Published in final edited form as:

Nat Rev Drug Discov. 2011 May ; 10(5): 365–376. doi:10.1038/nrd3444.

Anti-inflammatory therapeutics for the treatment of atherosclerosis

Israel F. Charo^{*} and Rebecca Taub[‡]

Rebecca Taub: icharo@gladstone.ucsf.edu

^{*}Gladstone Institute of Cardiovascular Disease, 1650 Owens Street #149, San Francisco, California 94158, USA

[‡]Research & Development, VIA Pharmaceuticals, 500 Office Center Drive, Suite 400, Fort Washington, Pennsylvania 19034, USA

Abstract

Atherosclerosis is the primary cause of heart disease and stroke and is thus the underlying pathology of the leading causes of death in the western world. Although risk can be reduced by lowering lipid levels, the equally important contribution of inflammation to the development of cardiovascular disease is not adequately addressed by existing therapies. Here, we summarize the evidence supporting a role for inflammation in the pathogenesis of atherosclerosis, discuss agents that are currently in the clinic and provide a perspective on the challenges faced in the development of drugs that target vascular inflammation.

Atherosclerosis is the primary cause of coronary artery disease, stroke and peripheral vascular disease, and it therefore represents the most common cause of morbidity and mortality worldwide¹. Atherosclerosis is a complex disease that is thought to be initiated by the trapping of lipids in the subendothelial layer of the arterial wall, followed by the generation of biologically active species — including oxidized low-density lipoprotein (LDL) — which stimulate vascular cells to produce inflammatory molecules and recruit monocytes and T cells to the arterial wall. Blood monocytes that are recruited to the vessel wall are the precursors of the lipid-laden macrophages that form fatty streaks — the anatomical hallmark of early atherosclerosis^{2–6}. Other leukocyte populations such as neutrophils have also been associated with atherosclerotic plaque development⁷.

Traditionally, atherosclerosis was attributed to progressive narrowing of the artery lumen owing to hyperlipidaemia. It is now clear that the vascular wall is a site of chronic inflammation, resulting from complex interactions between the environment and the genetic makeup of the host^{2–6,8}. Major adverse cardiovascular events (MACE), including stroke and myocardial infarction (MI), occur in patients who develop atherosclerotic plaques in their carotid and coronary arteries. Approximately two-thirds of these events arise when the fibrous cap of an unstable atherosclerotic plaque ruptures, leading to release of thrombogenic material and blockage of the artery owing to platelet aggregation^{2,9} (BOX 1).

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Competing interests statement

The authors declare [competing financial interests](#): see Web version for details.

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A diagnosis of acute coronary syndrome (ACS) encompasses patients with new-onset ischaemic symptoms, those with progressively worsening chest pain or anginal syndromes, and those with chest pain that is associated with specific types of electrocardiographic changes, such as ST-segment elevation or depression. As all of these portend the possibility of irreversible cardiac damage, including MI and perhaps death, aggressive therapeutic interventions are commonly indicated. These interventions range from platelet inhibitors, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, antihypertensives and thrombolytic agents, to percutaneous arterial dilatation, stenting and coronary artery bypass surgery¹⁰. Long-term control of the disease and its consequences — through aggressive reduction or elevation of selected lipid fractions in the serum and prevention of stimulated platelet aggregation and the resulting thromboses — has significant clinical benefit^{11,12}.

However, despite optimal treatment, the residual risk of another MACE in these patients is estimated to be 70–80%. This is believed to be due, in part, to the fact that current therapies do not address the inflammatory component of atherosclerosis. Indeed, several anti-inflammatory drugs that aim to reduce the risk of MACE are now being investigated. Here, we discuss recent clinical trials that support the notion that inflammation is an important driver of MACE, review clinical trials of novel, non-lipid-lowering drugs that are aimed at reducing inflammation in atherosclerotic plaques and highlight the challenges associated with developing drugs in this class.

Biomarkers and imaging for cardiovascular diseases

Establishing markers for accurately predicting clinical outcome and assessing disease risk and progression would greatly facilitate cardiovascular disease drug development and treatment. According to the US National Institutes of Health (NIH) Definition Working Group, markers are defined as follows:

- Biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention
- Clinical end point: a characteristic or variable that reflects how a patient feels, functions or survives
- Surrogate end point: a biomarker that is intended to be substituted for a clinical end point

A surrogate end point should predict clinical benefit or harm, or lack of benefit on the basis of epidemiological, therapeutic, pathophysiological or other scientific evidence¹³. Unfortunately, the identification of such markers has proven to be difficult in the field of cardiovascular disease.

Indeed, the only currently recognized surrogate end points in cardiovascular disease are the lowering of LDL cholesterol (LDL-C) levels and other lipid modifications such as lowering triglyceride levels and reducing blood pressure. In cardiovascular outcome studies, MACE have been reduced by lowering LDL levels with HMG-CoA reductase inhibitors — namely the statins — and by decreasing blood pressure with antihypertensives^{11,14}. However, there are considerable limitations to such studies, as more than 6,000–10,000 patients must typically be treated, for a year or longer, to demonstrate that an agent prevents MACE, including heart attacks, strokes and cardiovascular mortality. Because of these daunting numbers and high clinical development costs, efforts have been made to establish novel surrogate biomarkers — including the use of imaging technologies and the identification of soluble biomarkers — that correlate with successful clinical outcomes.

However, such efforts have largely been unsuccessful and the acceptance of new surrogate end points for cardiovascular trials has been hindered by the lack of correlation between cardiovascular outcomes and what is considered to be a possible surrogate end point. One example is the rise in high density lipoprotein (HDL) cholesterol that was observed in response to torcetrapib in the ILLUMINATE trial¹⁵ (see TABLE 1 for full definitions of the names of trials discussed in this Review) and development of this agent has now been discontinued. Indeed, although HDL is accepted as a risk factor for cardiovascular disease, it is not accepted as a surrogate end point.

Furthermore, the failure of a cholesterol-lowering agent to show a benefit in carotid intima medial thickness (IMT) in the ENHANCE trial¹⁶, and the lack of cardiovascular benefit associated with glucose lowering in diabetic patients (the ACCORD trial)¹⁷, has led to questions regarding the role of well-established surrogates such as LDL-C and glucose. However, the explanations for the findings in these trials are complex and may not in fact disqualify the end points. For example, in the ENHANCE trial, the baseline IMT may have been too low to allow for a measurable effect to be detected. In any case, as a result of these clinical trial failures, the only acceptable end points for potential cardiovascular drugs, such as anti-inflammatory agents that do not lower LDL or blood pressure, continue to be a reduction in morbidity and mortality in large trials.

