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Inflammation and cardiovascular diseases: lessons from seminal clinical trials

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Abstract

Inflammation has been long regarded as a key contributor to atherosclerosis. Inflammatory cells and soluble mediators play critical roles throughout arterial plaque development and accordingly, targeting inflammatory pathways effectively reduces atherosclerotic burden in animal models of cardiovascular (CV) diseases. Yet, clinical translation often led to inconclusive or even contradictory results. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) followed by the Colchicine Cardiovascular Outcomes Trial (COLCOT) were the first two randomized clinical trials (RCTs) to convincingly demonstrate the effectiveness of specific anti-inflammatory treatments in the field of CV prevention, while other phase III trials - including the Cardiovascular Inflammation Reduction Trial (CIRT) one using methotrexate - were futile. This manuscript reviews the main characteristics and findings of recent anti-inflammatory phase III trials in cardiology and discusses their similarities and differences in order to get further insights into the contribution of specific inflammatory pathways on CV outcomes. CANTOS and COLCOT demonstrated efficacy of two anti-inflammatory drugs (canakinumab and colchicine, respectively) in the secondary prevention of major adverse CV events (MACE) thus providing the first confirmation of the involvement of a specific inflammatory pathway in human atherosclerotic CV disease (ASCVD). Also, they highlighted the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome-related pathway as an effective therapeutic target to blunt ASCVD. In contrast, other trials interfering with a number of inflammasome-independent pathways failed to provide benefit. Lastly, all anti-inflammatory trials underscored the importance of balancing the risk of impaired host defence with an increase in infections and the prevention of MACE in CV patients with residual inflammatory risk.

1. Inflammation and Atherosclerosis

The inflammatory theory of atherosclerosis emerged in the early 80s after the first histological reports of plaque invasion by immune cells [1]. After that, a plethora of basic and clinical findings have reinforced this hypothesis. To date, all subtypes of inflammatory cells have been identified within atherosclerotic lesions isolated from experimental models and patients [2-4]. Meanwhile macrophage subgroups seem to be the first cells to invade early arterial lesions (i.e. fatty streaks) thereby transforming into culprit foam cells [5]. Later on, also neutrophils and lymphocytes (both T and B cells) concur in determining plaque fate (i.e. vulnerability and rupture risk) [6-8]. Indeed, once in the vessel wall, white blood cells release several mediators with different and sometimes opposite effects on plaque stability; such effects span from further leukocyte invasion, to oxidative stress [9], matrix degradation by collagenases (i.e. metalloproteinases, fibroblast activation protein) [10], vascular smooth muscle cells migration and proliferation [11], but also collagen deposition or polarization of leukocytes toward anti-inflammatory phenotypes [12]. Of interest, inflammatory mediators have shown pivotal roles also in determining the latest catastrophic thrombotic complications of atherosclerosis, i.e. plaque rupture or erosion and in turn myocardial infarction and ischemic stroke [13, 14]. Such evidence has prompted the clinical evaluation of inflammation as a therapeutic target in an attempt to further reduce the burden of cardiovascular (CV) and cerebrovascular (CBV) diseases [15-18]. Indeed, the encouraging results obtained from basic CV research endorsed their ready translation into the clinical setting which unfortunately failed on several occasions. This was the case for the inhibitor of purinergic signaling methotrexate [19], but also for losmapimod [20], varespladib [21] and darapladip [22, 23]: more specific inhibitors of p38 mitogen-associated protein (MAP) kinase and the secretory or

lipoprotein-associated forms of phospholipase A2 ($sPLA_2$ and $Lp\text{-}LPA_2$), respectively (Figure 1). Only recently, two randomized clinical trials (RCTs) have confirmed the efficacy of specific anti-inflammatory interventions in preventing secondary major adverse CV events (MACE) thus confirming the inflammatory theory of atherosclerosis in CV patients [24, 25] (Figure 1). This manuscript reviews the main characteristics and findings of Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) and Colchicine Cardiovascular Outcomes Trial (COLCOT). Similarities and differences among these two RCTs and those reaching neutral results will be discussed in order to get further insights into their contribution to cardiology.

2. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS)

CANTOS is a double-blind RCT investigating canakinumab, a monoclonal antibody specifically inhibiting the function of one of the pro-inflammatory cytokines, interleukin 1-β (IL-1β) [24]. IL-1β was firstly identified as the transferable sterile factor with pyrogen function, then it was recognized as the mediator of many processes involved in host defense as well as in a variety of pathological conditions [26]. Within the atherosclerotic plaque, different cell types including endothelial cells, smooth muscle cells and immune cells synthetize IL-1β. In their cytoplasm, pro-IL1β is cleaved into its active form by cholesterol crystal-dependent assembling of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome as detailed in paragraph n° 4 [27]. Other stimuli can induce IL-1β synthesis and activation during atherosclerosis including other pro-inflammatory cytokines (e.g. tumor necrosis factor-α) [28], oxidative stress [29], shear stress [30], hypoxia [31] and neutrophil extracellular traps [32]. Once activated, IL-1β induces a strong inflammatory response within the vessel wall and

fuels a vicious circle involving oxidative stress and cell death. In endothelial cells, this cytokine induces the production of adhesion molecules and triggers the expression of several chemokines and cytokines (including IL1β itself) resulting in the recruitment of circulating leukocytes in the vessel wall [33, 34]. Finally IL-1β can modulate the endothelial expression of several enzymes involved in reactive oxygen species resultin in increased oxidative stress [35]. In the media layer of the vessel IL-1β induces smooth muscle cell-dependent production of platelet-derived growth factor among others involved in their proliferation and migration [36], again processes deeply involved in atherogenesis. IL-1β induces the polarization of macrophages towards the pro-inflammatory phenotype M1 [37], facilitates neutrophil degranulation and NET formation [38, 39]. Furthermore, IL-1β triggers leukocytes and other cells to produce matrix metalloproteinases and other enzymes involved in plaques destabilization as well as IL-6 that causes the production of fibrinogen and plasmin activator inhibitor [40, 41], all mediators involved in the final catastrophic evolution of atherosclerosis: the formation of an arterial thrombus. Pro-atherogenic functions of IL-1β have been extensively investigated and confirmed in animal experiments: its deficiency or inhibition blunts plaque growth in atherosclerotic-prone murine models [42, 43], and the repeated injection of the recombinant protein in the perivascular space increased intima-media thickness in pigs [44]. Canakinumab is an approved treatment for different rare conditions where inflammasome is chronically activated including juvenile chronic arthritis, familial Mediterranean fever and Muckle-Wellis syndrome [26]. In the CANTOS trial, 10’061 patients with stable coronary artery disease (CAD) and high-sensitivity C-reactive protein (hs-CRP) levels >2 mg/L under optimal CV medical treatment have been randomized to receive either placebo or the monoclonal anti-IL-1β antibody. Canakinumab was administered subcutaneously every 3 months at

dosages of 50, 150 or 300 mg in three different groups of patients which have been followed up for a median period of 3.7 years [24]. While the lowest dose did not show efficacy as compared to placebo, the two groups receiving canakinumab at a higher dosage successfully met the primary composite endpoint including nonfatal myocardial infarction, nonfatal stroke, or CV death. Highlighting the specificity of these findings, treatment with canakinumab did not affect circulating levels of cholesterol, while hs-CRP showed a drastic reduction already after the first administration which was maintained along the entire follow-up period [24]. Of interest, patients with on-treatment levels of hs-CRP<2mg/L were those who benefitted the most from the antibody, thus further confirming inflammation as an independent CV risk factor [45]. Yet, canakinumab slightly increased the risk of infections during the follow up. As for adverse events, the canakinumab harm showed higher incidence of neutropenia as well as a small, but significant rise in deaths attributed to infections or sepsis [24].

Secondary analyses of results from CANTOS broadened the interest beyond the IL-1β/MACE connection and highlighted the importance of other IL-1β-related pathways in different CV diseases. Such exploratory analyses showed that, among downstream mediators of IL-1 signalling, IL-6 might play a role in determining MACE. Indeed, among patients treated with canakinumab those who reached lower levels of IL-6 after the first treatment were also less likely to experience MACE (baseline IL-6 was 2.53-2.61 ng/L), hospitalization for unstable angina requiring urgent revascularization or to die during the follow-up from CV or all-causes [46]. Of note, IL-6 is highly expressed at the site of coronary occlusion in patients with myocardial infarction [47]. Furthermore, treatment with canakinumab associated with reduced cancer risk and mortality, a finding that opened a new avenue for treatment of cancer

patients, specifically those with lung cancer [48]. Finally, in CANTOS IL-1β inhibition improved gout control (a disease also related to NLRP-3 activation) and dose-dependently reduced heart failure hospitalization further confirming the role of inflammation and specifically of the IL-1 pathway in these conditions [24].

3. Colchicine Cardiovascular Outcomes Trial (COLCOT)

More recently, the double-blind RCT COLCOT explored the potent anti-inflammatory drug colchicine which is currently recommended for the treatment of pericarditis and acute gout attacks, but also familial Mediterranean fever and Behçet disease [25]. Colchicine's anti-inflammatory properties are known for centuries when extracts of the autumn crocus (*Colchicum autumnale*), where this alkaloid was originally isolated, were already used to treat joint swelling. This drug acts on inflammation through different mechanisms among which the inhibition of microtubule polymerization by binding free tubule dimers, remains the best characterized [49]. By doing this, colchicine blunts monocyte and neutrophil invasion at the site of the insult, but also reduces intracellular trafficking and thus the release of cytokine and production of reactive oxygen species and a variety of proteolytic enzymes [50, 51]. Only recently colchicine was reported to suppress crystal-induced NLRP3 inflammasome activation (e.g. by urate crystals) thus reducing the release of pro-inflammatory IL-1β and IL-18, a finding which might explain its higher efficacy in diseases mediated by the innate immune system as opposed to the adaptive one [52, 53]. Furthemore, colchicine inhibits endothelial production of IL-1β and adhesion molecules [54], again reducing leukocyte vascular invasion. Finally, this drug reduces mast cell and T cell activation [55, 56], it hampers smooth muscle cell activation [57], reduces leukocyte-mediated platelet activation [58] and blunts the inflammatory response by promoting macrophage shifting

towards the healing subtype M2 producing anti-inflammatory IL-10 and transforming growth factor (TGF)-β [59]. Preclinical evidence of its action on atherosclerosis models yelded confilicting results, first experiments on rabbits showed anti-atherosclerotic effects when started at the time of high-fat diet [60], such a positive effect was not confirmed when administered after plaque development [61, 62].

