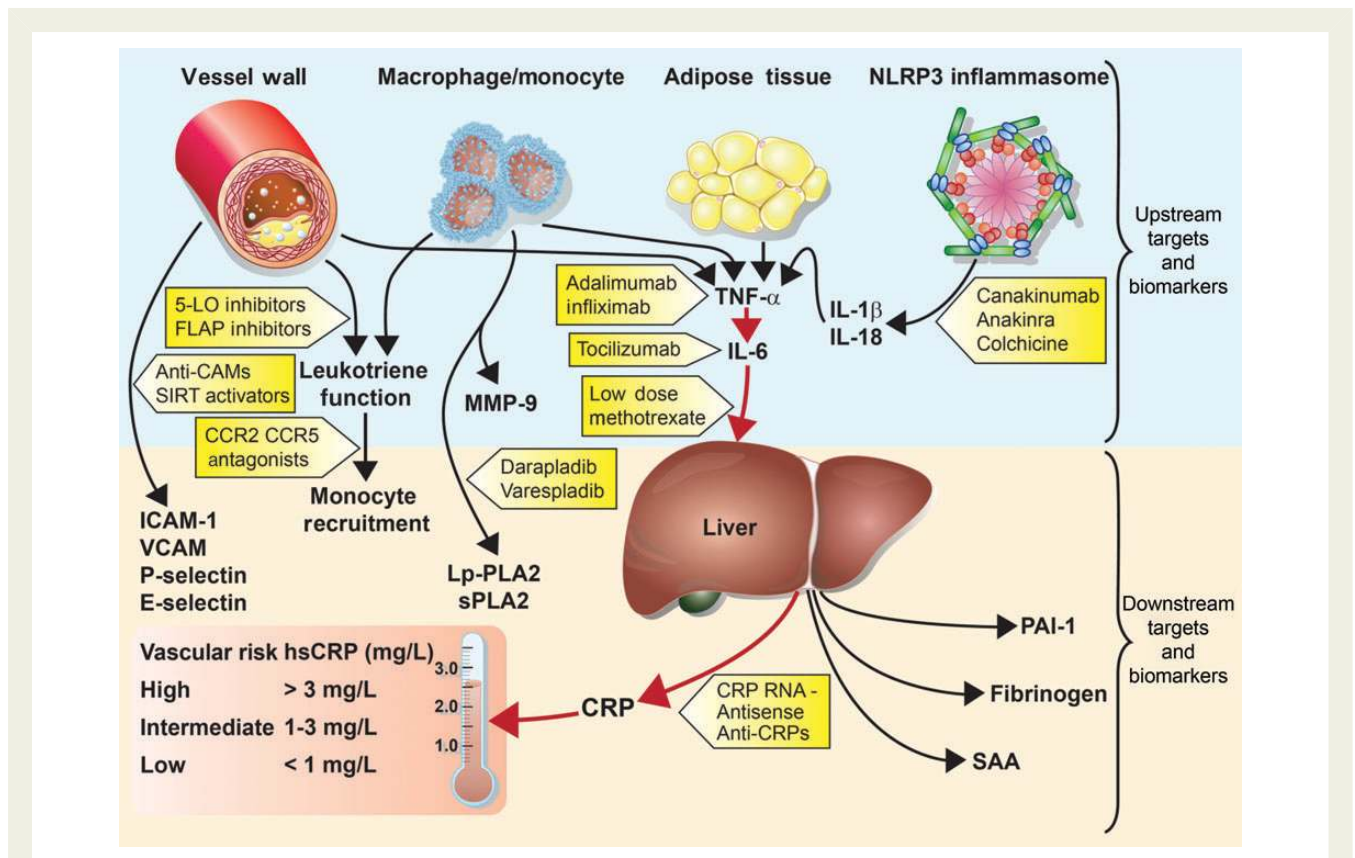
In addition to being a disorder of lipid accumulation, atherothrombosis is understood to have major inflammatory influences that interact with atherogenic lipoproteins to accelerate disease progression, ultimately leading to plaque rupture and clinical events.<sup>1</sup> Multiple cell types deriving from monocyte/macrophage lines have been implicated in atherogenesis, as have several chemokines, cytokines, and adhesion molecules that derive from the vessel wall or from tissue sources that associate with vascular risk such as central adipose.<sup>2,3</sup> Partly on this basis, it has been hypothesized that targeting different inflammatory pathways might have efficacy in the treatment and prevention of cardiovascular disease.<sup>4</sup> In particular, as outlined in *Figure 1*, several emerging lines of evidence support the hypothesis that inhibition of the central immune pathway linking interleukin-1 (IL-1),

tumour necrosis factor (TNF- $\alpha$ ), and IL-6 might serve as a potent target for atherothrombotic protection.

First, of data linking inflammatory biomarkers to future vascular events, evidence is most robust for the 'upstream' biomarkers TNF- $\alpha$  and IL-6<sup>5</sup> which lead to hepatic production of the clinically useful 'downstream' biomarker high-sensitivity (hs) C-reactive protein.<sup>4</sup> Indeed, IL-6 is highly up-regulated at the site of coronary occlusion in patients with ST-segment elevation infarction, while C-reactive protein is not<sup>6</sup> (*Figure 2*). In comprehensive meta-analyses, C-reactive protein has been shown to add as much to vascular risk prediction as either total or HDL-cholesterol. While hs C-reactive protein is more stable than IL-6 and thus easier to use in clinical practice, epidemiological data consistently show IL-6 levels to correlate with future vascular risk (*Figure 3*).<sup>7</sup> In recent overviews, of several inter-correlated measures of inflammation, only two

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**Figure 1** Inflammatory pathways as potential targets for atherosclerotic therapies. IL-1 $\beta$ , interleukin-1-beta; IL-18, interleukin-18; IL-6, interleukin-6; TNF- $\alpha$ , tumour necrosis factor-alpha; MMP-9, matrix metalloproteinase-9; Lp-PLA2, lipoprotein-associated phospholipase A2; sPLA2, secretory phospholipase A2; ICAM-1, intercellular adhesion molecule type 1; VCAM, vascular cellular adhesion molecule; PAI-1, plasminogen activator inhibitor type-1; SAA, serum amyloid A; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein.; 5-LO, 5-lipoxygenase; FLAP, 5-lipoxygenase-activating protein; SIRT1, sirtuin-1; CCR2/CCR5, chemokine receptor types 2 and 5.<sup>4</sup>

biomarkers—downstream C-reactive protein (hazard ratio 1.69 per 1 SD higher baseline level) and upstream TNF- $\alpha$  (hazard ratio 1.32 per 1 SD higher baseline level)—remained independently significant predictors of future vascular risk.<sup>7</sup>