Given the important contribution of inflammatory processes to atherosclerosis^{2,5,6}, there are various markers of inflammation that may be monitored to facilitate cardiovascular disease drug discovery. Indeed, there are a variety of soluble biomarkers and imaging modalities that may be affected by specific anti-inflammatory drug candidates, and could therefore be used to establish confidence that the therapeutic intervention will ultimately reduce the incidence of cardiovascular disease and may thus serve to support the decision to conduct large-scale cardiovascular outcome trials. Even though such biomarker and imaging end points are not accepted surrogate outcomes for Phase III studies, they provide important research tools to explore the mechanism of action of the drug, and shed light on its potential effectiveness.

Soluble biomarkers in patients with cardiovascular disease

Over the past several years, several known and novel biomarkers have been tested as correlates of risk for cardiovascular disease. Clinical assessments of cholesterol levels, diabetes, hypertension, smoking and other risk factors continue to be strongly associated with the risk of cardiovascular events. In many cases, modification of these risk factors, including hypertension and LDL-C levels, reduces the risk of MACE^{11,14}.

Among the soluble markers that have been studied, the marker that is most reproducibly associated with cardiovascular risk is C-reactive protein (CRP) or high-sensitivity CRP (hsCRP), which has been established both as an inflammatory biomarker and as a risk factor for cardiovascular disease^{18–20}. It is unknown whether the acute-phase protein simply represents a marker of inflammation or whether it is causally involved in atherogenesis. CRP is present in early atheromatous plaques and it colocalizes with activated complement. Modification of complement activation by CRP could either worsen atherosclerosis or facilitate LDL removal from atherosclerotic lesions¹⁸. However, in patients with genetically elevated CRP there appears to be no further increase in the risk of developing cardiovascular disease²¹. Nonetheless, increased CRP levels (>2 mg per litre) generally predict an increased risk of MACE^{19,20}. Moreover, statin therapy leads to a decrease in hsCRP levels and reduces the risk of MACE, even in the absence of reductions in LDL-C²².

Another inflammatory biomarker that has been consistently associated with cardiovascular disease risk is myeloperoxidase (MPO). MPO is an enzyme that is expressed in leukocytes

and is linked to both inflammation and oxidative stress; it has also been implicated in the initiation and propagation of atherosclerosis²³. In the past few years, evidence emerging from epidemiological studies has shown that higher concentrations of MPO are associated with an increased cardiovascular risk. However, the use of MPO as a biomarker and the interpretation of these studies is hampered by the fact that various assay methods and sampling procedures have been applied. Thus, there is a need for a robust MPO assay that can be applied uniformly²³.

In a recent study, investigators evaluated 30 biomarkers reflecting different components of the pathophysiology of atherosclerotic heart disease, including inflammation, in two cohorts totalling almost 10,000 individuals²⁴. Unfortunately, most of the novel biomarkers were either not associated with MACE or they were not reproduced in more than one cohort. Nonetheless, consistent — albeit modest — associations with incident events were seen across cohorts for three well-established cardiovascular biomarkers: hsCRP, B-type natriuretic peptide (BNP) and cardiac troponin I. BNP and its prohormone-derived amino terminal split product (NT-proBNP) appear to be particularly promising in diagnosing heart failure and more recently in predicting coronary artery disease. Concentrations of NT-proBNP are related to left ventricular filling pressures and wall stress²⁵. Although it is not an inflammatory biomarker like hsCRP, cardiac troponin I is a well-studied marker of myocardial damage²⁶. A weighted biomarker score that took into account several statistical assessments was developed in men from one of the cohorts and then validated in a second cohort of men. Adding the biomarker score to a standard risk factor model improved multiple metrics of risk prediction. Although the effect of therapeutic intervention on biomarkers was not evaluated, the size of the study and the overall approach provided significant insight into the value of such assays.

Imaging assessment of atherosclerotic disease

Several imaging technologies have been evaluated for their ability to predict cardiovascular risk (TABLE 2) and these have been extensively reviewed elsewhere^{13,27}. The most validated imaging techniques for coronary artery disease are coronary angiography and intravascular ultrasound (IVUS). These techniques accurately detect the presence of atherosclerotic disease but they are invasive. Modification of IVUS signals by various agents has been incorporated into expanded labelling for the prevention of atherosclerosis, but IVUS results are not accepted as a clinical end point. Likewise, carotid IMT is generally well accepted as a measure of the extent of atherosclerotic disease in the carotid artery, and agents that reduce the progression of IMT (for example, statins) have expanded label claims to include modification of atherosclerosis. However, even considerably well-validated imaging modalities such as these are not used as a substitute for clinical outcomes.

Another emerging technology that is used in the detection of cardiovascular diseases is fludeoxyglucose (FDG)-positron emission topography (PET), which is based on the finding that atherosclerotic plaques take up FDG, and the potential correlation between areas of high FDG uptake with macrophage density or inflammation within the plaque. This technique has not yet reached the same level of validation as IMT and IVUS. Other imaging technologies remain at various stages of development and validation.

Anti-inflammatory drug targets in atherosclerosis

The contribution of inflammation to the development of atherosclerotic plaques is now reasonably well-understood (FIG. 1) and several promising targets have been identified. Indeed, anti-inflammatory drugs that are aimed at reducing the risk of MACE are now in development. The most promising of these will be discussed below.

Notably, only a single drug in each target class is currently in clinical development (TABLE 3). In addition, statins, which were developed to reduce cholesterol synthesis, have been revealed to also exhibit an anti-inflammatory mechanism of action. Furthermore, it appears that some HDL-targeting therapeutic agents (for example, niacin and cholesteryl ester transfer protein inhibitors) may have direct anti-inflammatory actions; these have been extensively reviewed and so will not be discussed further here^{28,29}. However, the direct anti-inflammatory actions of HDL-targeting therapeutic agents have not yet been distinguished from their potential benefits in promoting cholesterol efflux from lipid-laden macrophages within atherosclerotic plaques.

HMG-CoA reductase inhibitors (statins)

Although statins were developed to specifically reduce cholesterol synthesis, clinical trials have indicated that their beneficial effects extend beyond lipid lowering. For example, in the CARE study, post-MI patients were randomly selected to determine whether subsequent treatment with pravastatin (Pravachol; Bristol-Myers Squibb) alters levels of the inflammatory biomarker CRP³⁰. After 5 years, patients in the placebo group exhibited a modest increase in CRP levels, whereas those in the pravastatin group exhibited decreased CRP levels. Of note, the decrease in CRP did not correlate directly with a reduction in cholesterol levels.