COLCOT tested for the first time colchicine at low dosage (0.5 mg *per os* once daily) as compared to placebo in 4'745 patients which suffered a myocardial infarction in the 30 days prior to the start of randomization; the median follow up was 1.8 years. The rate of the primary composite endpoint (CV death, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization) was reduced by 23% in the colchicine as compared to the placebo group [25]. Adverse events or serious adverse events showed similar rates in the two groups: the well-known gastrointestinal effects of colchicine which include nausea and abdominal discomfort were more frequent in the treatment arm as expected. Furthermore, chronic treatment with colchicine increased the frequency of pneumonia from 0.4 to 0.9% in COLCOT, however no deaths from infection were reported in the study and rates of septic shock in the treatment harm remained low and similar to those of the placebo group [25]. Although the analyses of the effect of colchicine treatment on hs-CRP levels and white blood cells were only available in a small subgroup of patients (median hs-CRP 4.28 mg/L), chronic colchicine treatment apparently did not associate with a significant reduction of those parameters as compared to placebo [25]. Finally, no data exploring the effects of such a treatment on lipid levels were to date reported, thus preventing any kind of conclusion on the specificity of the target.

4. Similarities and differences of CANTOS and COLCOT

CANTOS and COLCOT share similarities but also show important differences (Table 1) whose analysis may help in drawing conclusions on inflammation as a target for CV prevention [63].

The most obvious analogy between the two studies surely resides in the achievement of the primary composite endpoint which was similar and included a series of fatal and non-fatal acute CV and CBV events. In COLCOT the primary endpoint included hospitalization for unstable angina leading to urgent revascularization, while in CANTOS this outcome was only taken into consideration for the key secondary CV endpoint which confirmed the superiority of canakinumab treatment as compared to placebo in the prevention of CV events. Yet, the preliminary analyses of the components of the primary and secondary endpoints highlighted how the achieved results are radically different in the two trials. In CANTOS, the outcome was mainly driven by reduced rates of myocardial infarction, hospitalization for unstable angina leading to urgent revascularization and any coronary revascularization suggesting a major effect of such a treatment in the coronary circulation with blunted or no effect on the cerebral vasculature [24]. By contrast, colchicine mainly reduced the incidence of stroke and the frequency of urgent hospitalization for angina leading to revascularization [25]. Interestingly, similar findings on the protective effect of colchicine with respect to CBV events were reported in a meta-analysis of four RCTs thus suggesting a specific effect of colchicine on cerebral arteries which warrants further investigations [64]. Importantly, both studies failed to show a significant reduction in CV or all-cause mortality. Yet, in CANTOS, in patients achieving an on-treatment hs-CRP levels <2 mg/L, CV mortality after 3.7 years follow-up was 31% lower than placebo-treated ones [45]. Similarly, the

same subjects showed a 31% reduction in all-cause mortality, while effects of canakinumab on mortality were limited and nonsignificant among non-responders [45]. The lack of strong effectiveness on such a hard endpoint will probably hinder the routine use of colchicine and canakinumab for CV prevention, especially when taking into consideration the increased rates of infections that both treatments entail [24, 25].

Both trials investigated anti-inflammatory treatments in the setting of secondary CV prevention. Indeed, all enrolled patients had clinically overt CAD as they previously suffered from myocardial infarction (within 30 days in the COLCOT, not specified in the CANTOS). Accordingly, canakinumab and colchicine were added on top of optimal medical treatment with antithrombotic agents (95% in CANTOS, ~ 97-98% in COLCOT), lipid-lowering agents (93.4% in CANTOS, 99% in COLCOT), anti-ischemia drugs (91.4% in CANTOS, 88% for only beta-blockers in COLCOT) and inhibitors of the renin–angiotensin system (79.7% in CANTOS, not reported in COLCOT) [24, 25]. Of interest, statins and to a lesser extent proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors exert anti-inflammatory effects besides their actions on lipid levels [65, 66]. Indeed, low-density lipoprotein (LDL) cholesterol and inflammation show deep interconnections, hypercholesterolemia increases monocyte count by acting on cell survival and proliferation and specifically increasing pro-inflammatory monocyte/macropahge subsets that are known to facilitate plaque growth and destabilization [41, 67]. Furthermore, by acting on NLRP3 inflammasome LDL can induce epigenomic and transcriptional reprogramming of myeloid progenitors leading to long-term enhanced innate immune response to different stimuli, thus facilitating plaque progression (a process also known as “trained immunity”) [68]. Thus, the absolute beneficial effects of canakinumab and colchicine on plaque inflammation may have been at least in part masked by lipid-lowering agents

as their target pathways were already inhibited by such drugs. Yet, specific interactions between statins and the IL-1β pathway remain to be fully characterized as conflicting results have been reported to date [69]. Despite the broad application of anti-inflammatory drugs to prevent CV and CBV events remains undoubtedly more likely in the setting of secondary prevention, how to pharmacologically cope with the inflammation-related risk of primary MACE remains unclear [70-72]. This is particularly true when considering the recent trials investigating aspirin [73-75], which besides its anti-platelet properties also holds anti-inflammatory effects, at least at higher dosages [76, 77]. Indeed, in ASCEND, ARRIVE and ASPREE trials low-dose aspirin (ca. 100 mg daily) compared with placebo did not demonstrate CV benefit (reduction in myocardial infarction, stroke, or CV mortality), with aspirin treatment even being associated with increased risk of all-cause mortality and gastrointestinal malignancies in the elderly from ASPREE trial [73-75]. Specifically, the elderly may represent an interesting population for studying the role of inflammation inhibition on primary CV prevention as they show a chronic low-grade persistent increase in levels of pro-inflammatory mediators which is accompanied by a blunted inflammatory response to appropriate immunogenic triggers [78]. Such chronic low-grade inflammation is commonly referred to as inflammaging and is thought to underlie the progression of several age-dependent degenerative afflictions including CV diseases [78].

Although in both CANTOS and COLCOT patients with previous myocardial infarction were enrolled, the two cohorts show important differences. Specifically, in CANTOS patients have been selected to carry an increased “residual inflammatory risk”. Given the importance of inflammation in the pathophysiology of atherosclerosis and its thrombotic complications, this concept has developed in opposition to the classic

"residual cholesterol risk" and employs hs-CRP and not lipoprotein particles as a risk biomarker [79]. Selecting patients based on hs-CRP in CANTOS represented an important step toward precision medicine and surely enough increased the chances of success for this trial. Of interest, in patients treated with canakinumab, hs-CRP levels were greatly reduced already after the first administration of the drug and remained low throughout the whole follow-up period [24, 45]. Furthermore, patients showing lower hs-CRP levels after the first canakinumab injection were also those who benefitted the most while the rest of the treatment group showed a Kaplan-Meyer curve for the composite endpoint very similar to that of the control group [45]. In this setting, hs-CRP might serve as a very early marker of canakinumab efficacy, thus allowing a personalized therapy. In contrast, hs-CRP was assessed only in a small subgroup of 207 patients in COLCOT, thus limiting the interpretation of the results [24, 25]. Yet, its levels at the time of randomization were similar to those of CANTOS while, whether in CANTOS only the treatment arm showed a significant reduction, in COLCOT hs-CRP was blunted in all patients independently of the randomization. Although apparently counterintuitive, the authors address this finding by discussing the different cohorts enrolled by the trials: early (max 30 days) after myocardial infarction in COLCOT vs stable CAD in CANTOS [25].

Colchicine and canakinumab are very different from many points of view: the first is an inexpensive plant-derived chemical with broad targets used in many patients for several years and thus well characterized; the second is a biological of recent development which received FDA authorization for selected rare diseases and maintains high market cost. Indeed, comparison of cost-effectiveness analysis between the two medications yielded opposite results whereby, the addition of colchicine on top of standard of care therapy proved far more economically efficient

as compared to canakinumab [80, 81]. Yet, these two drugs find in NLRP3 inflammasome pathways a common target [82](Figure 2). Inflammasomes are multiprotein complexes involved in mediating inflammation [83]. NLRP3 is the most widely studied inflammasome and holds important functions in atherogenesis. Accordingly, NLRP3 components are highly expressed in human plaques and their inhibition was shown to blunt atherosclerosis in animal models [84, 85]. NLRP3 assembly and activation are finely tuned by a “two-hit” process: the first signal triggers the transcription of its components [namely, NLRP3, Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and pro-caspase] while a second stimulus activates caspase 1 and leads to secretion of active IL-1β and IL-18 thus fueling inflammation [86]. Of note, priming and activating stimuli include different mediators deeply involved in atherogenesis such as modified lipoproteins [87], cholesterol crystals [85], lipopolysaccharide (LPS) [88] and reactive oxygen species [89], further underscoring the relevance of this complex in plaque growths . Canakinumab and colchicine act at different levels of the NLRP3 pathways. Canakinumab specifically inhibits IL-1β preventing its binding to IL-1 receptor and thus the activation of intracellular nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and MAP kinase pathways [90]. On the other hand, colchicine is thought to inhibit the expression of pyrin gene, thus blunting NLRP3 assembly [91]. Furthermore, by acting on microtubule formation, colchicine also prevents the intracellular transport of the adaptor protein ASC, fundamental for NLRP3 proteins co-localization [92]. In addition, colchicine directly blocks caspase-1-mediated pro-IL-1β activation [93] alongside reducing P2X7-associated pore formation and decreasing potassium efflux, an important step in NLRP3 response [94]. NLRP3 has emerged as an important common denominator to the two successful anti-inflammatory trials in

cardiology differentiating them from negative ones such as Cardiovascular Inflammation Reduction Trial (CIRT) where the purine signalling inhibitor methotrexate was employed in patients at high CV risk (i.e. previous myocardial infarction, multivessel CAD, type 2 diabetes or metabolic syndrome) [19]. In this sense, beside IL-1β, also other mediators of the inflammasome pathway such as IL-18 and its downstream effector IL-6 should be further explored as potential therapeutic targets to reduce CV event rates.