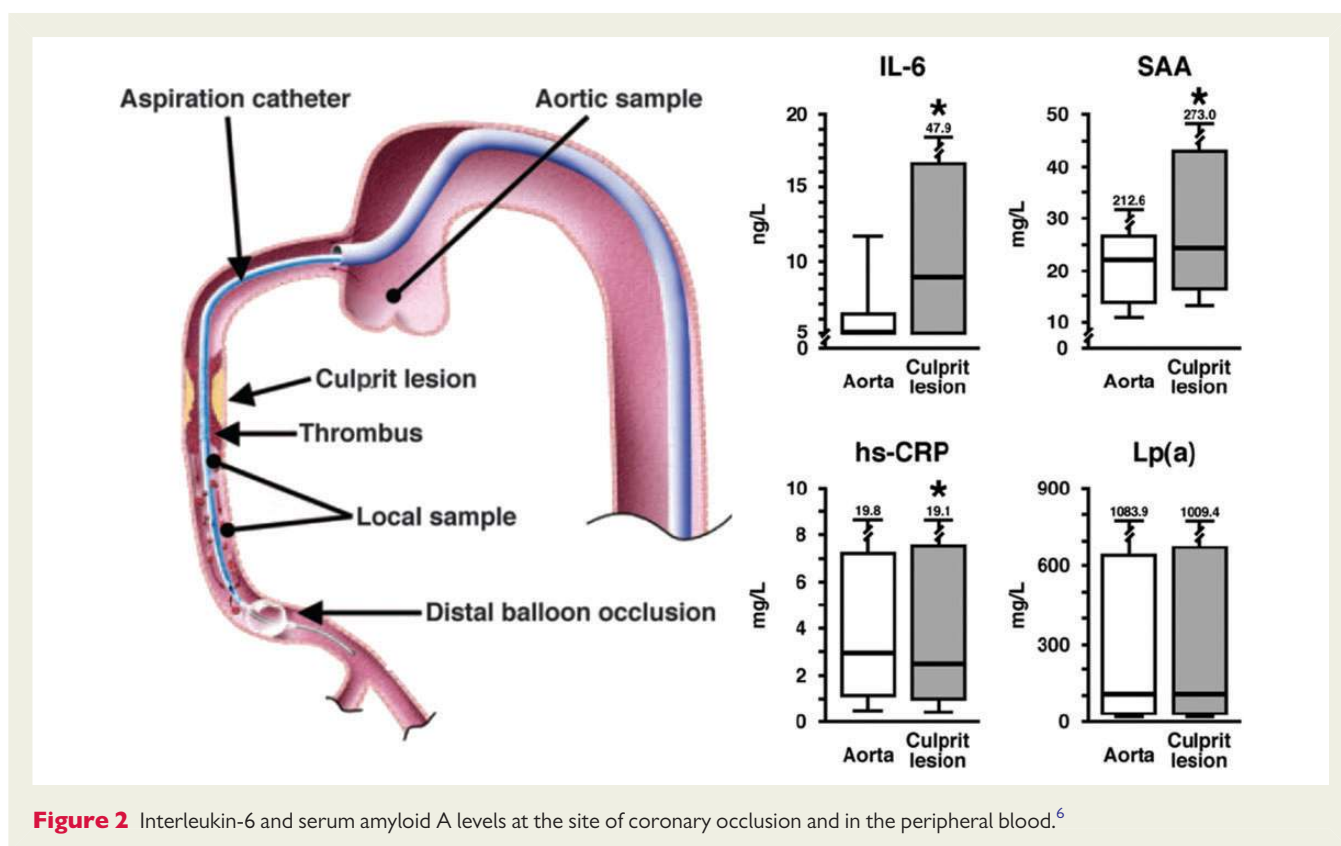
Secondly, two Mendelian randomization studies demonstrate that genetic polymorphism in the IL-6 receptor signalling pathway concordantly leads to reduced plasma levels of C-reactive protein and to reduced vascular risk<sup>8,9</sup> (Figure 4). While such data do not prove a causal relationship between inflammation and vascular events, they do provide elegant support for the concept that targeting IL-6 signalling may be a novel method for atheroprotection.

Thirdly, as will be discussed in greater detail, recent evidence has directly linked deposition of cholesterol crystals to activation of the IL-1 $\beta$  producing nucleotide-binding leucine-rich repeat-containing pyrin receptor (NLRP3) inflammasome.<sup>10,11</sup> Since IL-1 $\beta$  production leads to increased levels of IL-6 and C-reactive protein, these data provide a mechanistic link between early deposition of cholesterol crystals within the vessel wall to the macrophage–monocyte interactions that initiate fatty streaks and promote local atherosclerotic progression.

Fourthly, while statins are powerful LDL-lowering drugs proven to reduce vascular risk in many patient groups, these agents also reduce C-reactive protein.<sup>12,13</sup> Statins reduce C-reactive protein in relation to potency and across dose ranges, but the magnitude of C-reactive

protein reduction for individuals cannot be predicted on the basis of LDL reduction. Polymorphisms that influence statin-induced changes in LDL-cholesterol are separate and distinct from those that influence statin-induced changes in C-reactive protein.<sup>14,15</sup> The JUPITER primary prevention trial showed that providing statin therapy to individuals with high levels of inflammation prevents cardiovascular events and reduces all-cause mortality, even if those individuals already have low levels of LDL-cholesterol.<sup>16</sup> JUPITER also demonstrated that statins reduce venous thrombosis, an intriguing finding as there are no atherosclerotic plaques to rupture in the venous wall and LDL-cholesterol has little relation to stasis-induced thrombosis.<sup>17</sup> Further, the greatest absolute risk reductions associated with statin therapy in JUPITER were observed among those with the highest levels of inflammation at a trial entry.<sup>18</sup> As previously shown in the PROVE-IT TIMI 22 trial, the greatest relative risk reductions were also seen among those who not only reduced LDL-cholesterol <70 mg/dL, but who also reduced hs C-reactive protein below 1 mg/L.<sup>19</sup>

These and other observations surrounding C-reactive protein and statin therapy provide evidence that lipid lowering is in part an anti-inflammatory therapy.<sup>20</sup> Intensification of statin therapy results in rapid reduction of atherosclerotic inflammation as imaged by FDG-PET/CT.<sup>21</sup> Mechanisms for the anti-inflammatory effects of statin therapy include impaired prenylation of small G proteins and



the expression of transcription factors such as Kruppel-like factor 2 which promotes transcription of genes sets with anti-thrombotic, anti-inflammatory, and anti-proliferative functions.<sup>22,23</sup>

On the basis of these accumulating data, it is convenient to categorize anti-inflammatory agents undergoing evaluation as potential atheroprotective drugs into two groups, those that do and do not target the central IL-1, TNF- $\alpha$ , and IL-6 pathway. As shown in Figure 5, Phase III trials are now underway with anti-inflammatory agents that lead to marked reductions in IL-6 and C-reactive protein (such as canakinumab and methotrexate) as well as with agents that do not (such as varespladib and darapladib). Care must be taken when interpreting these studies as outcomes for drugs that target IL-6 signalling may be uninformative for agents that do not, and vice versa. Examples of these two groups of agents and their potential to impact on vascular risk are reviewed below.

