Atherosclerotic Plaque Destabilization
Mechanisms, Models, and Therapeutic Strategies

Carlos Silvestre-Roig, Menno P. de Winther, Christian Weber, Mat J. Daemen, Esther Lutgens, Oliver Soehnlein

Abstract: Understanding the pathophysiology of atherogenesis and the progression of atherosclerosis have been major goals of cardiovascular research during the previous decades. However, the complex molecular and cellular mechanisms underlying plaque destabilization remain largely obscure. Here, we review how lesional cells undergo cell death and how failed clearance exacerbates necrotic core formation. Advanced atherosclerotic lesions are further weakened by the pronounced local activity of matrix-degrading proteases as well as immature neovessels sprouting into the lesion. To stimulate translation of the current knowledge of molecular mechanisms of plaque destabilization into clinical studies, we further summarize available animal models of plaque destabilization. Based on the molecular mechanisms leading to plaque instability, we outline the current status of clinical and preclinical trials to induce plaque stability with a focus on induction of dead cell clearance, inhibition of protease activity, and dampening of inflammatory cell recruitment. (Circ Res. 2014;114:214-226.)

Key Words: atherosclerosis ■ efferocytosis ■ macrophages ■ necrotic core ■ neutrophils

Atherosclerosis is a chronic inflammatory disease of the arterial wall, arising from an imbalance in lipid metabolism and a maladaptive inflammatory response.1 In recent decades, atherosclerosis-related arterial diseases have become the leading cause of death and morbidity worldwide. Despite emerging anti-inflammatory and lipid-lowering treatments, which may reduce acute coronary syndrome (ACS), effective preventive approaches specifically targeting mechanisms leading to plaque destabilization remain elusive. Hence, a more detailed understanding of the mechanisms underlying plaque destabilization may help us to improve detection and treatment of plaque vulnerability. The aim of this review was to provide a comprehensive summary of mechanisms underlying plaque destabilization, to summarize various animal models allowing to mimic certain aspects of plaque destabilization, and to give an overview of emerging molecular strategies to target plaque destabilization.

Mechanisms Converting a Stable Plaque to a Vulnerable Plaque
Lesional Cell Death Triggers Plaque Vulnerability
Rupture-prone vulnerable plaques are typically associated with the presence of a highly inflammatory cell treatment and a large necrotic core (NC) covered by a thin fibrous cap, the latter being characterized by decreased smooth muscle cell (SMC) and extracellular matrix (ECM) content.2 Exacerbated macrophage and SMC apoptosis is a main contributor of NC expansion. In stable atherosclerotic lesions, SMCs are predominant in the fibrous cap and contribute to cap thickening by producing elastin, collagen, and other matrix components. During plaque destabilization, increased apoptosis of SMCs residing in the fibrous cap results in reduced ECM protein production. The induction of SMC apoptosis will give rise to plaques with thinner fibrous caps, reduced matrix protein deposition, exacerbated plaque inflammation, and expanded NC.3 Infiltration of inflammatory macrophages and lymphocytes induces SMC apoptosis through the release of proinflammatory tumor necrosis factor4 and tumor necrosis factor–related apoptosis-inducing ligand.5 The cytotoxic activity of plaque lymphocytes has been attributed to a subpopulation of CD8+ T cells, which mediate plaque destabilization by induction of macrophage, endothelial cell (EC), and SMC apoptosis.6 SMC integrity during advanced atherosclerosis is also compromised by mast cells, which contribute to SMC apoptosis by a mechanism involving tryptase and chymase release upon Toll-like receptor-4 activation.7 Loss of cell matrix and cell-to-cell interactions after matrix metalloproteinase (MMP)-dependent degradation is an alternative mechanism of induction of SMC apoptosis. Particularly, MMP-7–driven cleavage of N-cadherin–dependent cell-to-cell contacts promotes SMC apoptosis.8 In addition, the accumulation of ECM cleavage products, such as degraded collagen, induces SMC

Original received April 8, 2013; revision received October 10, 2013; accepted November 10, 2013. In October 2013, the average time from submission to first decision for all original research papers submitted to Circulation Research was 12.81 days.