5. Every cloud has a silver lining: learning from futile trials

5.1 Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) and Stabilization of Plaque Using Darapladib-Thrombolysis in Myocardial Infarction 52 (SOLID-TIMI 52) trials

STABILITY and SOLID-TIMI 52 are large double-blind trials investigating darapladib, a potent oral inhibitor of Lp-PLA_2 [22, 23]. Lp-PLA_2 is a serine lipase responsible for removing the acetyl group at the sn-2 position of platelet-activating factor, an active phospholipid with proven functions in different pathologic and physiologic processes. Lp-PLA_2 activity leads then to the production of lysophospholipids and oxidized phospholipids which have been identified within atherosclerotic plaques [95]. Most of Lp-PLA_2 circulates bound to LDL where it participates in its oxidative modification within the vascular wall generating oxidized phospholipids thus fueling vascular inflammation and atherosclerosis progression [95, 96]. Yet, as Lp-PLA_2 removes oxidized phospholipids, an anti-oxidative functions for this enzymes has also been demonstrated [97]. Despite of this, most of the recent literature concur on the pro-inflammatory role of such a molecule. Lysophospholipids generated by Lp-PLA_2 contributes to the inflammatory response by favouring neutrophil function [98], but also

acting as chemokines and attracting leukocytes [99] eventually contributing to plaque development [100, 101]. Similarly, oxidized non esterified fatty acids produced by Lp-PLA_2 facilitate the expression of adhesion molecules and attract macrophages to the arterial intima [102]. The trials found their rational in several experimental and clinical observations, among them the proof of darapladib's efficacy in blunting atherosclerosis in a swine model of disease together with the direct association between Lp-PLA_2 levels [103] and the risk of CAD after adjustment for conventional risk factors that was reported by a large meta-analysis of prospective studies [104]. As a result, STABILITY investigated the efficacy of darapladip 160 mg OD in 15'828 patients with chronic CAD, while SOLID-TIMI 52 used the same treatment to prevent major events in 13'026 patients recently hospitalized with acute coronary syndrome (ACS) [22, 23]. For STABILITY, the inclusion criteria were previous myocardial infarction, previous coronary reperfusion intervention or multivessel CAD, while in SOLID-TIMI 52 only patients hospitalized with ACS in the 30 days prior to randomization were enrolled. In addition, for inclusion in both studies, patients were required at least one traditional CV risk factors (age>60 years, diabetes, dyslipidemia, active smoking, polyvascular arterial disease or moderate kidney dysfunction). As a result, the populations enrolled were quite heterogeneous, thus possibly accounting at least in part for the neutral results achieved. Indeed, darapladip failed to prevent the primary endpoint that included CV death, myocardial infarction or stroke in STABILITY and CV death, myocardial infarction or urgent coronary revascularization for myocardial ischemia in SOLID-TIMI 52 [22, 23]. Of interest, in STABILITY the intervention lead to a modest nominal reduction of the prespecified secondary endpoints of MACE and total coronary events, a preliminary finding that did not find support in SOLID-TIMI 52. Consistently, adverse events of darapladib treatment were similar in the two trials

and consisted of increased rate of diarrhea and odor in skin, feces, and urine of treated patients due to the presence of a sulfhydryl group in the darapladib molecule [22, 23]. These two studies did not investigate the dose-dependent effect of the drug and the used protocol were based on earlier placebo-controlled trials showing a reduction of Lp-PLA_2 activity by 60-66% when 160 mg of darapladib were administered orally once a day [105, 106]. Although patients in STABILITY and SOLID-TIMI 52 were not selected based on the residual inflammatory risk resulting in quite low baseline levels of inflammatory markers (1.3 mg/L hs-CRP and 2.1 ng/L IL-6 for STABILITY[107] and IL-6 2.02 ng/L for SOLID-TIMI 52 sub cohort analysis[108]), the same preliminary investigations suggested that the effects of this protocol on such circulating mediators are very modest and at best leading to a reduction in IL-6 levels of 12.3% without significantly affecting hs-CRP [105, 106]. These weak effects are supposed to partially account for the disappointing results of the trials and definitively ceased the interest in Lp-PLA_2 inhibitors for preventing MACEs.

5.2 Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) trial

The secretory form of PLA_2 was instead investigated in the VISTA-16 trial [21]. The $sPLA_2$ family comprises several hydrolyzing enzymes acting on glycophospholipids (kindly refer to the previous paragraph) and generating different lipids (including prostaglandins) involved in atherogenesis with both pro- and anti-inflammatory functions [109]. $sPLA_2$ enzymes are constitutively expressed in inflammatory cells as well as in fibroblasts furthermore, their expression can be modulated by a variety of pro-inflammatory molecules including IL-1β, IL-6 and interferons. $sPLA_2$ promote the formation of lysophosphatidylcholine, arachidonic acid, docosahexaenoic acid, and

eicosapentaenoic acid, all precursors of multiple pro-inflammatory mediators [110]. Further, by acting on their membrane receptor, sPLA2 can mediate several cellular processes including proliferation, migration and production of cytokines [111]. Patients enrolled in VISTA-16 received varespladib, an oral pan-sPLA$_2$ inhibitor associated with blunted atherosclerosis in experimental models that previously showed to have important inhibitory effects on the pro-atherogenic sPLA2 isoforms of the group II [112]. A total of 5145 patients were randomized to receive varespladib 500 mg OD or matching placebo on top of optimal secondary cardio-preventive therapy before the trail was terminated for futility and possible harm on March 2012. In this case the study cohort consisted of patients within 96h from their hospitalization for ACS with one additional risk factor for recurrent events [21]. Although the primary composite endpoint including fatal and non-fatal CV ischemic events including stroke and unstable angina requiring hospitalization was equally distributed among patients in the two arms, the secondary outcome not including unstable angina occurred more often in patients receiving the sLPA$_2$ inhibitor as compared to the placebo group. Such an effect was mainly driven by higher rate of recurrent myocardial infarction [21]. Furthermore, adverse events occurred more often in the varespladib group with evidence of hepatotoxic effects requiring suspension of the treatment [21]. Whether the increased CV risk was due to sLPA$_2$ inhibition or to off-target effects of the compound remains to be fully elucidated. The fact that genetic deficiency of sPLA$_2$ is not associated with increased atherosclerosis in animal models may support the latter hypothesis [113]. Furthermore, the causative role of sLPA$_2$ for atherothrombosis has been recently questioned by Mendelian randomization studies which failed to find any association [114]. Differently from CANTOS and COLCOT, VISTA-16 trial and other studies investigating varespladib did not show robust effects of such a compound on

IL-1β/IL-6 pathway and hs-CRP levels (the latter being very high at baseline –median value 10.5 mg/L- due to the early enrollment after the acute events) [21, 115, 116]. Failing to show any effect on CV risk, trials targeting inflammatory pathways other than IL-1β/IL-6 cascade indirectly underscore the importance of such signalling in vascular inflammation.

5.3 Losmapimod to Inhibit p38 MAP Kinase as a Therapeutic Target and Modify Outcomes After an Acute Coronary Syndrome (LATITUDE)–TIMI 60 trial

LATITUDE-TIMI 60 trial employed losmapimod (7.5 mg twice daily) to inhibit p38 MAP kinase in patients hospitalized for myocardial infarction and treated with the standard of care therapy [20]. The p38 MAP kinase systems includes 4 proteins of which p38α and p38β are ubiquitously expressed in human cells and deeply involved in the intracellular signalling under inflammatory and environmental stress conditions. Activation of such signalling cascade as reported in atherosclerotic vessels, enhances the synthesis of different inflammatory mediators such as ILs, pro-oxidant enzymes and metalloproteinases thereby further amplifying the vascular inflammatory process [117]. Specifically, in endothelial cells p38 MAP kinase mediates cell adhesion molecules, chemotactic agents, migration, endothelial permeability and angiogenesis [118-121]. In smooth muscle cells this system associates with apoptosis, calcification, hypertrophy, migration and proliferation [122-124]. While in leukocytes, p38 MAPkinase is involved in TLR signalling, cytokine production, vascular wall infiltration, T-cell receptor/B-cell receptor signalling, macrophage LDL uptake and apoptosis (foam cell formation), but also dendritic cell maturation and antigen presentation [125-130]. Accordingly, preclinical data showed beneficial effects of losmapimod on endothelial function in hypertensive rats by blunting IL-1 production [131]. The study

was composed of two part; part A randomized to placebo or losmapimod 3’503 patients and was designed to assess the drug safety profile and explore its efficacy before proceeding with the larger part B (approximately 22 000 patients). Again, the primary endpoint was the secondary prevention of CV death, mycardial infarction, and severe recurrent ischemia requiring urgent coronary artery revascularization while the principal secondary endpoint included only fatal and non-fatal myocardial infarctions [20]. Investigators ceased the enrollment of patients after part A due to futility since losmapimod did not reduce the risk of recurrent major adverse CV events at week 24 of follow up. Exploratory analysis on secondary outcome and subgroup analysis further confirmed the main result [20]. Suprisingly, treatment with losmapimod led to a 30% reduction of hs-CRP at week 12 as compared to placebo (baseline levels 3.6-3.7 mg/L) and also lowered pro-brain natriuretic peptide (pro-BNP), both valid markers of CV outcomes. The short treatment duration (12 weeks) and the non-specific targeting of both p38α and p38β, which can play different roles in CV disease progression, were put forward as possible explanations for the disappointing results. Lastly, a recent randomized double-blind placebo-controlled trial employing this drug in patients with chronic obstructive pulmonary disease failed to show any effect of such treatment on arterial inflammation and endothelial function as assed by F-Fluorodeoxyglucose positron emission tomography and brachial artery flow-mediated dilatation, respectively [132].