## Agents that inhibit the central interleukin-1, tumour necrosis factor- $\alpha$ , interleukin-6 regulatory pathway

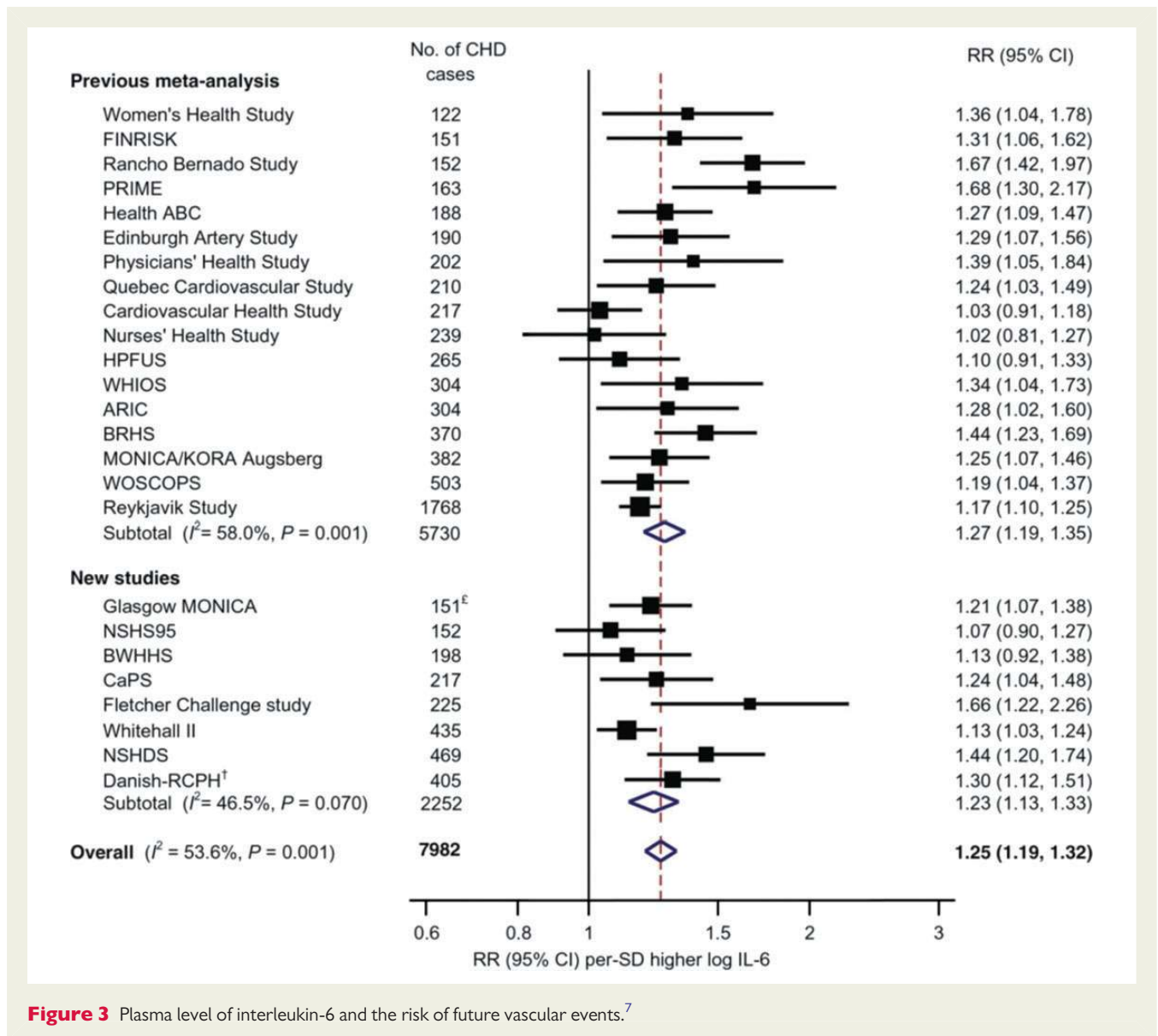
### Interleukin-1 antagonists and the nucleotide-binding leucine-rich repeat-containing pyrin receptor 3 inflammasome

Interleukins are critical mediators of the systemic anti-inflammatory response. Of inflammatory molecules implicated in atherothrombosis,

IL-1 plays a prominent role as it sits proximal to the classical IL-6 signalling cascade. Thus, IL-1 has long been considered a target for novel vasculoprotective agents.<sup>24</sup>

The IL-1 type 1 receptor is impacted upon by two activators (IL-1 $\alpha$  and IL-1 $\beta$ ) and by the naturally occurring inhibitor, IL-1 receptor antagonist (IL-1Ra) that competitively blocks binding of IL-1 $\alpha$  and  $\beta$  to the type 1 receptor. In a clinically important additional level of regulation, innate immune cells including monocytes initially produce IL-1 $\beta$  as an inactive precursor (pro-IL-1 $\beta$ ) that requires proteolytic cleavage to attain biological activity. This is typically mediated by a complex of intracellular proteins known as the NLRP3 inflammasome which, in response to the presence of crystalline structures, leads to activation of caspase-1 (also known as IL-1 $\beta$ -converting enzyme).<sup>25</sup> While this mechanism of IL-1 $\beta$  activation was originally described as a danger signal triggered by exogenous crystals such as alum, silica, or asbestos, the same NLRP3 inflammasome is also activated by endogenous agents such as uric acid and cholesterol when they move from a soluble to crystalline form.<sup>10,11</sup> The recognition that cholesterol crystals can activate IL-1 $\beta$  production has provided a common hypothesis linking hyperlipidaemia to vascular inflammation.