Further evidence for an important anti-inflammatory action of statins came from a retrospective analysis of another study: the AFCAPS/TexCAPS trial³¹. This primary prevention study in 6,500 patients demonstrated a statistically significant reduction in serious cardiovascular events in patients with no history of cardiovascular disease. Interestingly, no clinical benefit was observed in individuals with LDL-C levels <150 mg per dl and hsCRP levels <2 mg per litre, whereas a statistically significant benefit was observed in those with LDL-C levels <150 mg per dl and hsCRP levels >2 mg per litre. In conjunction with preclinical data, these results suggest that statins have a rapid anti-inflammatory effect that may be independent of their ability to lower LDL-C. More recent trials have sought to test this hypothesis.

The PROVE IT-TIMI 22 trial was designed to determine whether intensive lowering of blood cholesterol levels beyond the recommended level of 100 mg per dl (2.59 mmol per litre) would further increase the clinical benefits of statins¹⁹. Pravastatin (40 mg per day), which was expected to reduce LDL-C levels to ~100 mg per dl, was compared with atorvastatin (Lipitor; Pfizer) (80 mg per day), which was expected to lower LDL-C to ~70 mg per dl. A total of 4,162 patients were enrolled in the trial, and the primary outcomes that were measured were: the time from randomization to death from any cause; MI; documented unstable angina requiring rehospitalization; or revascularization (angioplasty or bypass grafting). The LDL-C levels during follow-up were 95 mg per dl in the pravastatin group and 62 mg per dl in the atorvastatin group. Median HDL cholesterol levels rose during follow-up by 8.1% in the pravastatin group and by 6.5% in the atorvastatin group ($P < 0.001$). Median CRP levels fell from 12.2 mg per litre at baseline in each group to 2.1 mg per litre in the pravastatin group and 1.3 mg per litre in the atorvastatin group ($P < 0.001$). For all randomized patients, the Kaplan-Meier event rates of the primary end point after 2 years were 26.3% in the pravastatin group versus 22.4% in the atorvastatin group — this was a 16% reduction in the hazard ratio favouring atorvastatin ($P = 0.005$; 95% confidence interval, 5–26%).

Importantly, a clinical benefit emerged as early as 30 days after starting treatment and was consistent over time; this strongly suggests that patients with ACS have active, unstable plaques that are amenable to statin treatment. The greatest reduction was in patients whose LDL-C levels were lowered to 70 mg per dl or lower and CRP levels were lowered to <2 mg

per litre. However, results of the PROVE IT-TIMI 22 trial were limited to secondary prevention, and did not prospectively determine whether lowering CRP in individuals with normal LDL-C levels would reduce the risk of MACE.

The JUPITER trial formally tested the hypothesis that lowering LDL-C with statins would reduce MACE in patients with 'normal' LDL-C (< 130 mg per dl), who are not candidates for lipid-lowering under current guidelines²². In a double-blind, placebo-controlled multi-centre trial, 11,001 men and 6,801 women with hsCRP levels > 2 mg per dl (median 4.2 mg per dl) and LDL-C levels < 130 mg per dl (median 108 mg per dl) but no history of cardiovascular disease were randomized to receive 20 mg of rosuvastatin (Crestor, AstraZeneca) or placebo daily. The primary end points were MI, stroke, hospitalization for unstable angina, arterial revascularization or cardiovascular-related death. The study was terminated early, after a mean follow-up of 1.9 years, because the primary end point of all vascular events was reduced by 41% ($P < 0.00001$; 251 first cardiovascular events in the placebo group versus 142 in the rosuvastatin group). On the basis of Kaplan–Meier estimates, 95 patients would have to be treated with rosuvastatin for 2 years to prevent one primary end point. Accordingly, if the results are projected forward, approximately 25 patients would have to be treated for 5 years to prevent one primary end point. These results compared very favourably to treatment of hyperlipidaemic patients with statins, in which the 5-year number needed to treat patients for cost effectiveness ranged between 44 and 63. Even more striking is the comparison to hypertension, in which the 5-year number needed to treat patients ranged between 86 and 140.

The JUPITER trial confirmed the results of the PROVE IT-TIMI 22 trial in that the hazard ratio of vascular events was reduced by 65% in patients in whom LDL-C was reduced to < 70 mg per dl and CRP was reduced to < 2 mg per litre; and it was reduced by 79% in patients in whom LDL-C was reduced to < 70 mg per dl and CRP was reduced to < 1 mg per litre.

What are the mechanisms of the anti-inflammatory activity of statins? Some of the reduction in inflammation may come from reducing LDL-C levels. At least in mice, hypercholesterolaemia is pro-inflammatory and leads to monocyteosis and elaboration of chemokines and cytokines^{32–34}. Although the anti-inflammatory activity of statins may be due, in part, to its lipid-lowering activity, there is considerable evidence showing that statins also have direct anti-inflammatory activity on virtually all of the cells that are involved in atherosclerotic plaque development and rupture. On endothelial cells, statins reduce the expression of vascular cell adhesion molecule 1 and intracellular adhesion molecule 1, which arrest and capture circulating monocytes³⁵. *In vivo*, statins reduce the growth of macrophages and lower their metalloproteinase activity, and these effects are likely to stabilize vulnerable plaques³⁶. Inhibition of interferon- γ production and T cell activation in response to statins is also likely to improve plaque stability³⁷.

Do statins exert anti-inflammatory effects independently of their lipid-lowering effects? The molecular target of all statins is HMG-CoA reductase — the enzyme responsible for the initial and rate-limiting step in cholesterol synthesis. Blocking HMG-CoA reductase also blocks the farnesylation and membrane localization of RAS³⁸. Other isoprenoids are also necessary for the attachment of RAC and RHO to the plasma membrane; RAC and RHO are important in regulating cell shape and motility. Although the extent to which isoprenoid production and the localization of small GTPases to the cell membrane is blocked by therapeutic doses *in vivo* is unclear, this is a potential mechanism for the direct anti-inflammatory activity of statins³⁹.

Antioxidants

Reducing the oxidation of lipoproteins to prevent their uptake into vessel wall cells is an emerging approach for anti-inflammatory therapy. The ARISE trial examined the effect of succinobucol (AGI-1067) — an antioxidant related to probucol — in a randomized prospective trial of 6,144 patients who had an MI or unstable angina within 2 weeks to 1 year before recruitment⁴⁰. The primary end point was a composite of time to cardiovascular death, resuscitated cardiac arrest, MI, stroke, unstable angina or coronary revascularization. Secondary end points included cardiovascular death, cardiac arrest, MI and stroke. Although there was no effect on the primary end point, the prespecified composite secondary end point occurred in fewer patients in the treated group (207 versus 252 events; $P=0.029$). The treated group also had a decrease in the incidence of new-onset diabetes, in agreement with the emerging appreciation of the role of inflammation in insulin resistance⁴¹. Adverse events included modest increases in LDL-C and blood pressure and infrequent elevations in liver enzymes. Despite its failure to meet its primary end points, this trial demonstrated that an anti-inflammatory drug can reduce the incidence of MACE without lowering lipid levels.