5.4 Cardiovascular Inflammation Reduction Trial (CIRT)

CIRT is a randomized, double-blind trial of methotrexate (at a target dose of 15 to 20 mg weekly) in patients with stable atherosclerosis (history of myocardial infarction or multivessel CAD and either diabetes or metabolic syndrome) [19]. Methotrexate is an

inexpensive and widely used broad-spectrum immunomodulatory agent targeting purinergic signalling and approved for the treatment of different rheumatological inflammatory conditions. Being an analogue of folic acid, methotrexate inhibits different key enzymes involved in the synthesis of purins and pyrimidines, thereby affecting DNA synthesis, cell proliferation and turnover. As such, methotrexate exerts its effects mainly on high turnover cells such as inflammatory ones. Furthermore, methotrexate also inhibits the enzymes involved in purine catabolism, thereby leading to intracellular accumulation of adenosine (deriving from AMP dephosphorilation) and activation of anti-inflammatory receptors A_{2A} and A_3 receptors (for a detailed discussion of methotrexate pharmacodynamics please refer to [133]). Of much interest, methotrexate can promote reverse cholesterol transport and limits macrophage transformation into foam cells, thereby having beneficial impact on atherosclerotic plaque development [134]. Differently from its effects on white blood cells, less is known about the putative role of methotrexate on vascular wall. *In vitro* characterization of methotrexate effects on endothelial cells has often reported conflicting results, methotrexate antiproliferative effects may negatively impact on endothelial functions causing cell swelling and membrane disruption [135, 136]. Yet, when given at lower dosages, it can reduce the TNF-α-mediated adhesion molecule upregulation [137, 138]. Also, by increasing AMPK phosphorylation, low-dose methotrexate was shown to induce the expression of manganese superoxide dismutase and heme oxygenase, thereby potentially reducing oxidative stress [139]. Even less is known about the effects of methothrexate on smooth muscle cells. Yet, preliminary reports suggest an anti-proliferative role [140] which requires additional confirmation and characterization. Treatment with methotrexate at different dosages yelded promising results in animal models of atherosclerosis [138, 141]. Nonetheless, the

cardioprotective role of methotrexate in patients with rheumatoid arthritis have been widely substaintiated [142, 143]. With this basis, the CIRT trial consisted of a run-in phase which allowed enrolling only patients that could tolerate methotrexate at the dosage of 15 mg *per* week. Only participants that completed the trial run-in phase proceeded to the second phase and were randomized to receive placebo or continuing methotrexate which was then increased at 20 mg weekly at month 4 of treatment [19]. The final primary endpoint - a composite of CV death, nonfatal myocardial and stroke, or hospitalization for unstable angina that led to urgent revascularization – was equally distributed among the study arms and reached a pre-specified boundary for futility leading to the premature termination of the investigation on March 2018 [19]. Also, methotrexate did not show any effect on further secondary endpoints or subgroup analysis in the 4'786 CIRT participants. As expected, methotrexate associated with the well-known side-effects of the drug (including mouth sores, oral pain, modest leukopenia and elevation of liver transaminases); however, the authors reported for the first time an increased risk for the development of non–basal-cell skin cancer in patients treated with methotrexate [19]. Among the limitations of this trial is the fact that patients enrolled in CIRT were not selected based on their residual inflammatory risk, thus showing lower baseline inflammation levels as compared to CANTOS (i.e. hs-CRP median level for CANTOS 4.2 mg/L vs 1.6 mg/L in the CIRT trial) [19, 24]. Furthermore, methotrexate did not reduce plasma levels of IL-1β, IL-6 or hs-CRP suggesting a low anti-inflammatory effect on the pathways that showed great promise in the CANTOS and COLCOT trials.

Besides offering insights on the specific drug that was investigated, neutral RCTs further underlined the importance of a correct stratification of patient based on the

residual inflammatory risk as indicated by circulating levels of inflammatory biomarkers. Indeed, differently from the CANTOS, none of the neutral RCTs included hs-CRP levels among the enrollment criteria. Future anti-inflammatory clinical trials in the CV setting will have to take into consideration baseline levels of inflammation, potentially advancing personalized cardiology by administrating secondary prevention agents for atherosclerotic protection only to the most appropriate patients. This aspect gains even more weight when considering the high price of some anti-inflammatory agents (i.e monoclonal antibodies), thereby improving the cost-effectiveness analysis of such interventions [80, 81].

6. Conclusions

The role of inflammation in the development and fate of an atherosclerotic plaque has been convincingly demonstrated in different pre-clinical models. The very high number of immune cells and mediators of inflammation within the human atheroma has long suggested a key role for inflammation in patients as well. Yet, preliminary studies provided limited evidence and often conflicting results. By using canakinumab and colchicine, CANTOS and COLCOT studies solidly confirmed the inflammation theory of atherosclerosis in humans and demonstrated for the first time that residual inflammatory risk is an effective target for secondary CV prevention thus representing an important opportunity to implement personalized medicine. Specifically, these trials put NLRP3 inflammasome pathway inhibition under the spotlight as a promising strategy for anti-inflammatory interventions in cardiology. Furthermore, they highlighted the need for a careful monitoring of host defense adverse effects of such interventions and the importance of finding safer targets which risks of infections. Findings from neutral trials on anti-inflammatory agents did not dispute the

importance of inflammation in atherosclerosis, but rather proved the importance of considering specific and often interconnected inflammatory pathways as therapeutic targets in CV patients with residual inflammatory risk. Although CANTOS and COLCOT may not immediately impact on everyday CV practice, they definitively opened the door for the clinical translation of immunomodulatory agents to lower CV risk.

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9. Conflict of interests

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this editorial.

10. References

1. Gerrity RG. The role of the monocyte in atherogenesis: I. Transition of blood-borne monocytes into foam cells in fatty lesions. *Am J Pathol* 1981;**103**:181-190.
2. Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir ED, Amadori L, Khan NS, Wong CK, Shamailova R, Hill CA, Wang Z, Remark R, Li JR, Pina C, Faries C, Awad AJ, Moss N, Bjorkegren JLM, Kim-Schulze S, Gnjatic S, Ma'ayan A, Mocco J, Faries P, Merad M, Giannarelli C. Single-cell immune landscape of human atherosclerotic plaques. *Nat Med* 2019;**25**:1576-1588.
3. Winkels H, Ehinger E, Vassallo M, Buscher K, Dinh HQ, Kobiyama K, Hamers AAJ, Cochain C, Vafadarnejad E, Saliba AE, Zernecke A, Pramod AB, Ghosh AK, Anto Michel N, Hoppe N, Hilgendorf I, Zirlik A, Hedrick CC, Ley K, Wolf D. Atlas of the Immune Cell Repertoire in Mouse Atherosclerosis Defined by Single-Cell RNA-Sequencing and Mass Cytometry. *Circ Res* 2018;**122**:1675-1688.
4. Cole JE, Park I, Ahern DJ, Kassiteridi C, Danso Abeam D, Goddard ME, Green P, Maffia P, Monaco C. Immune cell census in murine atherosclerosis: cytometry by time of flight illuminates vascular myeloid cell diversity. *Cardiovasc Res* 2018;**114**:1360-1371.
5. Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. *Arterioscler Thromb Vasc Biol* 2007;**27**:1159-1165.

6. Ammirati E, Moroni F, Magnoni M, Camici PG. The role of T and B cells in human atherosclerosis and atherothrombosis. *Clin Exp Immunol* 2015;**179**:173-187.

7. Bonaventura A, Montecucco F, Dallegri F, Carbone F, Luscher TF, Camici GG, Liberale L. Novel findings in neutrophil biology and their impact on cardiovascular disease. *Cardiovasc Res* 2019;**115**:1266-1285.

8. Hu D, Mohanta SK, Yin C, Peng L, Ma Z, Srikakulapu P, Grassia G, MacRitchie N, Dever G, Gordon P, Burton FL, Ialenti A, Sabir SR, McInnes IB, Brewer JM, Garside P, Weber C, Lehmann T, Teupser D, Habenicht L, Beer M, Grabner R, Maffia P, Weih F, Habenicht AJ. Artery Tertiary Lymphoid Organs Control Aorta Immunity and Protect against Atherosclerosis via Vascular Smooth Muscle Cell Lymphotoxin beta Receptors. *Immunity* 2015;**42**:1100-1115.

9. Lara-Guzman OJ, Gil-Izquierdo A, Medina S, Osorio E, Alvarez-Quintero R, Zuluaga N, Oger C, Galano JM, Durand T, Munoz-Durango K. Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. *Redox Biol* 2018;**15**:1-11.

10. Montecucco F, Lenglet S, Gayet-Ageron A, Bertolotto M, Pelli G, Palombo D, Pane B, Spinella G, Steffens S, Raffaghello L, Pistoia V, Ottonello L, Pende A, Dallegri F, Mach F. Systemic and intraplaque mediators of inflammation are increased in patients symptomatic for ischemic stroke. *Stroke* 2010;**41**:1394-1404.

11. Bennett MR, Sinha S, Owens GK. Vascular Smooth Muscle Cells in Atherosclerosis. *Circ Res* 2016;**118**:692-702.

12. Thomas AC, Eijgelaar WJ, Daemen MJ, Newby AC. Foam Cell Formation In Vivo Converts Macrophages to a Pro-Fibrotic Phenotype. *PLoS One* 2015;**10**:e0128163.

13. Crea F, Liuzzo G. Pathogenesis of acute coronary syndromes. *J Am Coll Cardiol* 2013;**61**:1-11.

14. Bonaventura A, Liberale L, Vecchie A, Casula M, Carbone F, Dallegri F, Montecucco F. Update on Inflammatory Biomarkers and Treatments in Ischemic Stroke. *Int J Mol Sci* 2016;**17**.