From the Departments of Pathology (C.S.-R., M.D., O.S.) and Biochemistry (C.S.-R., M.d.W., E.L.), Academic Medical Center, Amsterdam, the Netherlands; Institute for Cardiovascular Prevention, Ludwig Maximilian University of Munich, Munich, Germany (C.W., E.L., O.S.); and Deutsches Zentrum für Herz-Kreislauf-Forschung e.V. (DZHK) (German Centre for Cardiovascular Research), partner site of Munich Heart Alliance, Munich, Germany (C.W., O.S.).

Correspondence to Oliver Soehnlein or Carlos Silvestre-Roig, PhD, Department of Pathology, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, the Netherlands. E-mail oliver.soehnlein@gmail.com or c.silvestre-roig@amc.uva.nl

© 2014 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.114.302355

214
apoptosis. Collectively, continuous infiltration of inflammatory leukocytes in the fibrous cap mediates SMC apoptosis by cell-to-cell interaction or by protease-mediated alteration of the extracellular environment.

Macrophage apoptosis is detected throughout all stages of atherosclerosis. In animal models, genetic manipulation of proapoptotic and antiapoptotic factors in macrophages revealed opposite stage-dependent effects: enhanced macrophage apoptosis reduces early atheroma burden while accelerating atherosclerosis and plaque necrosis during advanced disease stages. Defective clearance of apoptotic macrophages during advanced atherosclerosis may explain their progressive accumulation, thereby contributing to enlarged NC size and plaque inflammation. Moreover, the analysis of human culprit specimens revealed that apoptotic macrophages are frequently present in rupture sites within the fibrous cap, suggesting a contribution of macrophage death not only to NC expansion but also to plaque rupture. Macrophage-engulfed lipoproteins are transported to and processed in the endoplasmic reticulum (ER) and the resulting free cholesterol is re-esterified, leading to the formation of lipid droplets, a characteristic of foam cells. During advanced atherosclerosis, accumulation of free cholesterol due to impaired esterification and exposure to oxidized cholesterol trigger a process known as unfolded protein response (UPR). UPR is an adaptive response to maintain ER homeostasis as a consequence of accumulation of misfolded proteins or other ER stressors. On prolonged activation, UPR results in the induction of cell apoptosis (Figure 1). Of note, although the importance of ER stress on macrophage apoptosis during advanced atherosclerosis has been clearly demonstrated in vivo, data on mechanisms triggering this process primarily stem from in vitro studies. One branch of the UPR pathway mediating cell apoptosis involves the activation of the transcription factor C/EBP homologous protein (CHOP). CHOP is activated in human vulnerable plaques, and its deficiency critically reduces atheroma burden and lesional apoptosis.

CHOP mediates mitochondria-dependent apoptosis through the downregulation of Bcl-2, whose absence in myeloid cells increases intimal apoptosis and NC formation in advanced atherosclerosis. In addition, CHOP triggers ER calcium release, thus activating the downstream effector calcium/calmodulin-dependent protein kinase IIy. Calcium/calmodulin-dependent protein kinase IIy induces various apoptosis pathways involving signal transducer and activator of transcription-1 activation, a decisive molecule in lesional macrophage apoptosis and NC expansion. Continuous exposure to ER stressors also induces alternative UPR-independent macrophage apoptosis mechanisms, which depend on the c-Jun N-terminal kinase pathway downstream of scavenger receptor A or Toll-like receptor-4 activation. Interestingly, although high doses of atherogenic lipids can directly trigger ER stress by activating the UPR response in vitro, at low doses a second hit mediated by CD36 and Toll-like receptor-2 is required to boost nicotinamide adenine dinucleotide phosphate (NADPH) oxidase–dependent reactive oxygen species production, ultimately inducing cell apoptosis. Finally, ER stress also triggers a p38 mitogen-activated protein kinase pathway, which exerts a prosurvival function that counterbalances ER-mediated macrophage apoptosis during atherosclerosis. However, unbalanced activity of ER stress pathways in favor of the signal transducer and activator of transcription-1/c-Jun N-terminal kinase/CHOP instead of p38 mitogen-activated protein kinase pathway may be crucial in promoting macrophage apoptosis, NC expansion, and plaque vulnerability. Alternatively, autophagy death processes have also been identified in atherosclerotic plaques as a way of macrophage removal (Figure 1). Under low oxidative or ER stress conditions, macrophage autophagy is induced, thereby preventing apoptosis and reactive oxygen species production and promoting their clearance by other phagocytes. However, during advanced atherosclerosis, the continuous exposure to such stressors inhibits autophagy in favor of an apoptosis-mediated macrophage death, ultimately leading to necrotic cell accumulation and enhanced plaque vulnerability. Despite the increasing knowledge about mechanisms underlying macrophage apoptosis, additional efforts are required to clarify which key molecules are responsible for its induction in vivo.