Selective phospholipase A2 inhibitors

Members of the phospholipase A2 (PLA2) superfamily are frequently associated with lipoproteins, such as LDL particles, and they modify phospholipids within these particles to generate atherogenic moieties. These moieties, which include pro-inflammatory lipids, might be important for attracting and maintaining inflammatory cells in the vascular wall^{42,43}. Phospholipases therefore represent promising targets for the prevention of atherosclerotic cardiovascular disease, and two phospholipases are currently being investigated for their therapeutic potential: lipoprotein-associated PLA2 (Lp-PLA2) and secretory PLA2 (sPLA2). sPLA2 is an acute-phase protein that is produced by hepatocytes and smooth muscle cells. Lp-PLA2 is a calcium-independent circulating phospholipase that is bound largely to lipoprotein particles, particularly LDL. Although only sPLA2 affects LDL turnover, both of these phospholipases interact with LDL particles and modify them into more atherogenic particles⁴². As a result of the action of these PLA2 enzymes, the level of oxidized LDL increases and biologically active products such as oxidized non-esterified fatty acids and lysophosphatidylcholine are generated, which activate inflammatory and proatherogenic pathways in the vascular wall (FIG. 2a). Owing to the proatherogenic actions of PLA2 enzymes, inhibitors of PLA2 could potentially be beneficial in the treatment of atherosclerotic cardiovascular disease. However, the complexity of the PLA2 system, including its multiple isozymes, makes it difficult to determine the overall contribution of any one isozyme to proatherogenic pathways. In addition, specific PLA2 inhibitors do not block the activity of other PLA2 isozymes that may also be proatherogenic, therefore the potential clinical benefit of these inhibitors may be limited.

Lp-PLA2 inhibitors

Darapladib is a selective Lp-PLA2 inhibitor that is being evaluated in large outcome studies in patients with established atherosclerotic cardiovascular disease. Epidemiological data suggest that elevated levels of Lp-PLA2 activity correlate with increased risk of atherosclerotic disease. In a large analysis of 32 prospective studies, associations between circulating Lp-PLA2 mass and activity and risk of coronary heart disease, stroke and mortality were assessed under different circumstances⁴⁴.

Individual records from 79,036 participants in these studies were examined in a meta-analysis to calculate risk ratios per 1 standard deviation higher value of Lp-PLA2 or other risk factors. Log-linear associations of Lp-PLA2 activity and mass with risk of coronary heart disease and vascular death risk ratios, (which were adjusted for conventional risk factors), were modest: approximately 1.10 (95% confidence interval, 1.05–1.16) with Lp-

PLA2 activity and 1.11 (confidence interval, 1.07–1.16) with Lp-PLA2 mass for coronary heart disease. Lp-PLA2 activity and mass each showed continuous associations with risk of coronary heart disease, which were similar in magnitude to those with non-HDL cholesterol or systolic blood pressure in this population. Associations of Lp-PLA2 mass and activity are not exclusive to vascular outcomes, and the vascular associations depend at least partly on lipid levels.

In Phase I and Phase II clinical trials of darapladib, circulating Lp-PLA2 activity was reduced by up to 95% in a dose-dependent fashion. In various *ex vivo* studies, darapladib inhibited monocyte chemotaxis and reduced the production of oxidized fatty acids. In patients, it had no effect on levels of plasma lipid, but levels of hsCRP and interleukin-6 (IL-6) were slightly lowered. In a preclinical pig model of atherosclerosis, darapladib reduced coronary atherosclerosis, primarily by reducing the size of the necrotic core of the coronary plaque, and this effect is hypothesized to correlate with plaque stabilization and reduction in plaque rupture events⁴⁵. In an IVUS study (IBIS-2)⁴⁶, after 12 months of treatment with darapladib, coronary plaque volume — which was the primary end point — was not affected; however, necrotic core growth was substantially reduced. Based on the clinical biomarker, preclinical and imaging results, darapladib is now being tested in two large cardiovascular outcome studies — STABILITY and SOLID-TIMI 52 — which will investigate the effect of darapladib on the incidence of MI, strokes and cardiovascular mortality in patients who are at high risk of MACE.

sPLA2 inhibitors

The most advanced inhibitor in this class is varespladib, which has been studied in Phase I and Phase II clinical trials. Varespladib is a nonselective inhibitor that has activity against the following isoforms of sPLA2: group IIA sPLA2, group V sPLA2 and group X sPLA2. Out of the more than ten secretory phospholipases, these isoforms are highly expressed in atherosclerotic lesions, and they are believed to have important roles in the modification of LDL to a more proatherogenic form, and in the generation of inflammatory lipids and atherogenic macrophage foam cells.

Preclinical animal data support the impact of sPLA2 inhibitors in general and particularly of varespladib on atherosclerosis, primarily in apolipoprotein E (APOE)- deficient mice. In three different studies in *ApoE* knockout mice, when varespladib was given alone or in combination with a statin, it substantially reduced the level of atherosclerotic lesions⁴⁷. In addition, sPLA2-IIA is elevated during MI and can therefore predict cardiovascular events⁴⁸. In Phase II trials of patients with recent MIs, varespladib modestly reduced hsCRP and LDL-C levels, and did not change levels of oxidized lipids⁴⁹. Because of the elevation of sPLA2 activity during an MI and the potential role of this enzyme in myocardial ischaemic events, varespladib is being studied in a 16-week Phase III cardiovascular outcome study (VISTA-16) in patients who have suffered from an ACS event.

Leukotriene pathway

Leukotrienes (LTs) are a class of eicosanoids that exert pro-inflammatory effects. By interacting with their receptors, LTs promote the accumulation and function of virtually all subgroups of leukocytes at sites of inflammation. LTs mediate a variety of inflammatory and allergic conditions, including rheumatoid arthritis, inflammatory bowel disease, psoriasis and allergic rhinitis, and their most relevant pathophysiological implication has been a link to bronchial asthma⁵⁰. Within the LT pathway, arachidonate 5-lipoxygenase (5-LO) catalyses the conversion of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid and, subsequently, to the unstable intermediate leukotriene A4 (LTA4) with the aid of 5-LO-activating protein (FLAP). 5-LO is selectively expressed in bone marrow-derived cells —

that is, neutrophils, monocytes, macrophages, dendritic cells and mast cells. LTA4 is further metabolized to the inflammatory cell chemoattractant leukotriene B4 (LTB4) or to cysteinyl LTs — such as leukotriene C4 (LTC4), leukotriene D4 (LTD4) or leukotriene E4 (LTE4) — by downstream enzymes (FIG. 2b).