15. Liberale L, Holy EW, Akhmedov A, Bonetti NR, Nietlispach F, Matter CM, Mach F, Montecucco F, Beer JH, Paneni F, Ruschitzka F, Libby P, Luscher TF, Camici GG. Interleukin-1beta Mediates Arterial Thrombus Formation via NET-Associated Tissue Factor. *J Clin Med* 2019;**8**.

16. Liberale L, Diaz-Canestro C, Bonetti NR, Paneni F, Akhmedov A, Beer JH, Montecucco F, Luscher TF, Camici GG. Post-ischaemic administration of the murine Canakinumab-surrogate antibody improves outcome in experimental stroke. *Eur Heart J* 2018;**39**:3511-3517.

17. Carbone F, Liberale L, Bonaventura A, Cea M, Montecucco F. Targeting Inflammation in Primary Cardiovascular Prevention. *Curr Pharm Des* 2016;**22**:5662-5675.

18. Montecucco F, Liberale L, Bonaventura A, Vecchie A, Dallegri F, Carbone F. The Role of Inflammation in Cardiovascular Outcome. *Curr Atheroscler Rep* 2017;**19**:11.

19. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, Gupta M, Tsigoulis M, Verma S, Clearfield M, Libby P, Goldhaber SZ, Seagle R, Ofori C, Saklayen M, Butman

S, Singh N, Le May M, Bertrand O, Johnston J, Paynter NP, Glynn RJ, Investigators C. Low-Dose Methotrexate for the Prevention of Atherosclerotic Events. *N Engl J Med* 2019;**380**:752-762.

20. O'Donoghue ML, Glaser R, Cavender MA, Aylward PE, Bonaca MP, Budaj A, Davies RY, Dellborg M, Fox KA, Gutierrez JA, Hamm C, Kiss RG, Kovar F, Kuder JF, Im KA, Lepore JJ, Lopez-Sendon JL, Ophuis TO, Parkhomenko A, Shannon JB, Spinar J, Tanguay JF, Ruda M, Steg PG, Theroux P, Wiviott SD, Laws I, Sabatine MS, Morrow DA, Investigators L-T. Effect of Losmapimod on Cardiovascular Outcomes in Patients Hospitalized With Acute Myocardial Infarction: A Randomized Clinical Trial. *JAMA* 2016;**315**:1591-1599.
21. Nicholls SJ, Kastelein JJ, Schwartz GG, Bash D, Rosenson RS, Cavender MA, Brennan DM, Koenig W, Jukema JW, Nambi V, Wright RS, Menon V, Lincoff AM, Nissen SE, Investigators V-. Varespladib and cardiovascular events in patients with an acute coronary syndrome: the VISTA-16 randomized clinical trial. *JAMA* 2014;**311**:252-262.
22. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, Hochman JS, Bode C, Maggioni AP, Im K, Shannon JB, Davies RY, Murphy SA, Crugnale SE, Wiviott SD, Bonaca MP, Watson DF, Weaver WD, Serruys PW, Cannon CP, Investigators S-T, Steen DL. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. *JAMA* 2014;**312**:1006-1015.
23. Investigators S, White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, Budaj A, Harrington RA, Steg PG, Ardissino D, Armstrong PW, Avezum A, Aylward PE, Bryce A, Chen H, Chen MF, Corbalan R, Dalby AJ, Danchin N, De Winter RJ, Denchev S, Diaz R, Elisaf M, Flather MD, Goudev AR, Granger

CB, Grinfeld L, Hochman JS, Husted S, Kim HS, Koenig W, Linhart A, Lonn E, Lopez-Sendon J, Manolis AJ, Mohler ER, 3rd, Nicolau JC, Pais P, Parkhomenko A, Pedersen TR, Pella D, Ramos-Corrales MA, Ruda M, Sereg M, Siddique S, Sinnaeve P, Smith P, Sritara P, Swart HP, Sy RG, Teramoto T, Tse HF, Watson D, Weaver WD, Weiss R, Viigimaa M, Vinereanu D, Zhu J, Cannon CP, Wallentin L. Darapladib for preventing ischemic events in stable coronary heart disease. *N Engl J Med* 2014;**370**:1702-1711.

24. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ, Group CT. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* 2017;**377**:1119-1131.

25. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, Pinto FJ, Ibrahim R, Gamra H, Kiwan GS, Berry C, Lopez-Sendon J, Ostadal P, Koenig W, Angoulvant D, Gregoire JC, Lavoie MA, Dube MP, Rhainds D, Provencher M, Blondeau L, Orfanos A, L'Allier PL, Guertin MC, Roubille F. Efficacy and Safety of Low-Dose Colchicine after Myocardial Infarction. *N Engl J Med* 2019;**381**:2497-2505.

26. Libby P. Interleukin-1 Beta as a Target for Atherosclerosis Therapy: Biological Basis of CANTOS and Beyond. *J Am Coll Cardiol* 2017;**70**:2278-2289.

27. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, Eklund KK. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* 2010;**5**:e11765.

28. McGeough MD, Wree A, Inzaugarat ME, Haimovich A, Johnson CD, Pena CA, Goldbach-Mansky R, Broderick L, Feldstein AE, Hoffman HM. TNF regulates transcription of NLRP3 inflammasome components and inflammatory molecules in cryopyrinopathies. *J Clin Invest* 2017;**127**:4488-4497.

29. Lavieri R, Rubartelli A, Carta S. Redox stress unbalances the inflammatory cytokine network: role in autoinflammatory patients and healthy subjects. *J Leukoc Biol* 2016;**99**:79-86.

30. Pan L, Hong Z, Yu L, Gao Y, Zhang R, Feng H, Su L, Wang G. Shear stress induces human aortic endothelial cell apoptosis via interleukin1 receptorassociated kinase 2induced endoplasmic reticulum stress. *Mol Med Rep* 2017;**16**:7205-7212.

31. Folco EJ, Sukhova GK, Quillard T, Libby P. Moderate hypoxia potentiates interleukin-1beta production in activated human macrophages. *Circ Res* 2014;**115**:875-883.

32. Hu Z, Murakami T, Tamura H, Reich J, Kuwahara-Arai K, Iba T, Tabe Y, Nagaoka I. Neutrophil extracellular traps induce IL-1beta production by macrophages in combination with lipopolysaccharide. *Int J Mol Med* 2017;**39**:549-558.

33. Mako V, Czucz J, Weiszhar Z, Herczenik E, Matko J, Prohaszka Z, Cervenak L. Proinflammatory activation pattern of human umbilical vein endothelial cells induced by IL-1beta, TNF-alpha, and LPS. *Cytometry A* 2010;**77**:962-970.

34. Myers CL, Wertheimer SJ, Schembri-King J, Parks T, Wallace RW. Induction of ICAM-1 by TNF-alpha, IL-1 beta, and LPS in human endothelial cells after downregulation of PKC. *Am J Physiol* 1992;**263**:C767-772.

35. Asehnoune K, Strassheim D, Mitra S, Kim JY, Abraham E. Involvement of reactive oxygen species in Toll-like receptor 4-dependent activation of NF-kappa B. *J Immunol* 2004;**172**:2522-2529.

36. Eun SY, Ko YS, Park SW, Chang KC, Kim HJ. IL-1beta enhances vascular smooth muscle cell proliferation and migration via P2Y2 receptor-mediated RAGE expression and HMGB1 release. *Vascul Pharmacol* 2015;**72**:108-117.

37. Moratal C, Raffort J, Arrighi N, Rekima S, Schaub S, Dechesne CA, Chinetti G, Dani C. IL-1beta- and IL-4-polarized macrophages have opposite effects on adipogenesis of intramuscular fibro-adipogenic progenitors in humans. *Sci Rep* 2018;**8**:17005.

38. Prince LR, Allen L, Jones EC, Hellewell PG, Dower SK, Whyte MK, Sabroe I. The role of interleukin-1beta in direct and toll-like receptor 4-mediated neutrophil activation and survival. *Am J Pathol* 2004;**165**:1819-1826.

39. Bonaventura A, Liberale L, Carbone F, Vecchie A, Diaz-Canestro C, Camici GG, Montecucco F, Dallegri F. The Pathophysiological Role of Neutrophil Extracellular Traps in Inflammatory Diseases. *Thromb Haemost* 2018;**118**:6-27.

40. Dong J, Fujii S, Imagawa S, Matsumoto S, Matsushita M, Todo S, Tsutsui H, Sobel BE. IL-1 and IL-6 induce hepatocyte plasminogen activator inhibitor-1 expression through independent signaling pathways converging on C/EBPdelta. *Am J Physiol Cell Physiol* 2007;**292**:C209-215.

41. Liberale L, Dallegri F, Montecucco F, Carbone F. Pathophysiological relevance of macrophage subsets in atherogenesis. *Thromb Haemost* 2017;**117**:7-18.

42. Kirii H, Niwa T, Yamada Y, Wada H, Saito K, Iwakura Y, Asano M, Moriwaki H, Seishima M. Lack of interleukin-1beta decreases the severity of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 2003;**23**:656-660.

43. Vromman A, Ruvkun V, Shvartz E, Wojtkiewicz G, Santos Masson G, Tesmenitsky Y, Folco E, Gram H, Nahrendorf M, Swirski FK, Sukhova GK, Libby P. Stage-dependent differential effects of interleukin-1 isoforms on experimental atherosclerosis. *Eur Heart J* 2019;**40**:2482-2491.

44. Shimokawa H, Ito A, Fukumoto Y, Kadokami T, Nakaike R, Sakata M, Takayanagi T, Egashira K, Takeshita A. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest* 1996;**97**:769-776.

45. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ, Group CT. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet* 2018;**391**:319-328.

46. Ridker PM, Libby P, MacFadyen JG, Thuren T, Ballantyne C, Fonseca F, Koenig W, Shimokawa H, Everett BM, Glynn RJ. Modulation of the interleukin-6 signalling pathway and incidence rates of atherosclerotic events and all-cause mortality: analyses from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). *Eur Heart J* 2018;**39**:3499-3507.