**Defective Cell Efferocytosis Primes for Accumulation of Necrotic Cells**

Clearance of apoptotic cells by phagocytes, involving a process termed efferocytosis, impedes the accumulation of necrotic corpses and triggers phagocyte reprogramming toward anti-inflammatory phenotypes, boosting the resolution of inflammation. During atherosclerosis, effective efferocytosis is hampered, resulting in the accumulation of apoptotic cells and the delay of resolution of inflammation. Apoptotic cells produce a plethora of find-me and eat-me signals that mediate phagocyte attraction, interaction, and, finally, engulfment. Consequently, an insufficient production of attraction and recognition molecules or an altered interaction and phagocytosis may explain defective efferocytosis during atherosclerosis. Find-me signals released by apoptotic cells include lysophosphatidylcholine and sphingosine-1-phosphate (Figure 1). During apoptosis, a caspase-3–dependent activation of phospholipase A₂ results in the release of lysophosphatidylcholine, triggering phagocyte migration and engulfment. During hypercholesterolemia, oxidized low-density lipoprotein or lysophosphatidylcholine reduces apoptotic cell clearance by competing with the same receptors. On the other hand, sphingosine-1-phosphate acts as a find-me and eat-me signal with atheroprotective functions because the administration of a synthetic homolog induces plaque stability by reducing NC formation. Taken together, the importance of find-me signals in end-stage phagocyte attraction...
and locomotion may facilitate lesional efferocytosis, thereby reducing apoptotic cell accumulation.

Once phagocytes are adjacent to the apoptotic cell, eat-me molecules expressed in the apoptotic membranes enable their recognition\(^2\) (Figure 1). Neutrophil-borne pentraxin 3 is exposed on neutrophil apoptosis, promoting clearance by macrophages. Its depletion results in enhanced plaque size.\(^2\) Neutrophil apoptosis is mediated by ER stressors such as lipoprotein (Lp)-a, oxidized low-density lipoprotein (oxLDL), or fatty acids (FA) that activate lesional macrophage apoptosis. Alternative pathways for induction of macrophage apoptosis mediated by ER stress involve the activation of c-Jun N-terminal kinase pathway via SR-A and TLR4. Finally, a pathway that engages CD36 and TLR2 and TLR4 activates nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase, boosting reactive oxygen species production and triggering cell apoptosis. However, macrophage apoptosis is an atheroprotective process that is inhibited by oxLDL. B to D, Apoptotic cells are cleared by phagocytes in a process called efferocytosis. During advanced atherosclerosis, efferocytosis is impaired, leading to apoptotic cell accumulation, secondary necrosis, and formation and expansion of the NC. Cell efferocytosis involves phagocyte attraction by find-me signals released by the apoptotic cell (B), recognition of the apoptotic cell by eat-me molecules (C), and phagocyte receptors that mediate cell engulfment (D).

Figure 1. Implication of cell death and defective efferocytosis during plaque destabilization. A, Death of macrophages is a major contributor to necrotic core (NC) expansion and fibrous cap thinning. Endoplasmic reticulum (ER) stressors such as lipoprotein (Lp)-a, oxidized low-density lipoprotein (oxLDL), or fatty acids (FA) accelerate lesional macrophage apoptosis. Alternative pathways for induction of macrophage apoptosis mediated by ER stress involve the activation of c-Jun N-terminal kinase pathway via SR-A and TLR4. Finally, a pathway that engages CD36 and TLR2 and TLR4 activates nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase, boosting reactive oxygen species production and triggering cell apoptosis. However, macrophage apoptosis is an atheroprotective process that is inhibited by oxLDL. B to D, Apoptotic cells are cleared by phagocytes in a process called efferocytosis. During advanced atherosclerosis, efferocytosis is impaired, leading to apoptotic cell accumulation, secondary necrosis, and formation and expansion of the NC. Cell efferocytosis involves phagocyte attraction by find-me signals released by the apoptotic cell (B), recognition of the apoptotic cell by eat-me molecules (C), and phagocyte receptors that mediate cell engulfment (D).
Extracellular Proteases, Reactive Oxygen Species, and Lesional Angiogenesis Weaken Atherosclerotic Lesions