Numerous human and animal studies have shown that the LT pathway is important in the development and progression of atherosclerosis^{51,52}. LTB4 is a potent chemoattractant that induces chemotaxis and adhesion of neutrophils, macrophages and other inflammatory cells to the vascular endothelium. The cysteinyl LTs increase vascular permeability and contractility, through direct effects on leukocytes, smooth muscle cells and endothelial cells^{53–55}. Resident macrophages can perpetuate a vicious cycle of local inflammation by releasing inflammatory cytokines, matrix-degrading metalloproteinases (which contribute to plaque rupture) and tissue factors (which increase plaque thrombogenicity), and by producing more LTs⁵⁶. Recent studies have also revealed that hyperlipidaemia induces infiltration of neutrophils into atherosclerotic lesions⁷. 5-LO and downstream LTs are expressed in mouse^{57,58} and human atherosclerotic plaques^{59,60}, and 5-LO expression is higher in unstable atherosclerotic plaques⁵³. Genetic studies also implicate 5-LO in human coronary artery disease, MI and stroke^{56,61–63}. Therapeutic strategies aimed at modulating components of the LT pathway are therefore under investigation.

FLAP inhibitors

Additional evidence of the contribution of LTs to atherosclerosis was demonstrated in a study involving individuals at risk for coronary artery disease who had a genetic predisposition to LT pathway activation and were treated with DG-031 (veliflapon) — a FLAP inhibitor⁶⁴. In response to 750 mg per day of DG-031, LTB4 production was reduced by 26% ($P=0.003$), and MPO activity was reduced by 12% ($P=0.02$). The higher two doses of DG-031 produced a nonsignificant reduction in CRP (16%; $P=0.07$) at 2 weeks. The minor inhibition of LT production demonstrated by this weak FLAP inhibitor makes it difficult to draw conclusions on the potential of FLAP inhibitors in modifying inflammatory biomarkers or ultimately in the treatment of cardiovascular diseases.

5-LO inhibitors

The leading 5-LO inhibitor, VIA-2291 (atreleuton), is a potent 5-LO inhibitor that was studied in 1,100 patients in clinical trials for asthma, another inflammatory disease⁶⁵. A study was undertaken to test the safety and efficacy of 5-LO inhibition by VIA-2291 in a dose-ranging, double-blind study in patients with ACS, to provide insights into its effects on hsCRP and coronary atherosclerosis.

Three weeks after an ACS, 191 patients were randomized to receive VIA-2291 at 25 various doses or placebo daily for 12 weeks in addition to standard care therapy⁶⁶. The primary end point — whole blood-stimulated LTB4 at trough drug level — was reduced in all VIA-2291 groups ($P<0.0001$) in a dose-dependent fashion; approximately 80% inhibition was achieved in >90% of patients in the 100 mg dose group. Urine levels of LTE4 were substantially reduced in all dose groups.

A subset of patients underwent serial 64-slice coronary computed tomography examination at baseline and after 24 weeks of therapy. Among the 60 patients with two evaluable scans, new coronary plaques were observed in 5 out of 18 (27.8%) placebo-treated patients and in 2 out of 42 (4.8%) VIA-2291-treated patients ($P=0.01$). At 24 weeks, non-calcified plaque volume, which was analysed in 34 of the patients, was smaller in VIA-2291-treated than in placebo-treated patients ($P<0.01$). hsCRP levels were also reduced by 66% ($P<0.01$) relative to placebo at 24 weeks but not at 12 weeks. Preliminary data from the computed

tomography sub-study suggest that such a reduction in LT production influences atherosclerosis; however, this requires confirmation in a larger study. Nonetheless, these data support the potential of LT inhibitors to provide clinical benefit in patients with established atherosclerotic disease.

CCL2–CCR2 pathway

Another therapeutic approach is based on interrupting the accumulation of monocytes and macrophages in vulnerable plaques. Plaque-destabilizing macrophages are derived from circulating blood monocytes and chemokine CC motif ligand 2 (CCL2; also known as MCP1), is an important signal for their recruitment. CCL2 is synthesized by cells lining the vascular wall, such as smooth muscle cells, endothelial cells and macrophages, in response to pro-inflammatory mediators, such as lipopolysaccharide and tumour necrosis factor (TNF)^{33,67}. Exposure of endothelial cells to oxidized lipids also potentially upregulates CCL2 production, thus implicating oxidized LDL as a molecular link between hypercholesterolaemia and foam cell formation⁶⁸. Furthermore, genetic deletion in mice of the receptor for CCL2 — chemokine receptor 2 (CCR2)⁶⁹ — drastically reduces monocyte recruitment and the development of diet-induced atherosclerosis⁷⁰. Other chemokines have also been implicated in atherogenesis⁷¹ and are of potential interest as clinical targets; these include fractalkine (CX3CL1), chemokine CC motif ligand 5 (CCL5; also known as RANTES), IL-8 (also known as CXCL8) and a scavenger receptor that binds phosphatidylserine and oxidized lipoproteins (chemokine CXC motif 16 (CXCL16); also known as SRPSOX).

Chemokine receptors are downregulated when monocytes differentiate into mature macrophages⁷². Therefore, the turnover rate of macrophages in established plaques is likely to determine whether these intriguing results can be translated into novel therapeutics for human atherosclerosis. Nonetheless, at least one encouraging clinical study has been reported. MLN1202 is a humanized monoclonal antibody that blocks the activation of CCR2 by CCL2 (REF. 73). In a small Phase II trial, 112 patients at high- risk for cardiovascular disease with CRP >3.0 mg per litre were randomized to receive a single dose of MLN1202 or placebo and monitored for 16 weeks. CRP was reduced by 26.7% on day 57 after dosing, and remained substantially reduced (19.1%) at day 85. CRP reductions were much more likely in patients with a single nucleotide polymorphism (an alanine to guanine mutation) at position 2,485 of CCL2; in patients with this common mutation, the median CRP reduction was ~35%. Interestingly, MLN1202 achieved similar reductions in CRP regardless of whether the patients were taking statins.

The data in this small study provide evidence that specific antagonism of CCR2 reduces CRP levels in a patient population at risk for cardiovascular disease. However, the mechanisms and in particular the anatomical location of the target monocytes and macrophages remain unknown. Larger studies will be required to determine whether this approach will reduce the rate of cardiovascular events. Although this is a promising approach, a recent preclinical study illustrates the challenge of treating atherosclerosis with chemokine antagonists⁷⁴. In this study, blocking CCR2 with a small molecule at concentrations that exceed the effector concentration for half-maximal response (EC₅₀) for at least 16 hours per day failed to reduce atherosclerotic lesion formation in APOE-deficient mice. This implies, therefore, that extremely high levels of target coverage are required to treat vascular inflammation and indeed perhaps inflammation in general. Other novel approaches, such as the use of peptides that interrupt chemokine binding to glycosaminoglycans⁷⁵ or peptides that interrupt chemokine–chemokine interactions⁷⁶ will also face these challenges. It should also be noted that CCR2 is required for the efficient mobilization of inflammatory monocytes from the bone marrow^{32,77}, which suggests that CCR2 antagonists may increase the risk of infection.