Activated macrophages within atheromatous plaques express IL-1 resulting in smooth muscle proliferation, recruitment of additional inflammatory cell lines into the plaque and endothelial wall, a triggering of an auto-inductive pro-inflammatory process, and critical regulation of upstream induction of IL-6 and downstream production of C-reactive protein.<sup>7,26</sup> Atheroprone oscillatory flow has recently been shown to additionally increase NLRP3 expression, activation of caspase-1, and IL-1 $\beta$  production.<sup>27</sup>



**Figure 3** Plasma level of interleukin-6 and the risk of future vascular events.<sup>7</sup>

On the basis of these data, a pivotal trial in secondary prevention has been initiated known as the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS).<sup>28</sup> Canakinumab is a human monoclonal anti-human IL-1 $\beta$  antibody indicated for the treatment of several rare IL-1 $\beta$  over-expression disorders. In high vascular risk patients, canakinumab produces dose-dependent reductions of >50% for IL-6 and C-reactive protein, as well as a 20% reduction in fibrinogen, a third mediator of thrombosis and inflammation<sup>29</sup> (Figure 6). In clinical data among those with rheumatoid arthritis (RA) and diabetes, canakinumab has shown little effect on lipid levels or platelet function. Thus, canakinumab provides a novel method to directly test the inflammatory hypothesis of atherothrombosis by inhibiting the central IL-1, TNF- $\alpha$ , and IL-6 pathway without confounding effect on lipids or coagulation.

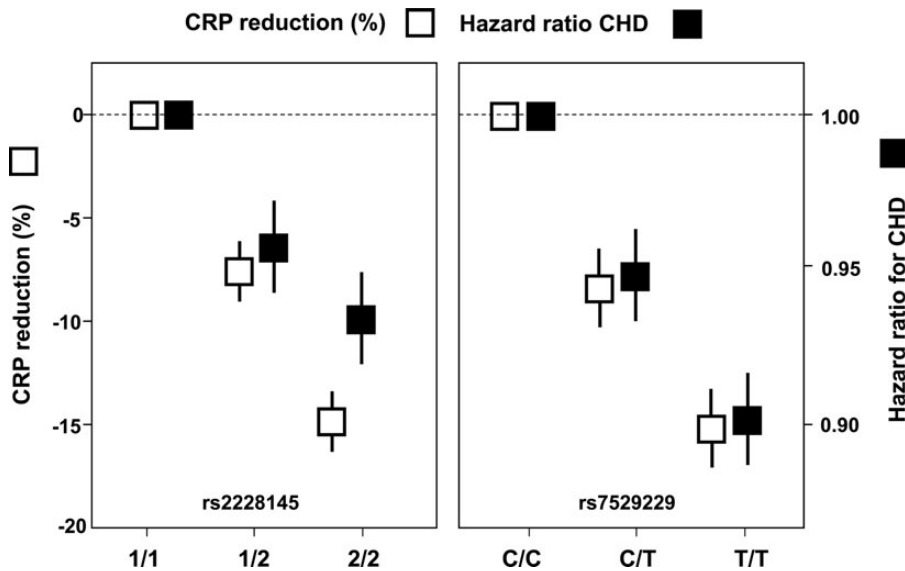
Now fully enrolled, CANTOS includes 10 000 participants with stable coronary artery disease who remain at high inflammatory risk due to a persistent elevation of C-reactive protein (>2 mg/L)

despite usual therapy, including statins. CANTOS is an event-driven trial in which the primary endpoint is a reduction in rates of recurrent major cardiovascular events. Critical secondary endpoints include evaluations of all-cause mortality and a slowing of diabetic progression. This latter endpoint reflects the recognition that diabetes is also an inflammatory disorder driven in part by IL-1 activation.<sup>30</sup>

At least two other IL-1 antibodies are in development as potential agents for atherothrombosis. In addition, the exogenous IL-1Ra anakinra (an approved agent for RA which has been shown to improve vascular function in that setting).<sup>31,32</sup> Anakinra also has efficacy in diabetes by reducing HbA1c, the pro-insulin:insulin, IL-6, and C-reactive protein.<sup>33</sup>

### Methotrexate and the tumour necrosis factor and interleukin-6 inhibitors

Patients with systemic inflammatory disorders such as RA have TNF- $\alpha$ -induced endothelial dysfunction which can be reversed by



**Figure 4** Genetic polymorphism in the IL-6 regulatory pathway associate with lifelong lower levels of C-reactive protein and with concordantly lower vascular event rates.<sup>8,9</sup>

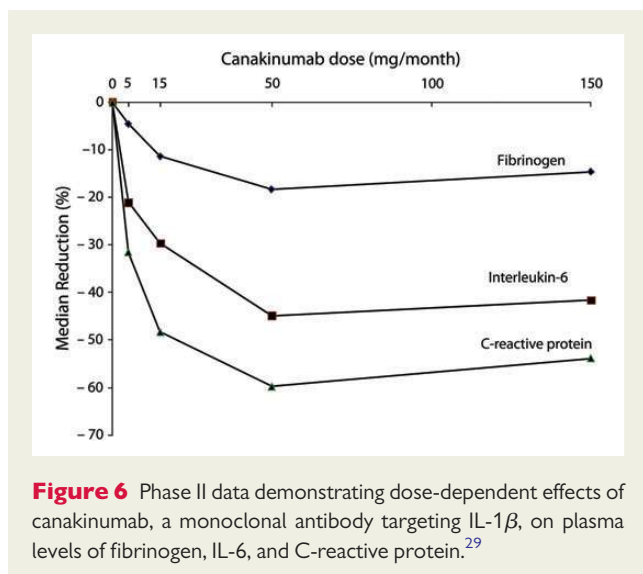
Clinical trials of anti-inflammatory therapy in cardiovascular disease

Drug	Target	Trial	Size	Sponsor	Status
<b>A. Agents impacting on the IL-6 signalling pathway</b>					
Canakinumab	IL-1 $\beta$	CANTOS	10 000	Novartis	Enrolling
Methotrexate	IL-6, TNF	CIRT	7 000	NHLBI	Enrolling
Anakinra	IL-1Ra	IL-HEART	190	UK-MRC	Completed
Colchicine	multiple	LoDoCo	532	HRS, Aus	Positive
Tocilizumab	IL-6	Entracte	3 000	Hoffmann	Enrolling
Etanercept	TNF	Entracte	3 000	Hoffmann	Enrolling
<b>B. Agents impacting on alternative inflammatory pathways</b>					
Succinobucol	Ox-LDL	ARISE	6 144	AtheroGenics	Negative
Varespladib	sPLA <sub>2</sub>	VISTA-16	5 000	Anthera	Negative
Darapladib	Lp-PLA <sub>2</sub>	STABILITY	15 000	GSK	Enrolled
Darapladib	Lp-PLA <sub>2</sub>	SOLID-TIMI-52	13 000	GSK	Enrolled
Inclacumab	P-Selectin	SELECT-ACS	544	Roche	Completed
Inclacumab	P-Selectin	SELECT-CABG	380	Roche	Enrolled