Mechanical weakening of the fibrous cap is an important step that precedes plaque rupture and consequent secondary thrombotic events. Several factors influence these structural changes in which protease-driven degradation of ECM is a dominant process37 (Figure 2). Lesional expression of MMP family members, such as MMP-8, MMP-9, or MMP-12, correlates with plaque instability and risk for ACS.38,39 However, results from studies using MMP transgenic or knockout mice are not conclusive with regard to their beneficial or detrimental effects on plaque stability.37 For instance, the deficiency of MMP-3 was associated with increased plaque instability, whereas the absence of MMP-12 led to more stable plaques.40 Overexpression of an active form of MMP-9 by macrophages induced a vulnerable plaque phenotype with higher incidence of plaque disruption, probably by promoting fibrous cap weakening.41 Other MMPs such as MMP-8 and MMP-13 have also been suggested to control plaque macrophage and collagen content.42,43 In addition to macrophages, neutrophils are also an important source of MMP-8 and MMP-9 and may contribute to plaque instability. In fact, lesional MMP-8 and MMP-9 levels44 were mainly associated with increased intraplaque neutrophil infiltration and were inversely correlated with plaque stability traits. In contrast, the beneficial function attributed to MMP-2 and MMP-3 may be based on their importance in regulating SMC migration and fibrous cap formation.45,46 Altogether, the divergent effect of MMP family members may be explained by their cellular origin and the various functions they control.

Reaction of O2 with an uncoupled electron gives rise to superoxide anion in a process catalyzed by NADPH, xanthine oxidases, or endothelial nitric oxide synthase.47 Superoxide anions and their main producers, the NADPH and xanthine oxidases, are abundantly present and active in human atherosclerotic plaques.48 NADPH oxidase is an enzymatic complex formed by membrane-bound cytochrome b558 (gp91phox/Nox2 and p22phox) and the cytosolic members p67phox, p47phox, p40phox, and Rac.47 The expression of Nox2 and its homolog Nox449 is significantly increased in human coronary artery disease (CAD) and correlates with signs of plaque instability. However, experimental studies investigating the implication of NADPH oxidase members in plaque destabilization...
are unclear. SMC-specific expression of the p22phox subunit displays accelerated atherosclerosis, increased plaque neovascularization, and expansive remodeling\(^4\) (Figure 2). Furthermore, patients with angina pectoris subjected to intravascular ultrasound imaging showed a positive correlation between p22phox and coronary arterial expansive remodeling.\(^5\) Likewise, genetic depletion or pharmacological inhibition of the gp9phox homolog Nox1 inhibits diabetes mellitus–accelerated atherosclerosis.\(^6\) However, decreased reactive oxygen species production by genetic depletion of p47phox resulted in conflicting data.\(^7,8\) Although p47phox absence reduced superoxide levels, other sources of superoxide production, such as vascular lipoxygenases, may compensate for this reduction and explain the observed results. Because most experimental studies focus on early atherosclerosis, additional work is necessary to clarify the contribution of NADPH oxidases at advanced stages of atherosclerosis and during plaque destabilization.