Other targets and pathways

In addition to the agents that have been evaluated in randomized prospective clinical trials, there are other interesting inflammatory pathway drug targets that are supported by clinical outcome data from retrospective analyses (TABLE 3). Autoimmune and inflammatory diseases, such as rheumatoid arthritis and gout, are associated with an increased incidence of MI, stroke and other cardiovascular events. Rheumatoid arthritis is treated with potent anti-inflammatory agents⁷⁸, including anti-TNF agents such as etanercept (Enbrel; Amgen/Pfizer), infliximab (Remicade; Centocor Ortho Biotech) and adalimumab (Humira; Abbott). All of these agents are efficacious in improving activity, function and quality of life and/or retarding radiographic progression when used alone, or in combination with methotrexate. These agents also reduce biomarkers of inflammation (such as hsCRP) in patients with cardiovascular disease⁷⁹.

In one study of matched rheumatoid arthritis patients, those treated with anti-TNF agents had a lower incidence of cardiovascular events than untreated patients⁸⁰. Likewise, low-dose methotrexate treatment is associated with a lower incidence of cardiovascular events, and a large cardiovascular inflammation reduction trial (CIRT) of low-dose methotrexate has been planned in cardiovascular patients⁸¹. Although a vascular-specific drug would be optimal, reduction of MACE in response to an anti-TNF drug or methotrexate would provide important clinical evidence that effective treatment of inflammation can reduce cardiovascular mortality.

Another anti-inflammatory drug that is under investigation is an IL-1 antagonist. The MRC-ILA-HEART study is a randomized, double-blind, placebo-controlled clinical trial that is funded by the UK's Medical Research Council, and this study is investigating the effect of an IL-1 receptor antagonist on non-ST elevation myocardial infarction (NSTEMI). The aim is to determine whether treatment of NSTEMI ACS with an IL-1 receptor antagonist alters the inflammatory process involved in this condition. Preliminary data suggested that IL-1 inhibition improves vascular function⁸².

Finally, various adhesion molecules have been found to be highly expressed in atherosclerotic lesions, and their modulation leads to attenuation of atherosclerosis in animal models⁸³. In particular, P-selectin has been targeted as a prominent player in vascular injury, but clinical trials using antibodies directed against P-selectin and other adhesion molecules are at very early stages.

Conclusions and future directions

A substantial body of work clearly shows that atherosclerosis and atherosclerotic plaque formation, including plaque rupture that leads to MACE, are inflammatory processes that are poorly treated by existing cardiovascular medications — which include antithrombotics, statins and antihypertensives. Clinical trials with statins have generated the most compelling evidence that reducing MACE is dependent on both antilipidaemic and anti-inflammatory activities. However, at most, statins and other cardiovascular therapeutic agents only delay the progression of atherosclerotic disease. It is therefore clear that the next generation of therapeutic strategies must target atherosclerotic plaque modification in order to directly treat the underlying inflammation.

Inflammation is caused by complex interactions involving multiple cell types, multiple mediators and multiple cellular processes. It is not yet clear which anti-inflammatory targets will yield the greatest effect in preventing, reversing or delaying the atherosclerotic process. Some of the therapeutic agents that are most advanced in the field include the PLA2 inhibitors, which seem to have direct effects on atherogenic lipoproteins and foam cells

within the plaque. Other therapeutic agents are more general antioxidants that may affect several points in inflammatory pathways. A variety of molecules that interrupt chemokine and LT pathways have shown some benefit in clinical trials. However, the effects of these agents have been assessed mainly by measuring imaging outcomes and inflammatory biomarkers that may provide some proof of concept for conducting large and expensive cardiovascular outcome studies.

All of the drugs that are currently under investigation are supported by clinical biomarker data as well as valuable preclinical studies that predict efficacy in reducing the inflammatory component of the atherosclerotic plaque. Based on the weight of evidence, it can be predicted that PLA2 inhibitors will show some benefit in reducing MACE, particularly the PLA2 inhibitor darapladib, which is currently being investigated in longer term trials. It is not clear, however, whether this effect will be robust; that is, with a >25% reduction in MACE. This is partly because patients in these trials are taking a number of other agents that are standard care therapies — such as antihypertensives, statins and antiplatelet agents — all of which show some therapeutic benefit in reducing the risk of MACE. As demonstrated in FIG. 1, another concern is that interrupting only a single inflammatory pathway among the multitude of pathways that are involved in atherosclerotic plaque formation may not yield much effect. In addition, depending on their genetic background, not all patients may respond equally well to modification of a particular pathway.

Currently, only two Phase III trials are ongoing with anti-inflammatory drugs (darapladib and varespladib), although others are being planned (for example, CIRT). The reason why cardiovascular outcome trials are so large (>6,000 patients) and of a long duration (>2 years) is that only ~10% of the subjects will have a measureable MACE during the trial. Until biomarkers or surrogate markers are validated as clinical end points that correctly predict the progression of atherosclerotic disease, the requirement for large cardiovascular outcome studies will remain the greatest obstacle in testing new anti-inflammatory targets for the treatment of atherosclerotic cardiovascular diseases.

Current imaging or soluble biomarker assessments have little chance of being validated and recognized by regulatory agencies as surrogates for clinical end points, in part because recent large trials have failed to show a correlation between clinical benefit and the effect on biomarkers or imaging. Those clinical end points that offer the most promise are well-validated inflammatory biomarkers (for example, hsCRP) and robust imaging techniques (for example, angiography, IVUS and IMT) that are supported by a large amount of clinical trial data. Although they are also unlikely to be recognized as surrogate markers, these imaging and soluble biomarker assessments during Phase II trials provide important evidence to build confidence that the agent is worth advancing into a large Phase III cardiovascular outcome study. Nonetheless, even these techniques have substantial limitations in their utility owing to a lack of sensitivity or specificity or owing to issues such as radiation exposure, cost and invasiveness.