**Figure 5** Phase III trials of anti-inflammatory agents under evaluation in cardiovascular disease. Trials are grouped by agents that do and do not impact primarily on the central IL-1, TNF- $\alpha$ , IL-6 signalling system.

the specific antibody infliximab<sup>34</sup> as well as increased vascular event rates. Observational data suggest that these rates decline when patients are treated with agents that inhibit the IL-6 signalling system. A recent meta-analysis reported that patients with RA or

psoriatic arthritis (PsA) taking low-dose methotrexate (LDM) have a 21% lower risk of future cardiovascular events.<sup>35</sup> Similar data have been presented for RA patients taking TNF-inhibitors such as etanercept and infliximab or the IL-6 inhibitor tocilizumab.



Interleukin -6 inhibition with tocilizumab has been reported to improve endothelial function and reduces arterial stiffness.<sup>36</sup> However, as tocilizumab increases LDL-cholesterol levels, studies of this agent to date have largely focused on cardiovascular safety rather than efficacy. Among RA patients treated with TNF-inhibitors, modest improvements in cardiovascular risk profile have been observed in some studies.<sup>37</sup> In the setting of heart failure, however, etanercept failed to improve clinical outcomes and was associated with infection and adverse lipoprotein changes.<sup>38</sup>

To address some of these issues, the ENTRACTE investigators are undertaking a randomized, open-label clinical trial comparing the effects of IL-6 receptor blockade with tocilizumab to TNF-inhibition with etanercept on the rate of vascular events among 3000 patients with moderate-to-severe RA (ClinicalTrials.gov Identifier: NCT01331837). Rheumatoid arthritis patients 50 years of age or greater with an inadequate clinical response to at least one non-biological disease-modifying anti-rheumatic drug and a history of coronary heart disease or multiple cardiovascular risk factors are eligible for ENTRACTE. This trial has no placebo group as all enrolled patients have a systemic inflammatory disorder requiring treatment; whether results from ENTRACTE will generalize to populations without clinically manifest inflammatory disorders is thus uncertain.

Similarly, everolimus used in transplantation medicine has the potential to reduce inflammation via the mTOR pathways. The CLEVER-ACS trial (ClinicalTrials.gov Identifier: NCT01529554) is about to enroll patients with ST-segment elevation myocardial infarction to determine whether such an anti-inflammatory therapy will reduce infarct size, remodelling and clinical events in this patient population.

In 2013, the National Heart Lung and Blood Institute initiated the Cardiovascular Inflammation Reduction Trial (CIRT) in which 7000 patients with chronic atherosclerosis and either diabetes or metabolic syndrome will be randomized to usual care plus placebo or to usual care plus LDM.<sup>39</sup> Low-dose methotrexate (15–20 mg per week) is the treatment of choice for RA and PsA, and is taken safely on a long-term basis by several hundred thousand patients worldwide. Among those with RA and PsA, LDM reduces IL-6 and C-reactive protein and thus impacts directly on the central IL-6 signalling pathway. Emerging

evidence suggests that at least part of the anti-inflammatory and atheroprotective effects of LDM result from increased adenosine release and subsequent antagonism of the adenosine A<sub>2A</sub> receptor.<sup>40</sup> Stimulation of this receptor induces the expression of several proteins associated with reverse cholesterol transport including 27-hydroxylase and the ATP-binding cassette transporter, preventing formation of foam cells from human macrophages.<sup>41</sup> A<sub>2A</sub> stimulation also attenuates inflammation directly and reduces the expression of adhesion molecules such as intercellular adhesion molecule type 1 (ICAM) and vascular cellular adhesion molecule (VCAM) commonly associated with atheroma progression.<sup>42</sup> Methotrexate also may have direct anti-atherosclerotic effects; in the cholesterol-fed rabbit model, weekly i.v. methotrexate reduced new atheroma formation by 75% and reduced multiple biomarkers of macrophage function within the vessel wall.<sup>43</sup> The primary endpoint of CIRT, like that of CANTOS and ENTRACTE, is recurrent non-fatal cardiovascular events and cardiovascular mortality. An important pre-specified secondary endpoint of CIRT is a reduction in the progression to diabetes (among those with metabolic syndrome) and reduced need for hypolipidaemic agents (among those who are already diabetic). Cardiovascular Inflammation Reduction Trial employs a central titration algorithm to ensure identical allocation of LDM (or placebo) at all study sites, and has ongoing monitoring for side effects such as infection or bone-marrow suppression.

## Agents that inhibit alternative inflammatory pathways

### Secretory phospholipase inhibitors

Members of the phospholipase A<sub>2</sub> superfamily including lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) and secretory PLA<sub>2</sub> (sPLA<sub>2</sub>) are characterized by their ability to hydrolyse phospholipid molecules at the sn-2 position, a process that results in the production of potentially atherogenic lipid fractions and enhances oxidant stress.<sup>44</sup> Both Lp-PLA<sub>2</sub> and sPLA<sub>2</sub> derive largely from macrophages and circulate in plasma complexed with LDL- and HDL-cholesterol. sPLA<sub>2</sub>-modified lipoprotein and Lp-PLA<sub>2</sub>-modified lipoproteins have been shown to associate with oxidized LDL particles capable of activating multiple inflammatory pathways with potential relevance for atherogenesis and plaque rupture.<sup>45</sup> Clinically, plasma measurement of sPLA<sub>2</sub> activation as well as Lp-PLA<sub>2</sub> mass and Lp-PLA<sub>2</sub> activity associate with elevated cardiovascular risk.<sup>46,47</sup> However, the utility of commercial measures for these biomarkers remains controversial.<sup>48</sup> Once patients are treated with high-dose statin therapy, there is little evidence that levels of Lp-PLA<sub>2</sub> associate with residual vascular risk.<sup>49</sup> Whether secretory phospholipases play a causal role in atherothrombosis has also recently been challenged; as with C-reactive protein, Mendelian randomization studies for sPLA<sub>2</sub> have not found evidence, suggesting a direct causal relationship.<sup>50</sup> In contrast to canakinumab, anakinra, and methotrexate, it is unlikely that secretory phospholipase inhibitors impact directly on the central IL-6 regulatory pathway as neither varespladib (a non-specific sPLA<sub>2</sub> inhibitor) nor darapladib (a targeted LpPLA<sub>2</sub> inhibitor) has robust effects on IL-6 or C-reactive protein. In the FRANCIS trial,<sup>51</sup> on-treatment IL-6 levels were 3.2 pg/mL in the varespladib group and 3.0 pg/mL in the placebo group, a non-significant difference.