47. Schieffer B, Schieffer E, Hilfiker-Kleiner D, Hilfiker A, Kovanen PT, Kaartinen M, Nussberger J, Harringer W, Drexler H. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. *Circulation* 2000;**101**:1372-1378.

48. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ, Group CT. Effect of interleukin-1beta inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet* 2017;**390**:1833-1842.

49. Sternlicht H, Ringel I. Colchicine inhibition of microtubule assembly via copolymer formation. *J Biol Chem* 1979;**254**:10540-10550.

50. Martinez GJ, Robertson S, Barraclough J, Xia Q, Mallat Z, Bursill C, Celermajer DS, Patel S. Colchicine Acutely Suppresses Local Cardiac Production of Inflammatory Cytokines in Patients With an Acute Coronary Syndrome. *J Am Heart Assoc* 2015;**4**:e002128.

51. Korkmaz S, Erturan I, Naziroglu M, Uguz AC, Cig B, Ovey IS. Colchicine modulates oxidative stress in serum and neutrophil of patients with Behcet disease through regulation of Ca(2)(+) release and antioxidant system. *J Membr Biol* 2011;**244**:113-120.

52. Park YH, Wood G, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat Immunol* 2016;**17**:914-921.

53. Robertson S, Martinez GJ, Payet CA, Barraclough JY, Celermajer DS, Bursill C, Patel S. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clin Sci (Lond)* 2016;**130**:1237-1246.

54. Cronstein BN, Molad Y, Reibman J, Balakhane E, Levin RI, Weissmann G. Colchicine alters the quantitative and qualitative display of selectins on endothelial cells and neutrophils. *J Clin Invest* 1995;**96**:994-1002.

55. Lagunoff D, Chi EY. Effect of colchicine on rat mast cells. *J Cell Biol* 1976;**71**:182-195.

56. Ercolani L, Schulte WE. Metabolic and morphologic effects of colchicine on human T-lymphocyte expression of Fc mu and Fc gamma receptors. *Cell Immunol* 1983;**77**:222-232.

57. Bauriedel G, Heimerl J, Beinert T, Welsch U, Hofling B. Colchicine antagonizes the activity of human smooth muscle cells cultivated from arteriosclerotic lesions after atherectomy. *Coron Artery Dis* 1994;**5**:531-539.

58. Shah B, Allen N, Harchandani B, Pillinger M, Katz S, Sedlis SP, Echagarruga C, Samuels SK, Morina P, Singh P, Karotkin L, Berger JS. Effect of Colchicine on Platelet-Platelet and Platelet-Leukocyte Interactions: a Pilot Study in Healthy Subjects. *Inflammation* 2016;**39**:182-189.

59. Wang Y, Viollet B, Terkeltaub R, Liu-Bryan R. AMP-activated protein kinase suppresses urate crystal-induced inflammation and transduces colchicine effects in macrophages. *Ann Rheum Dis* 2016;**75**:286-294.

60. Wojcicki J, Hinek A, Jaworska M, Samochowiec L. The effect of colchicine on the development of experimental atherosclerosis in rabbits. *Pol J Pharmacol Pharm* 1986;**38**:343-348.

61. Hollander W, Paddock J, Nagraj S, Colombo M, Kirkpatrick B. Effects of anticalcifying and antifibrobrotic drugs on pre-established atherosclerosis in the rabbit. *Atherosclerosis* 1979;**33**:111-123.

62. Kaminiotis VV, Agrogiannis G, Konstantopoulos P, Androutsopoulou V, Korou LM, Vlachos IS, Dontas IA, Perrea D, Iliopoulos DC. Per os colchicine administration in cholesterol fed rabbits: Triglycerides lowering effects without affecting atherosclerosis progress. *Lipids Health Dis* 2017;**16**:184.

63. Wohlford GFt, Van Tassell BW, Ravindra K, Abbate A. COLCOT and CANTOS: piecing together the puzzle of inflammation and cardiovascular events. *Minerva Cardioangiol* 2020;**68**:5-8.

64. Khandkar C, Vaidya K, Patel S. Colchicine for Stroke Prevention: A Systematic Review and Meta-analysis. *Clin Ther* 2019;**41**:582-590 e583.

65. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. *Nat Rev Drug Discov* 2005;**4**:977-987.

66. Liberale L, Montecucco F, Camici GG, Dallegri F, Vecchie A, Carbone F, Bonaventura A. Treatment with Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors to Reduce Cardiovascular Inflammation and Outcomes. *Curr Med Chem* 2017;**24**:1403-1416.

67. Swirski FK, Libby P, Aikawa E, Alcaide P, Luscinskas FW, Weissleder R, Pittet MJ. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J Clin Invest* 2007;**117**:195-205.

68. Christ A, Gunther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, Scholz CJ, Oosting M, Haendler K, Bassler K, Klee K, Schulte-Schrepping J, Ulas T, Moorlag S, Kumar V, Park MH, Joosten LAB, Groh LA, Riksen NP, Espevik T, Schlitzer A, Li Y, Fitzgerald ML, Netea MG, Schultze JL, Latz E. Western Diet Triggers NLRP3-Dependent Innate Immune Reprogramming. *Cell* 2018;**172**:162-175 e114.

69. Liberale L, Carbone F, Camici GG, Montecucco F. IL-1beta and Statin Treatment in Patients with Myocardial Infarction and Diabetic Cardiomyopathy. *J Clin Med* 2019;**8**.

70. Selak V, Elley CR, Wells S, Rodgers A, Sharpe N. Aspirin for primary prevention: yes or no? *J Prim Health Care* 2010;**2**:92-99.

71. Byrne P, Cullinan J, Smith A, Smith SM. Statins for the primary prevention of cardiovascular disease: an overview of systematic reviews. *BMJ Open* 2019;**9**:e023085.

72. Gelbenegger G, Postula M, Pecen L, Halvorsen S, Lesiak M, Schoergenhofer C, Jilma B, Hengstenberg C, Siller-Matula JM. Aspirin for primary prevention of cardiovascular disease: a meta-analysis with a particular focus on subgroups. *BMC Med* 2019;**17**:198.

73. Group ASC, Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, Barton J, Murphy K, Aung T, Haynes R, Cox J, Murawska A, Young A, Lay M, Chen F, Sammons E, Waters E, Adler A, Bodansky J, Farmer A, McPherson R, Neil A, Simpson D, Peto R, Baigent C, Collins R, Parish S, Armitage J. Effects of Aspirin for Primary Prevention in Persons with Diabetes Mellitus. *N Engl J Med* 2018;**379**:1529-1539.

74. Gaziano JM, Brotons C, Coppolecchia R, Cricelli C, Darius H, Gorelick PB, Howard G, Pearson TA, Rothwell PM, Ruilope LM, Tendera M, Tognoni G, Committee AE. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. *Lancet* 2018;**392**:1036-1046.

75. McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, Kirpach B, Shah RC, Ives DG, Storey E, Ryan J, Tonkin AM, Newman AB, Williamson JD, Margolis KL, Ernst ME, Abhayaratna WP, Stocks N, Fitzgerald SM, Orchard SG, Trevaks RE, Beilin LJ, Donnan GA, Gibbs P, Johnston CI, Radziszewska

B, Grimm R, Murray AM, Group AI. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. *N Engl J Med* 2018;**379**:1519-1528.

76. Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, Newson J, Bellingan G, Gilroy DW. Effects of low-dose aspirin on acute inflammatory responses in humans. *J Immunol* 2009;**183**:2089-2096.

77. Amann R, Peskar BA. Anti-inflammatory effects of aspirin and sodium salicylate. *Eur J Pharmacol* 2002;**447**:1-9.

78. Liberale L, Montecucco F, Tardif JC, Libby P, Camici GG. Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease. *Eur Heart J* 2020. [Epub ahead of print] doi: 10.1093/eurheartj/ehz961.

79. Everett BM. Residual Inflammatory Risk: A Common and Important Risk Factor for Recurrent Cardiovascular Events. *J Am Coll Cardiol* 2019;**73**:2410-2412.

80. Sehested TSG, Bjerre J, Ku S, Chang A, Jahansouz A, Owens DK, Hlatky MA, Goldhaber-Fiebert JD. Cost-effectiveness of Canakinumab for Prevention of Recurrent Cardiovascular Events. *JAMA Cardiol* 2019;**4**:128-135.

81. Samuel M, Tardif JC, Khairy P, Roubille F, Waters DD, Gregoire JC, Pinto FJ, Maggioni AP, Diaz R, Berry C, Koenig W, Ostadal P, Lopez-Sendon J, Gamra H, Kiwan GS, Dube MP, Provencher M, Orfanos A, Blondeau L, Kouz S, L'Allier PL, Ibrahim R, Bouabdallaoui N, Mitchell D, Guertin MC, Lelorier J. Cost-Effectiveness of Low-Dose Colchicine after Myocardial Infarction in the Colchicine Cardiovascular Outcomes Trial (COLCOT). *Eur Heart J Qual Care Clin Outcomes* 2020. [Epub ahead of print] doi: 10.1093/ehjqcco/qcaa045

82. Ridker PM. From CANTOS to CIRT to COLCOT to Clinic: Will All Atherosclerosis Patients Soon Be Treated With Combination Lipid-Lowering and Inflammation-Inhibiting Agents? *Circulation* 2020;**141**:787-789.

83. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016;**16**:407-420.

84. Zheng F, Xing S, Gong Z, Mu W, Xing Q. Silence of NLRP3 suppresses atherosclerosis and stabilizes plaques in apolipoprotein E-deficient mice. *Mediators Inflamm* 2014;**2014**:507208.

85. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010;**464**:1357-1361.

86. Swanson KV, Deng M, Ting JP. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol* 2019;**19**:477-489.

87. Rhoads JP, Lukens JR, Wilhelm AJ, Moore JL, Mendez-Fernandez Y, Kanneganti TD, Major AS. Oxidized Low-Density Lipoprotein Immune Complex Priming of the Nlrp3 Inflammasome Involves TLR and FcgammaR Cooperation and Is Dependent on CARD9. *J Immunol* 2017;**198**:2105-2114.