Additional issues for drug development include the need for an excellent safety profile. Atherosclerotic cardiovascular disease is a chronic disease, and most drug therapies for treating or preventing it will therefore have to be administered chronically. In addition to being specific for the molecular target, anti-inflammatory drugs — by their very nature — may predispose patients to opportunistic infections and issues with wound healing. TNF-targeted drugs increase the incidence of tuberculosis, and natalizumab (Tysabri; Elan/Biogen Idec), which interrupts leukocyte trafficking, predisposes patients (albeit at a very low rate) to viral infections of the brain⁸⁴. In addition, drugs may possess other off-target safety liabilities that may make it difficult to establish a positive benefit–risk balance. Finding the

right balance between safety and efficacy will probably require a number of large trials assessing a variety of anti-inflammatory mechanisms.

Acknowledgments

The authors would like to thank D. Jones for his assistance in the preparation of this manuscript. This work was supported by the US National Institutes of Health grant R01 HL052773 (I.C.).

Glossary

Low-density lipoprotein (LDL)	LDL is one of the five major groups of lipoproteins that enable lipids such as cholesterol and triglycerides to be transported within the bloodstream.
Major adverse cardiovascular events (MACE)	A composite of cardiovascular events, typically including unstable angina, myocardial infarction, stroke and cardiovascular-related death.
Myocardial infarction (MI)	An event caused by occlusion of coronary blood flow, typically resulting from the rupture of a coronary atherosclerotic plaque and subsequent thrombosis leading to damage to the heart muscle.
Acute coronary syndrome (ACS)	Acute cardiovascular ischaemia resulting in unstable angina pectoris or myocardial infarction owing to impairment of blood flow within a coronary artery.
ST-segment elevation	An ST-segment elevation refers to a finding on a 12-lead electrocardiogram, in which the trace in the ST-segment is abnormally high above the isoelectric line. The finding of ST-segment elevations in select leads is consistent with myocardial infarction.
LDL cholesterol (LDL-C)	A measurement of the amount of cholesterol in the blood that is associated with low-density lipoprotein (LDL) particles. High LDL-C is associated with atherosclerotic cardiovascular disease.
High density lipoprotein (HDL)	A dense lipoprotein that forms complexes with cholesterol, which is typically composed of a number of lipoproteins such as apolipoprotein A1, apolipoprotein A2 and other components. HDL transports cholesterol from the peripheral tissue in the body to the liver where it is secreted as biliary cholesterol or bile salts.
Intima medial thickness (IMT)	The thickness of the intima within the carotid arteries as measured by an echocardiogram is correlated with the level of atherosclerotic disease.
C-reactive protein [CRP]	An acute phase protein that correlates with inflammatory disease processes, including atherosclerotic cardiovascular disease.
High-sensitivity CRP (hsCRP)	An assay that was developed to more accurately measure levels of C-reactive protein (CRP) in the blood, particularly relatively low levels of CRP.
Myeloperoxidase (MPO)	A protein that is expressed in the granules of most leukocytes, including neutrophils and monocytes. It is linked to inflammation and oxidative stress, and correlates with cardiovascular risk. MPO is released by leukocytes in a state of

Troponin

inflammation and it catalyses the formation of several reactive species. It has a role in the host defence against microorganisms.

A complex of three proteins (troponin T, troponin I and troponin C) that bind to the thin filament (actin) of striated muscle, which regulates muscle contraction. Following injury to heart muscle cells, the intact troponin complex, along with free troponin subunits, is released into the blood. The amino acid sequences for cardiac troponin T and troponin I isoforms are considerably different from their skeletal muscle counterparts and levels of troponin T can therefore be used to measure heart damage.

Intravascular ultrasound (IVUS)

An invasive technique in which an ultrasound device is inserted into the coronary arteries via a catheterization procedure.

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Box 1 | MACE caused by unstable atherosclerotic plaques

A model of an artery depicting a ruptured unstable plaque is shown in the figure. Approximately two-thirds of heart attacks and strokes are caused by the rupture of an unstable, rupture-prone atherosclerotic plaque. Unstable plaques are characterized by high numbers of inflammatory cells and high levels of inflammatory mediators such as leukotrienes, active proteases and cytokines. Histologically, rupture-prone plaques contain thin fibrous caps and large necrotic cores. Plaque rupture occurs when the thin fibrous cap is eroded; this leads to leakage of prothrombotic materials that result in thrombus formation at the site of plaque rupture, which impairs blood flow in the distal portion of the artery. As a consequence, major adverse cardiovascular events (MACE) can occur. As shown, stable plaques that are less prone to rupture contain fewer inflammatory cells and lower levels of inflammatory mediators, thick caps and a smaller necrotic core.

Figure 1. Model of an atherosclerotic plaque showing some of the key inflammatory mechanisms involved

Monocytes are first recruited to developing plaques by chemoattractants, such as chemokine CC motif ligand 2 (CCL2; also known as MCP1) and leukotriene B4 (LTB4). Following this, they differentiate into macrophages, which accumulate oxidized low-density lipoprotein (LDL) and become foam cells. The macrophages release chemotactic factors, which attract additional monocytes and thus set up a positive feedback loop, as well as leukotriene C4 (LTC4), which increases vascular permeability. Activated macrophages and foam cells secrete metalloproteinases and tissue factor, which degrade the fibrous plaque, leading to plaque rupture and thrombosis. CCR2, chemokine CC motif receptor 2; CysLTR, cysteinyl leukotriene receptor; Lp-PLA2, lipoprotein-associated phospholipase A2; LTB4R, leukotriene B4 receptor 1; sPLA2, secretory phospholipase A2.

Figure 2. The phospholipase A2 pathways that result in the generation of foam cells and atherogenic lipids, and the leukotriene pathway with enzymatic steps depicted

a | Secretory phospholipase A2 (sPLA2) interacts with circulating low-density lipoprotein (LDL) particles and modifies LDL, thereby reducing liver uptake of LDL by the LDL receptor. Both sPLA2 and lipoprotein-associated PLA2 (Lp-PLA2) interact with more oxidized forms of LDL to generate bioactive lipids. **b** | The cysteinyl leukotrienes increase vascular permeability and induce smooth muscle cell proliferation. Leukotriene B4 (LTB4) induces leukocyte chemotaxis into the atherosclerotic plaque. 5-LO, arachidonate 5-lipoxygenase; cPLA2, cytoplasmic PLA2; FLAP, 5-LO-activating protein; LTA4, leukotriene A4; LTA4H, LTA4 hydrolase; LTC4, leukotriene C4, LTC4S; LTC4 synthase; LTD4, leukotriene D4; LTE4, leukotriene E4; Lyso-PC, lysophosphatidylcholine; Ox-FA, oxidized fatty acids.