Similarly, no significant differences in on-treatment C-reactive protein levels were observed in the PLASMA I<sup>52</sup> or PLASMA II studies.<sup>53</sup>

With regard to darapladib, no significant effects on C-reactive protein when compared with placebo were found in phase II dose finding studies<sup>54</sup> or in the larger 300 patient Integrated Biomarker and Imaging Study (IBIS-2) which evaluated the effects of darapladib on IVUS-based plaque characteristics.<sup>55</sup> In IBIS-2, no effect of darapladib was observed on the co-primary endpoints of plasma C-reactive protein concentration or coronary atheroma deformability as evaluated by palpography. However, a secondary endpoint of lipid necrotic core progression as assessed by virtual histology was improved in the active treatment group ( $P = 0.01$ ). Thus, varespladib and darapladib are effective agents for reducing sPLA<sub>2</sub> and Lp-PLA<sub>2</sub>, respectively, but do not appear to impact greatly on the central IL-6 to C-reactive protein signalling pathway. As such, trials of these agents are of biological importance as they target a specific non-IL-6-dependent pathway hypothesized to be relevant to vascular inflammation.

To date, three major Phase III outcome trials have been initiated testing the impact of either varespladib or darapladib on recurrent vascular events. One of these trials, the 5000 participant VISTA-16 was recently stopped early by its Data and Safety Monitoring Board for futility.<sup>56</sup> The 15 000 participant STABILITY trial found a small but non-significant reduction in vascular events among those with stable atherosclerosis, while the SOLID-TIMI-52 trial of 13 000 patients with acute ischaemia is ongoing.<sup>57,58</sup>

### Vascular-targeted anti-oxidants

Part of the atherosclerotic hazard associated with secretory phospholipases is the result of production of lipid oxidation products and oxidative stress. An additional agent with anti-oxidant and potential anti-inflammatory properties to reach Phase II trial evaluation has been succinobucol, a monosuccinic acid ester of probucol, itself a lipid-lowering agent that failed to gain wide clinical use in part due to adverse effects on the QT segment.

Investigators in the Aggressive Reduction in Inflammation Stops Events (ARISE) trial randomly allocated 6144 patients with recent ischaemia to either succinobucol or to placebo and followed participants for the primary endpoint of cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, unstable angina, or revascularization.<sup>59</sup> No benefit on this primary endpoint was observed (HR: 1.00, 95% CI: 0.89–1.13,  $P = 0.96$ ), but adverse events in terms of haemorrhage, lipid levels, hypertension, and atrial fibrillation were increased. A modest benefit was present for succinobucol for a secondary endpoint limited to cardiovascular death, myocardial infarction, stroke, and cardiovascular arrest (HR: 0.81, 95% CI: 0.68–0.98,  $P = 0.029$ ) and a tertiary endpoint of new-onset diabetes developed in fewer patients without diabetes at baseline in the succinobucol group than in the placebo group (HR: 0.37, 95% CI: 0.24–0.56,  $P < 0.001$ ). Succinobucol modestly reduced haemoglobin A1c. In a second trial conducted among 232 patients undergoing elective percutaneous coronary interventions, succinobucol 280 mg daily had no effect on plaque volume or atherosclerotic regression as evaluated by intravascular ultrasound.<sup>60</sup> In this study, as in ARISE, succinobucol was not shown to reduce either IL-6 or C-reactive protein. Thus, trial data for succinobucol are not informative as a test of the central IL-6 regulatory pathway. Due largely to

the adverse effect profile observed in ARISE, no further development of this agent is underway.

### Adhesion molecule inhibitors

Adhesion molecules such as ICAM-1 and VCAM-1 serve critical roles in the adhesion and transmigration of leucocytes across the endothelial wall, an early step in the formation of the atherosclerotic plaque. Epidemiological data have long shown strong positive associations between soluble levels of these adhesion molecules and future vascular events and thus multiple adhesion molecules serve as potential vasculoprotective targets for atherothrombosis.<sup>61</sup>

Leucocyte tethering and rolling along the vascular endothelium is also mediated by a related class of cell surface glycoproteins known as selectins that are more typically expressed by platelets.<sup>62</sup> In particular, P-selectin has been shown to mediate multiple cell–cell interactions relevant to the initiation and progression of atherosclerotic plaques, an effect postulated to be of greatest importance at the time of plaque rupture.<sup>63</sup> In man, the expression of P-selectin is increased in atherosclerotic plaque, is found in higher levels in the plasma of those with unstable angina, and at least in the setting of dialysis, is associated with increased vascular mortality.<sup>64</sup> P-selectin antagonism has also been found to reduce thrombus formation in humans.<sup>65</sup>

Following this lead, 544 patients with non-ST-segment elevation myocardial infarction scheduled for angiography were randomly allocated in the SELECT-ACS trial to placebo or to inclacumab (a recombinant monoclonal antibody against P-selectin) at a dose of either 5 or 20 mg/kg given as a single 1-hour infusion. While there was no effect compared with placebo at the 5 mg inclacumab dose, those allocated to 20 mg inclacumab had moderately reduced myocardial damage as measured by indices of troponin and CK-MB release.<sup>66</sup> These Phase II data provide optimism that novel anti-thrombotic approaches that additionally impact on cell–cell interactions might prove efficacious in some cardiovascular settings. A similar Phase II trial of inclacumab is ongoing among patients scheduled for coronary artery bypass surgery (SELECT-CABG).