88. Ghonime MG, Shamaa OR, Das S, Eldomany RA, Fernandes-Alnemri T, Alnemri ES, Gavrilin MA, Wewers MD. Inflammasome priming by lipopolysaccharide is dependent upon ERK signaling and proteasome function. *J Immunol* 2014;**192**:3881-3888.

89. Usui F, Shirasuna K, Kimura H, Tatsumi K, Kawashima A, Karasawa T, Yoshimura K, Aoki H, Tsutsui H, Noda T, Sagara J, Taniguchi S, Takahashi M.

Inflammasome activation by mitochondrial oxidative stress in macrophages leads to the development of angiotensin II-induced aortic aneurysm. *Arterioscler Thromb Vasc Biol* 2015;**35**:127-136.

90. Rondeau JM, Ramage P, Zurini M, Gram H. The molecular mode of action and species specificity of canakinumab, a human monoclonal antibody neutralizing IL-1beta. *MAbs* 2015;**7**:1151-1160.

91. Nidorf SM, Eikelboom JW, Thompson PL. Targeting cholesterol crystal-induced inflammation for the secondary prevention of cardiovascular disease. *J Cardiovasc Pharmacol Ther* 2014;**19**:45-52.

92. Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, Akira S. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nat Immunol* 2013;**14**:454-460.

93. Otani K, Watanabe T, Shimada S, Takeda S, Itani S, Higashimori A, Nadatani Y, Nagami Y, Tanaka F, Kamata N, Yamagami H, Tanigawa T, Shiba M, Tominaga K, Fujiwara Y, Arakawa T. Colchicine prevents NSAID-induced small intestinal injury by inhibiting activation of the NLRP3 inflammasome. *Sci Rep* 2016;**6**:32587.

94. Marques-da-Silva C, Chaves MM, Castro NG, Coutinho-Silva R, Guimaraes MZ. Colchicine inhibits cationic dye uptake induced by ATP in P2X2 and P2X7 receptor-expressing cells: implications for its therapeutic action. *Br J Pharmacol* 2011;**163**:912-926.

95. Mannheim D, Herrmann J, Versari D, Gossl M, Meyer FB, McConnell JP, Lerman LO, Lerman A. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. *Stroke* 2008;**39**:1448-1455.

96. Wang WY, Li J, Yang D, Xu W, Zha RP, Wang YP. OxLDL stimulates lipoprotein-associated phospholipase A2 expression in THP-1 monocytes via PI3K and p38 MAPK pathways. *Cardiovasc Res* 2010;**85**:845-852.

97. Kono N, Inoue T, Yoshida Y, Sato H, Matsusue T, Itabe H, Niki E, Aoki J, Arai H. Protection against oxidative stress-induced hepatic injury by intracellular type II platelet-activating factor acetylhydrolase by metabolism of oxidized phospholipids in vivo. *J Biol Chem* 2008;**283**:1628-1636.

98. Frasch SC, Zemski-Berry K, Murphy RC, Borregaard N, Henson PM, Bratton DL. Lysophospholipids of different classes mobilize neutrophil secretory vesicles and induce redundant signaling through G2A. *J Immunol* 2007;**178**:6540-6548.

99. Lauber K, Bohn E, Krober SM, Xiao YJ, Blumenthal SG, Lindemann RK, Marini P, Wiedig C, Zobywalski A, Baksh S, Xu Y, Autenrieth IB, Schulze-Osthoff K, Belka C, Stuhler G, Wesselborg S. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. *Cell* 2003;**113**:717-730.

100. Herrmann J, Mannheim D, Wohlert C, Versari D, Meyer FB, McConnell JP, Gossl M, Lerman LO, Lerman A. Expression of lipoprotein-associated phospholipase A(2) in carotid artery plaques predicts long-term cardiac outcome. *Eur Heart J* 2009;**30**:2930-2938.

101. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007;**115**:2715-2721.

102. Silva IT, Mello AP, Damasceno NR. Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A(2) (Lp-PLA(2)): a review. *Lipids Health Dis* 2011;**10**:170.

103. Wilensky RL, Shi Y, Mohler ER, 3rd, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Hoffman BE, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nat Med* 2008;**14**:1059-1066.

104. Lp PLASC, Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet* 2010;**375**:1536-1544.

105. Mohler ER, 3rd, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, Zalewski A, Darapladib I. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. *J Am Coll Cardiol* 2008;**51**:1632-1641.

106. Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheye S, Aschermann M, Duckers H, Bleie O, Dudek D, Botker HE, von Birgelen C, D'Amico D, Hutchinson T, Zambanini A, Mastik F, van Es GA, van der Steen AF, Vince DG, Ganz P, Hamm CW, Wijns W, Zalewski A, Integrated B, Imaging Study I. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor

darapladib on human coronary atherosclerotic plaque. *Circulation* 2008;**118**:1172-1182.

107. Held C, White HD, Stewart RAH, Budaj A, Cannon CP, Hochman JS, Koenig W, Siegbahn A, Steg PG, Soffer J, Weaver WD, Ostlund O, Wallentin L, Investigators S. Inflammatory Biomarkers Interleukin-6 and C-Reactive Protein and Outcomes in Stable Coronary Heart Disease: Experiences From the STABILITY (Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy) Trial. *J Am Heart Assoc* 2017;**6**.

108. Fanola CL, Morrow DA, Cannon CP, Jarolim P, Lukas MA, Bode C, Hochman JS, Goodrich EL, Braunwald E, O'Donoghue ML. Interleukin-6 and the Risk of Adverse Outcomes in Patients After an Acute Coronary Syndrome: Observations From the SOLID-TIMI 52 (Stabilization of Plaque Using Darapladib-Thrombolysis in Myocardial Infarction 52) Trial. *J Am Heart Assoc* 2017;**6**.

109. Talmud PJ, Holmes MV. Deciphering the Causal Role of sPLA2s and Lp-PLA2 in Coronary Heart Disease. *Arterioscler Thromb Vasc Biol* 2015;**35**:2281-2289.

110. Quach ND, Arnold RD, Cummings BS. Secretory phospholipase A2 enzymes as pharmacological targets for treatment of disease. *Biochem Pharmacol* 2014;**90**:338-348.

111. Lambeau G, Ancian P, Barhanin J, Lazdunski M. Cloning and expression of a membrane receptor for secretory phospholipases A2. *J Biol Chem* 1994;**269**:1575-1578.

112. Fraser H, Hislop C, Christie RM, Rick HL, Reidy CA, Chouinard ML, Eacho PI, Gould KE, Trias J. Varespladib (A-002), a secretory phospholipase A2

inhibitor, reduces atherosclerosis and aneurysm formation in ApoE-/- mice. *J Cardiovasc Pharmacol* 2009;**53**:60-65.

113. Burton CA, Patel S, Mundt S, Hassing H, Zhang D, Hermanowski-Vosatka A, Wright SD, Chao YS, Detmers PA, Sparrow CP. Deficiency in sPLA(2) does not affect HDL levels or atherosclerosis in mice. *Biochem Biophys Res Commun* 2002;**294**:88-94.

114. Holmes MV, Simon T, Exeter HJ, Folkersen L, Asselbergs FW, Guardiola M, Cooper JA, Palmen J, Hubacek JA, Carruthers KF, Horne BD, Brunisholz KD, Mega JL, van Iperen EPA, Li M, Leusink M, Trompet S, Verschuren JJW, Hovingh GK, Dehghan A, Nelson CP, Kotti S, Danchin N, Scholz M, Haase CL, Rothenbacher D, Swerdlow DI, Kuchenbaecker KB, Staines-Urias E, Goel A, van 't Hooft F, Gertow K, de Faire U, Panayiotou AG, Tremoli E, Baldassarre D, Veglia F, Holdt LM, Beutner F, Gansevoort RT, Navis GJ, Mateo Leach I, Breitling LP, Brenner H, Thiery J, Dallmeier D, Franco-Cereceda A, Boer JMA, Stephens JW, Hofker MH, Tedgui A, Hofman A, Uitterlinden AG, Adamkova V, Pitha J, Onland-Moret NC, Cramer MJ, Nathoe HM, Spiering W, Klungel OH, Kumari M, Whincup PH, Morrow DA, Braund PS, Hall AS, Olsson AG, Doevendans PA, Trip MD, Tobin MD, Hamsten A, Watkins H, Koenig W, Nicolaides AN, Teupser D, Day INM, Carlquist JF, Gaunt TR, Ford I, Sattar N, Tsimikas S, Schwartz GG, Lawlor DA, Morris RW, Sandhu MS, Poledne R, Maitland-van der Zee AH, Khaw KT, Keating BJ, van der Harst P, Price JF, Mehta SR, Yusuf S, Witteman JCM, Franco OH, Jukema JW, de Knijff P, Tybjaerg-Hansen A, Rader DJ, Farrall M, Samani NJ, Kivimaki M, Fox KAA, Humphries SE, Anderson JL, Boekholdt SM, Palmer TM, Eriksson P, Pare G, Hingorani AD, Sabatine MS, Mallat Z, Casas JP, Talmud PJ. Secretory

phospholipase A(2)-IIA and cardiovascular disease: a mendelian randomization study. *J Am Coll Cardiol* 2013;**62**:1966-1976.

115. Rosenson RS, Elliott M, Stasiv Y, Hislop C, Investigators PI. Randomized trial of an inhibitor of secretory phospholipase A2 on atherogenic lipoprotein subclasses in statin-treated patients with coronary heart disease. *Eur Heart J* 2011;**32**:999-1005.

116. Rosenson RS, Hislop C, McConnell D, Elliott M, Stasiv Y, Wang N, Waters DD, Investigators P. Effects of 1-H-indole-3-glyoxamide (A-002) on concentration of secretory phospholipase A2 (PLASMA study): a phase II double-blind, randomised, placebo-controlled trial. *Lancet* 2009;**373**:649-658.