Table 1

Acronyms of clinical trials discussed in this Review

Clinical trial acronym	Clinical trial name	Drugs tested	Refs
ACCORD	Action to control cardiovascular risk in diabetes	Multiple diabetic agents	17
AFCAPS/TexCAPS	Air Force/Texas coronary atherosclerosis prevention study	Statins	31
ARISE	Aggressive reduction of inflammation stops events	Succinobucol (AGI-1067)	40
CARE	Cholesterol and recurrent events	Pravastatin (Pravachol; Bristol-Myers Squibb)	30
CIRT	Cardiovascular inflammation reduction trial	Methotrexate	81
ENHANCE	Simvastatin with or without ezetimibe in familial Hypercholesterolaemia	Simvastatin, ezetimibe (Ezetrol; Merck)	16
IBIS-2	Integrated biomarker imaging study 2	Darapladib	46
ILLUMINATE	Investigation of lipid level management to understand its impact in atherosclerotic events	Torcetrapib	15
JUPITER	Justification for the use of statin in prevention: an intervention trial evaluating rosuvastatin	Rosuvastatin (Crestor; AstraZeneca)	22
MRC-ILA-HEART	Medical research council interleukin-1 receptor antagonist — HEART study	Interleukin-receptor 1 antagonist	85
PROVE IT-TIMI 22	The pravastatin or atorvastatin evaluation and infection therapy thrombolysis in myocardial infarction 22 trial	Pravastatin, atorvastatin (Lipitor; Pfizer)	19
SOLID-TIMI 52	The stabilization of plaques using darapladib — thrombolysis in myocardial infarction 52 trial	Darapladib	ClinicalTrials.gov identifier: NCT01000727
STABILITY	The stabilization of atherosclerotic plaque by initiation of darapladib therapy trial	Darapladib	86
VISTA-16	Vascular inflammation suppression to treat acute coronary syndrome for 16 weeks	Varespladib	ClinicalTrials.gov identifier: NCT01130246

Table 2

Imaging technologies used for the detection of atherosclerotic disease *

Technology	Validation	Advantages	Limitations
Coronary angiography	<ul style="list-style-type: none"> Associated with cardiovascular events Accepted surrogate end point 	<ul style="list-style-type: none"> Experience in clinical trials Common procedure in the clinic 	<ul style="list-style-type: none"> Invasive Only provides dimensions of lumen Therapy-induced changes occur slowly Radiation exposure
Coronary angiography and intravascular ultrasound	<ul style="list-style-type: none"> Associated with cardiovascular events 	<ul style="list-style-type: none"> Experience in clinical trials Standard protocols Directly images disease Potentially shows rapid therapeutic response 	<ul style="list-style-type: none"> Invasive Difficult to assess plaque composition Does not measure function
Carotid intima medial thickness	<ul style="list-style-type: none"> Associated with cardiovascular events and coronary artery disease risk factors 	<ul style="list-style-type: none"> Experience in clinical trials Excellent availability Low cost Non-invasive 	<ul style="list-style-type: none"> Technically demanding Therapy-induced changes occur slowly Non-coronary assessment Lack of specificity
Carotid magnetic resonance imaging	<ul style="list-style-type: none"> Experience in image analysis Not validated in clinical trials 	<ul style="list-style-type: none"> Possible plaque characterization Non-invasive 	<ul style="list-style-type: none"> Standard protocols necessary Noncoronary assessment
Computed tomography coronary angiography	<ul style="list-style-type: none"> Not validated in clinical trials 	<ul style="list-style-type: none"> May show rapid response Non-invasive Plaque characterization 	<ul style="list-style-type: none"> Radiation exposure Non-standardized protocols Equipment still evolving
Fludeoxyglucose-positron emission tomography	<ul style="list-style-type: none"> Not validated in clinical trials 	<ul style="list-style-type: none"> Non-invasive Potentially measures inflammation 	<ul style="list-style-type: none"> Technique still evolving Limited experience in clinical trials

* Table is adapted, with permission, from REF. 13 © (2006) American Heart Association.

Table 3

Anti-inflammatory drugs for the treatment of cardiovascular disease

Compound; company	Target	Target pathway	Anti-inflammatory mechanism	Preclinical and clinical validation	Highest stage of clinical development*	Refs
Multiple compounds	HMG-CoA reductase	Cholesterol biosynthesis	Unknown; linked to lowering of hsCRP levels	Multiple large clinical trials showing improved cardiovascular outcomes	Marketed	11,14, 19,22
Succinobucol (AG-1067); AtheroGenics	Succinobucol	Antioxidant	Antioxidant mechanism not specified	Phase III MACE end point missed; Phase II imaging; preclinical models	Phase III (ARISE)	40
Varespladib; Anthera	sPLA2	Phospholipase inhibitor	Lipoprotein modification; release of bioactive lipids	No clinical MACE; Preclinical animal model	Phase III (VISTA-16)	47–49
Darapladib; GlaxoSmithKline	Lp-PLA2	Phospholipase inhibitor	Lipoprotein modification; release of bioactive lipids	No clinical MACE; Phase II imaging; preclinical animal models	Phase III (STABILITY, SOLID-TIMI 52)	44–46
Veliflapon (DG-031); DeCODE genetics	FLAP	Leukotriene	Inhibits leukotrienes; reduces leukocyte activity in plaque	No clinical morbidity and mortality; Phase II biomarker study	Phase II	64
Atreleuton (VIA-2291); VIA Pharmaceuticals	5-LO	Leukotriene	Inhibits leukotrienes; reduces leukocyte activity in plaque	No clinical MACE; Phase II biomarker; imaging; preclinical models	Phase II	66
MLN1202; Millennium Pharmaceuticals	CCR2	CCL2 –CCR2 pathway	Inhibits monocyte trafficking into atherosclerotic plaque	No clinical MACE; biomarkers; preclinical models	Phase II	67–70,73
Academic	Methotrexate	Antiproliferative	Antiproliferative agent blocks inflammatory response	Rheumatoid arthritis; high level of atherosclerosis; biomarkers	Phase III (CIRT)	81
Unspecified	TNF	TNF pathway	TNF-mediated effects	Retrospective clinical outcomes	Unknown	80,82
Academic	IL-1	IL-1 pathway	IL-1 mediated effects	Genetic; biomarkers	Phase II (MRC-ILA-HEART)	85
Roche; RO4905417	P-selectin	Cellular migration — endothelial cells	Blocks monocyte and/or leukocyte penetration into intimal space	Preclinical models	Phase II	83, NCT01245634

5-LO, arachidonate 5-lipoxygenase; CCL2, chemokine CC motif ligand 2 (also known as MCP1); CCR2, chemokine CC motif receptor 2; FLAP, 5-LO-activating protein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; hsCRP, high-sensitivity C-reactive protein; IL-1, interleukin 1; Lp-PLA2, lipoprotein-associated phospholipase A2; MACE, major adverse cardiovascular events; sPLA2, secretory PLA2; TNF, tumour necrosis factor.

* See Table 1 for full definitions of acronyms of the names of clinical trials mentioned here.