### Leukotriene inhibitors

Derived from arachidonic acid, the leukotrienes comprise a group of eicosanoids capable of activating multiple inflammatory pathways. Among potential leukotriene modifiers being considered in vascular disease, inhibitors of the 5-lipoxygenase (5-LO) pathway have been most aggressively pursued.<sup>67,68</sup> This reflects, in part, observations of increased 5-LO expression in those with unstable atheroma and among those with chronic ischaemia. Interest in inhibiting 5-LO also increased after reports that polymorphism in the 5-LO activating protein gene results in an increased risk of myocardial infarction.<sup>69</sup> To date, one clinical 5-LO inhibitor (Atreleuton, VIA-2291) has been evaluated in the setting of carotid disease and in an imaging trial conducted in the setting of acute coronary ischaemia.<sup>70</sup> In the carotid study, 50 patients undergoing elective carotid endarterectomy were treated for 12 weeks with either 100 mg of VIA-2291 or placebo; in this study, the primary endpoint of per cent reduction in macrophage inflammatory cells in excised plaque tissue did not differ between the two treatment groups. In the acute coronary ischaemia study, 191 patients were randomly assigned to receive 25, 50, or 100 mg VIA-2291 or placebo daily for 12 weeks. The

primary study endpoint, whole blood stimulated leukotriene LTB<sub>4</sub> at a trough drug level, was reduced in all active treatment groups ( $P < 0.001$ ) and a significant reduction of urine leukotriene LTE<sub>4</sub> was observed compared with placebo. In a subset limited to 60 compliant patients who had coronary CT exams before and after treatment, new coronary plaques were less frequent in the active treatment group when compared with placebo.

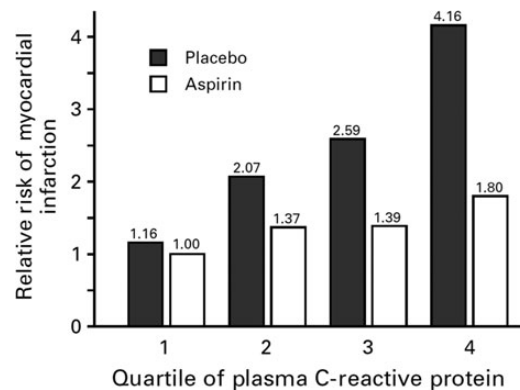
## Serpins and sirtuins

Serine protease inhibitors (serpins) are a family of common glycoproteins that exhibit complex effects on thrombosis in man, but in virus vectors exhibit anti-thrombotic and anti-inflammatory properties.<sup>71</sup> In particular, infusion of the viral serpin Serp-1 results in inhibition of tissue-type and urokinase type plasminogen activation and in animal models has resulted in reduced neointimal proliferation and inhibition of monocytes/macrophage recruitment to the site of tissue injury following experimental balloon angioplasty.<sup>72</sup> Recently, Tardif *et al.* performed a Phase II study of the myxoma virus-derived serpin Serp-1 among 48 patients with ACS undergoing percutaneous coronary intervention.<sup>73</sup> Treatment was given by i.v. bolus of 5 or 15  $\mu\text{g}/\text{kg}$  Serp-1 (or placebo) before intervention and 24–48 h after. In this study, the primary endpoint of in-stent neointimal hyperplasia evaluated by intravascular ultrasound did not differ after 6 months. However, Serp-1 did reduce markers of cardiac damage such as troponin I and CKMB levels and showed a trend towards clinical benefit although the number of events recorded was small. Of interest Serp-1 did not have impact on circulating inflammatory biomarkers in this initial evaluation.

Sirtuins comprise a family of NAD<sup>+</sup>-dependent deacetylases that regulate multiple cellular processes related to ageing, apoptosis, atherosclerosis, and inflammation.<sup>74,75</sup> Of these enzymes, SIRT1, a histone deacetylase, has gained interest in vascular disease as it exerts anti-inflammatory effects via NF $\kappa$ B, inhibits atherothrombosis, and promotes angiogenesis.<sup>76,77</sup> In apo-E-deficient mice, overexpression of SIRT1 decreases atherosclerotic progression.<sup>78</sup> On the other hand, pharmacological SIRT1 activation in ApoE<sup>-/-</sup> mice provides atheroprotection by reducing hepatic PCSK9 secretion and enhancing LDL-R expression.<sup>79</sup> Consistent with the common soil hypothesis linking atherothrombosis to insulin resistance, genetic variation in sirtuins has been associated with type 2 diabetes and obesity.<sup>80</sup> On this basis, sirtuin activators have been hypothesized as a potential novel strategy to prevent atherosclerosis and treat diabetes,<sup>81</sup> an intriguing issue as small molecule activators of SIRT1 are available. In addition, as the metabolite NAD<sup>+</sup> has been shown to result in sirtuin activation, there is renewed interest in the administration of NAD<sup>+</sup> precursors such as nicotinic acid and nicotinamide as therapeutic agents in the prevention of recurrent vascular disease.

## The re-purposing of generic anti-inflammatory agents

As described above, the generic agent LDM is currently being evaluated by the National Heart Lung and Blood Institute as a potential anti-atherosclerotic agent. Several other generic anti-inflammatory drugs including aspirin, salsalate, colchicine, and hydroxychloroquine have also been considered for evaluation in the setting of atherosclerosis.



**Figure 7** Effects of aspirin when compared with placebo in the primary prevention of future vascular events, stratified by the baseline level of C-reactive protein.<sup>82</sup>

## Aspirin and salsalate

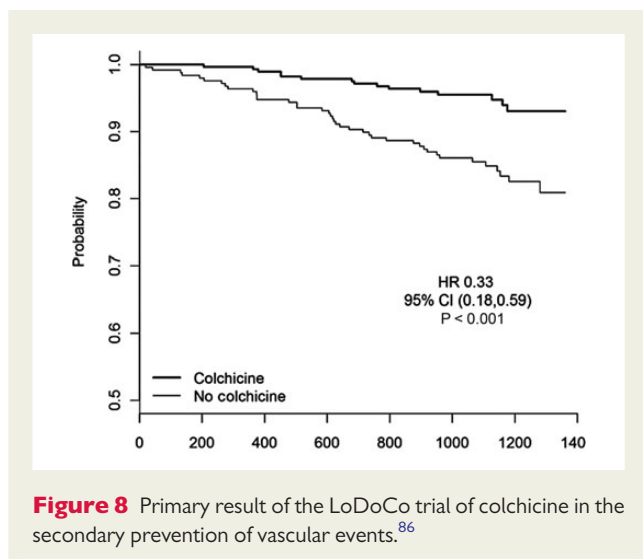
Aspirin, an acetylated form of salicylate, has both anti-thrombotic and anti-inflammatory effects, the latter due to the fact that the acetyl group covalently modifies serine at the active site of cyclo-oxygenase enzymes. In 1997, an analysis of inflammation performed within the Physicians Health Study demonstrated that the magnitude of cardiovascular risk reduction associated with random allocation to aspirin when compared with placebo was greatest among those with the highest baseline levels of C-reactive protein and became sequentially smaller as the levels of C-reactive protein dropped ( $P$ -trend across quartiles  $< 0.001$ )<sup>82</sup> (Figure 7). This differential benefit of aspirin on first-ever vascular events was not related to anti-platelet effects as these did not vary across levels of C-reactive protein. Thus, this study of one of the oldest drugs used in medicine effectively became the first clinical demonstration that anti-inflammatory therapies might be effective for the prevention of cardiovascular disease.