117. Cheriyan J, Webb AJ, Sarov-Blat L, Elkhawad M, Wallace SM, Maki-Petaja KM, Collier DJ, Morgan J, Fang Z, Willette RN, Lepore JJ, Cockcroft JR, Sprecher DL, Wilkinson IB. Inhibition of p38 mitogen-activated protein kinase improves nitric oxide-mediated vasodilatation and reduces inflammation in hypercholesterolemia. *Circulation* 2011;**123**:515-523.

118. Borbiev T, Birukova A, Liu F, Nurmukhambetova S, Gerthoffer WT, Garcia JG, Verin AD. p38 MAP kinase-dependent regulation of endothelial cell permeability. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L911-918.

119. Diaz-Canestro C, Merlini M, Bonetti NR, Liberale L, Wust P, Briand-Schumacher S, Klohs J, Costantino S, Miranda M, Schoedon-Geiser G, Kullak-Ublick GA, Akhmedov A, Paneni F, Beer JH, Luscher TF, Camici GG. Sirtuin 5 as a novel target to blunt blood-brain barrier damage induced by cerebral ischemia/reperfusion injury. *Int J Cardiol* 2018;**260**:148-155.

120. Goebeler M, Kilian K, Gillitzer R, Kunz M, Yoshimura T, Brocker EB, Rapp UR, Ludwig S. The MKK6/p38 stress kinase cascade is critical for tumor necrosis

factor-alpha-induced expression of monocyte-chemoattractant protein-1 in endothelial cells. *Blood* 1999;**93**:857-865.

121. Zhu Y, Liao H, Wang N, Ma KS, Verna LK, Shyy JY, Chien S, Stemerman MB. LDL-activated p38 in endothelial cells is mediated by Ras. *Arterioscler Thromb Vasc Biol* 2001;**21**:1159-1164.

122. Jing Q, Xin SM, Cheng ZJ, Zhang WB, Zhang R, Qin YW, Pei G. Activation of p38 mitogen-activated protein kinase by oxidized LDL in vascular smooth muscle cells: mediation via pertussis toxin-sensitive G proteins and association with oxidized LDL-induced cytotoxicity. *Circ Res* 1999;**84**:831-839.

123. Ushio-Fukai M, Alexander RW, Akers M, Griendling KK. p38 Mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. Role in vascular smooth muscle cell hypertrophy. *J Biol Chem* 1998;**273**:15022-15029.

124. Liao L, Zhou Q, Song Y, Wu W, Yu H, Wang S, Chen Y, Ye M, Lu L. Ceramide mediates Ox-LDL-induced human vascular smooth muscle cell calcification via p38 mitogen-activated protein kinase signaling. *PLoS One* 2013;**8**:e82379.

125. Zhang J, Salojin KV, Gao JX, Cameron MJ, Bergerot I, Delovitch TL. p38 mitogen-activated protein kinase mediates signal integration of TCR/CD28 costimulation in primary murine T cells. *J Immunol* 1999;**162**:3819-3829.

126. Khiem D, Cyster JG, Schwarz JJ, Black BL. A p38 MAPK-MEF2C pathway regulates B-cell proliferation. *Proc Natl Acad Sci U S A* 2008;**105**:17067-17072.

127. Zhao M, Liu Y, Wang X, New L, Han J, Brunk UT. Activation of the p38 MAP kinase pathway is required for foam cell formation from macrophages exposed to oxidized LDL. *APMIS* 2002;**110**:458-468.

128. Cheng F, Twardowski L, Fehr S, Aner C, Schaeffeler E, Joos T, Knorpp T, Dorweiler B, Laufer S, Schwab M, Torzewski M. Selective p38alpha MAP kinase/MAPK14 inhibition in enzymatically modified LDL-stimulated human monocytes: implications for atherosclerosis. *FASEB J* 2017;**31**:674-686.

129. Senokuchi T, Matsumura T, Sakai M, Matsuo T, Yano M, Kiritoshi S, Sonoda K, Kukidome D, Nishikawa T, Araki E. Extracellular signal-regulated kinase and p38 mitogen-activated protein kinase mediate macrophage proliferation induced by oxidized low-density lipoprotein. *Atherosclerosis* 2004;**176**:233-245.

130. Meng Z, Yan C, Deng Q, Dong X, Duan ZM, Gao DF, Niu XL. Oxidized low-density lipoprotein induces inflammatory responses in cultured human mast cells via Toll-like receptor 4. *Cell Physiol Biochem* 2013;**31**:842-853.

131. Willette RN, Eybye ME, Olzinski AR, Behm DJ, Aiyar N, Maniscalco K, Bentley RG, Coatney RW, Zhao S, Westfall TD, Doe CP. Differential effects of p38 mitogen-activated protein kinase and cyclooxygenase 2 inhibitors in a model of cardiovascular disease. *J Pharmacol Exp Ther* 2009;**330**:964-970.

132. Fisk M, Cheriyan J, Mohan D, Forman J, Maki-Petaja KM, McEniery CM, Fuld J, Rudd JHF, Hopkinson NS, Lomas DA, Cockcroft JR, Tal-Singer R, Polkey MI, Wilkinson IB. The p38 mitogen activated protein kinase inhibitor losmapimod in chronic obstructive pulmonary disease patients with systemic inflammation, stratified by fibrinogen: A randomised double-blind placebo-controlled trial. *PLoS One* 2018;**13**:e0194197.

133. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. *Nat Rev Rheumatol* 2020;**16**:145-154.

134. Reiss AB, Carsons SE, Anwar K, Rao S, Edelman SD, Zhang H, Fernandez P, Cronstein BN, Chan ES. Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages. *Arthritis Rheum* 2008;**58**:3675-3683.

135. Fuskevag OM, Kristiansen C, Olsen R, Aarbakke J, Lindal S. Microvascular perturbations in rats receiving the maximum tolerated dose of methotrexate or its major metabolite 7-hydroxymethotrexate. *Ultrastruct Pathol* 2000;**24**:325-332.

136. Merkle CJ, Moore IM, Penton BS, Torres BJ, Cueny RK, Schaeffer RC, Jr., Montgomery DW. Methotrexate causes apoptosis in postmitotic endothelial cells. *Biol Res Nurs* 2000;**2**:5-14.

137. Yamasaki K, Takeyoshi M, Sawaki M, Imatanaka N, Shinoda K, Takatsuki M. Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. *Toxicology* 2003;**183**:93-115.

138. Bulgarelli A, Martins Dias AA, Caramelli B, Maranhao RC. Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits. *J Cardiovasc Pharmacol* 2012;**59**:308-314.

139. Thornton CC, Al-Rashed F, Calay D, Birdsey GM, Bauer A, Mylroie H, Morley BJ, Randi AM, Haskard DO, Boyle JJ, Mason JC. Methotrexate-mediated activation of an AMPK-CREB-dependent pathway: a novel mechanism for vascular protection in chronic systemic inflammation. *Ann Rheum Dis* 2016;**75**:439-448.

140. Amarnani D, Machuca-Parra AI, Wong LL, Marko CK, Stefater JA, Stryjewski TP, Eliott D, Arboleda-Velasquez JF, Kim LA. Effect of Methotrexate on an In Vitro Patient-Derived Model of Proliferative Vitreoretinopathy. *Invest Ophthalmol Vis Sci* 2017;**58**:3940-3949.

141. Zhang R, Chen S, Zhang H, Liu Q, Xing J, Zhao Q, Wang Y, Yu B, Hou J. Effects of Methotrexate in a Rabbit Model of In-Stent Neoatherosclerosis: An Optical Coherence Tomography Study. *Sci Rep* 2016;**6**:33657.

142. Micha R, Imamura F, Wyler von Ballmoos M, Solomon DH, Hernan MA, Ridker PM, Mozaffarian D. Systematic review and meta-analysis of methotrexate use and risk of cardiovascular disease. *Am J Cardiol* 2011;**108**:1362-1370.

143. Roubille C, Richer V, Starnino T, McCourt C, McFarlane A, Fleming P, Siu S, Kraft J, Lynde C, Pope J, Gulliver W, Keeling S, Dutz J, Bessette L, Bissonnette R, Haraoui B. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. *Ann Rheum Dis* 2015;**74**:480-489.

11. Figure legend

Figure 1: Recent phase III randomized clinical trials investigating anti-inflammatory agents in cardio- and cerebrovascular prevention. Hazard ratios of the different anti-inflammatory interventions with respect to secondary prevention of major adverse cardiovascular events. Boxes indicate the targets of the different trials and basic findings suggesting their potential mechanisms of action. HR: hazard ratio.

Figure 2: NLRP3 pathway as a common denominator of canakinumab and colchicine effects. While canakinumab specifically inhibit IL-1β-mediated activation of IL-1 receptor, colchicine inhibits different steps of NLRP3 inflammasome assembling, priming and activation thus affecting all its downstream mediators. Beside their direct effects on the vasculature, pro-inflammatory cytokines also trigger the acute phase response thereby increasing circulating levels of CRP. When measured by a hig-sensitivity (hs) assay, hs-CRP can be used as biomarker of residual inflammatory risk. CRP: C-reactive protein; IL: interleukin; LPS: lipopolysaccharide; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; ROS: reactive oxygen species.

Table 1: Main characteristics of the 2 positive randomized clinical trials

	Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS)	**Colchicine Cardiovascular Outcomes Trial (COLCOT)**
Population	Previous myocardial infarction and hs CRP ≥ 2 mg/L under intensive secondary CV prevention therapy	Myocardial infarction within 30 days with percutaneous revascularization procedures under intensive secondary CV prevention
Drug	Canakinumab	Colchicine
Target	Interleukin-1β	Microtubule assembly
Route of administration	Subcutaneous	Oral
Frequency of administration	Every 3 months	Once daily
Dosage	50-100-150 mg	0.5 mg
Primary composite endpoint	Nonfatal myocardial infarction, any nonfatal stroke, or cardiovascular death	Cardiovascular death, resuscitated cardiac arrest, myocardial infarction, stroke, or urgent hospitalization for angina leading to coronary revascularization
Adverse reactions	Neutropenia, fatal infections or sepsis, uncomplicated thrombocytopenia	Nausea, flatulence and pneumonia

Figure 1