Unlike aspirin, salicylate (salsalate) is not acetylated. Thus, salsalate is not a cyclo-oxygenase inhibitor and does not impact upon bleeding times or platelet aggregation. Salsalate, however, is an effective anti-inflammatory widely used to treat joint pain and that modestly reduces leucocyte counts likely as a result of nuclear factor- $\kappa$ B effects. Whether or not salsalate might reduce cardiovascular event rates is unknown. However, as shown in stages 1 and 2 of the TINSAL-T2D trials conducted among diabetics, salsalate safely reduces haemoglobin A1c as well as C-reactive protein, and has systemic anti-inflammatory effects as reflected in reduced circulating leucocyte, neutrophil, and lymphocyte.<sup>83,84</sup> Modest but not dose-limiting tinnitus was observed in these studies. As an inexpensive and otherwise well-tolerated generic agent, salsalate remains a candidate anti-inflammatory agent worthy of testing in future vascular inflammation reduction trials.

## Colchicine and hydroxychloroquine

Other than methotrexate, perhaps the most provocative data supporting the concept of 're-purposing' old anti-inflammatory medications for a new role in vasculoprotection derives from recent data regarding colchicine, an agent long used to treat gout. Colchicine





has several anti-inflammatory properties including anti-tubulin effects that inhibit neutrophil function as well as modest effects on the NLRP3 inflammasome, a mechanism consistent with its effects on gout, a classical disorder of crystal deposition. After demonstrating that colchicine reduces C-reactive protein independent of aspirin and statins,<sup>85</sup> the LoDoCo Investigators assigned 532 patients with stable coronary artery disease to receive either colchicine 0.5 mg per day for at least 30 days or to non-placebo control treatment using a PROBE open-label study design.<sup>86</sup> Although >20% of those allocated to colchicine were intolerant of treatment due to anticipated adverse gastrointestinal effects and stopped treatment either early ( $n = 32$ ) or late ( $n = 30$ ) in the trial, active treatment nonetheless markedly reduced the primary trial endpoint of recurrent acute coronary syndrome, cardiac arrest or non-embolic stroke (HR: 0.33, 95% CI: 0.18–0.59,  $P = 0.001$ ), with most of the effect due to reduction in hospitalization for ACS (Figure 8). Benefits appeared early after trial initiation, were sustained throughout the median 3-year follow-up period, and were consistent in all major subgroups considered. Large-scale, fully blinded trials of colchicine in secondary prevention are thus warranted.

Finally, the anti-malarial drug hydroxychloroquine has long been used to reduce inflammation in patients with RA and lupus. Part of this anti-inflammatory effect is related to acidic alteration of the pH within lysosomes, an effect that diminishes proteolysis and may result in reduced immune cell function. Hydroxychloroquine also inhibits stimulation of toll-like receptor-9 and can produce an inhibition of the innate immune response.<sup>87</sup> Like salsalate, hydroxychloroquine has been shown to reduce haemoglobin A1c in refractory diabetic patients.<sup>88</sup> However, rare reports in lupus patients of hydroxychloroquine-induced cardiomyopathy have limited further evaluation of this agent in vascular patients.

## High-density lipoprotein

HDL-cholesterol has a variety of anti-inflammatory effects such as reduction of the interaction of white blood cells with the endothelium, reduction in VCAM and ICAM expression in the presence of cytokines such as TNF- $\alpha$ , reduction in the production of reactive

oxygen species by endothelial cells and inhibition of apoptosis.<sup>89,90</sup> However, these anti-inflammatory effects are lost in HDL-C obtained from patients with coronary artery disease, acute coronary syndromes, and renal insufficiency,<sup>91</sup> at least in part due to reduced PON-1 activity. To date, trials aimed at increasing HDL-C in order to reduce clinical events have been negative<sup>92</sup> or neutral.<sup>93</sup>

## Adaptive immunity and vaccination against atherosclerosis

In addition to innate immunity, adaptive immunity plays a substantive role in atherothrombosis and antigen-presenting dendritic cells, T lymphocytes, B lymphocytes, and immunoglobulins are all found within vascular plaque.<sup>94</sup> Several laboratories have thus aggressively pursued vaccination strategies as a novel method to reduce atherosclerosis. While early targets for these vaccines used LDL as an immunogen,<sup>95</sup> more recent work has focused on epitopes to oxidized LDL or to specific peptide sequences of apolipoprotein B 100, the principle apoprotein carried within LDL-cholesterol. In mouse models of hypercholesterolaemia, it has also recently become apparent that TH1 cells appear to increase atherosclerotic progression, whereas T regulatory cells may have a role in disease regression.<sup>96,97</sup> All of these observations, as well as initial success in mouse models, have provided impetus to take potential atherosclerosis vaccines based on apo B peptides and other antigens into human clinical testing.<sup>98</sup>

## Limitations and future directions

As several commentators have noted, the inflammatory system is simultaneously redundant, compensatory, and crucial for survival.<sup>99</sup> As such, anti-inflammatory therapies in the setting of chronic disorders pose substantive challenges. Further, evaluation of risks as well as benefits must drive the development of agents in this class. As shown by adverse data for TNF-inhibitors in heart failure and the finding of increased vascular risk among those taking COX-2 inhibitors, there may be unintended consequences of inflammation suppression. However, as proven among these with RA and inflammatory bowel disease, long-term treatment with systemic anti-inflammatory agents can be accomplished safely. In addition to the therapies described here, multiple alternative approaches to inflammation inhibition are being developed. These include targeted steroid delivery systems such as Nanocort; the infusion of reconstituted HDL-cholesterol; and emerging imaging-based approaches to inflammation detection and targeted intervention.<sup>100–102</sup> Genetic studies are also pointing to new directions for vascular inflammation inhibition. In one recent genome-wide association study, major histocompatibility complex genes that regulate inflammation and T-cell responses were found to significantly associate with vascular risk.<sup>103</sup> The next 5 years will see publication of several massive trials directly testing the inflammatory hypothesis of atherosclerosis.<sup>104</sup> The core results of these trials—including those that do and do not inhibit the central IL-1, TNF- $\alpha$ , and IL-6 pathway—will tell us a great deal about whether anti-inflammatory therapies will eventually become a cornerstone of vascular risk reduction. If successful, these trials will usher in a new era in which the treatment of chronic vascular disease moves beyond the reduction of LDL-cholesterol alone.

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