Neoangiogenesis is a signature process of advanced atherosclerotic plaques and may significantly contribute to weakening of the plaque. Newly formed plaque vasculature originating from vasa vasorum in the adventitia sustains the influx of inflammatory leukocytes and the accumulation of cholesterol and erythrocyte-derived phospholipids, thus contributing to plaque instability. Neovascularization and intraplaque hemorrhage have recently been established as independent predictors of future adverse cardiovascular outcomes.\(^9\) Intraplaque vessels are characterized by an immature structure with a reduced number of mural pericytes and SMCs covering the ECs, hence contributing to increased leakiness and rupture susceptibility.\(^10\) Moreover, intravital imaging of advanced atherosclerotic plaques in the apolipoprotein E–deficient (Apoe\(^{-/-}\)) mouse revealed that the intimal microvasculature is a major contributor to leukocyte plaque recruitment\(^11\) (Figure 2). Degranulation of neutrophils and mast cells after activation significantly increases endothelial permeability, which may promote intraplaque leukocyte infiltration.\(^10,12\) Moreover, mast cell activation correlates with a higher incidence of intraplaque hemorrhage, an important destabilizing factor.\(^11\) Reduced oxygen supply in growing lesions and augmented oxygen demand through continuous influx of leukocytes generate a hypoxic environment likely responsible for intraplaque neangiogenesis. Hypoxia has been detected in macrophages within human atherosclerotic plaques and correlates with angiogenesis and the hypoxia-inducible transcription factor.\(^11\) Interestingly, a major target of hypoxia-inducible transcription factor, that is, vascular endothelial growth factor, enhances plaque instability through increasing leukocyte influx without affecting neovascularization.\(^13\) Other angiogenic factors such as Ets2 or the Notch ligand β-like 4 have recently been implicated in atherosclerosis progression and destabilization via induction of neovessel formation and leukocyte infiltration.\(^14,15\) Because neangiogenesis is observed in advanced stages of plaque progression, future studies are needed to clearly determine to what degree hypoxia initiates and drives lesional angiogenesis and which additional factors may be involved herein.

**Animal Models of Plaque Destabilization**

In recent decades, the study of the pathophysiology of plaque destabilization and rupture has been hampered by the absence of proper animal models that could mimic processes occurring during advanced phases of human atherosclerosis. Genetically modified hyperlipidemic mice have extensively been used to understand the mechanisms underlying the initiation and progression of atherosclerosis.\(^16\) With a high cholesterol diet, these mice experience development of atheromata and exhibit features similar to those of early human atherosclerosis and some features of advanced plaques. The development of foam cell–rich lesions, followed by the formation of a NC, SMC migration to the fibrous cap, and accumulation of fibrotic tissue, can be studied with these mouse models. Although these events are similar to those occurring in humans, others such as plaque rupture and superimposed thrombosis are rarely observed in murine atherosclerosis and, when observed, are different.\(^16\) Hence, various approaches to generate plaque destabilization based on surgical or genetic manipulation have been proposed and are described here and in Table 1.

The alteration of blood flow by the use of perivascular devices accelerates atherosclerosis and plaque vulnerability. The implantation of a silastic collar around both common carotid arteries accelerates the progression of the disease.\(^16\) In this setup, Apoe\(^{-/-}\) mice displayed more heterogenic and acellular lesions with defined fibrous caps and enlarged NCs as compared with low-density lipoprotein receptor–null (Ldlr\(^{-/-}\)) mice. Increased inflammatory cell recruitment and endothelial activation were observed, whereas intraplaque hemorrhage was rarely present after a longer duration of collar placement. This model was extended by the local overexpression of the proapoptotic protein p53, resulting in thinner fibrous caps due to increased SMC apoptosis and enlarged NCs. In addition, plaque disruption with partial extrusion of the NC and intraplaque hemorrhages was observed.\(^16\) Using a similar approach, overexpression of MMP-9 after collar implantation enhanced plaque vulnerability by promoting fibrous cap thinning and NC expansion. The incidence of intraplaque hemorrhage was also dramatically increased in this model.\(^16\) A more recent study proposed a model combining perivascular collar placement with a mouse model of genetically imposed hypercoagulability.\(^16\) Hypercoagulability induces vulnerable plaques characterized by increased lesion sizes, outward remodeling, larger NCs, thinner fibrous caps, enhanced neointimal apoptosis, and increased macrophage and neutrophil infiltration. Unlike in other mouse models of plaque vulnerability, events such as plaque disruption with superimposed thrombosis, repeated plaque disruptions, and intramural thrombi were evidenced.\(^16\) A variant of the perivascular collar model was developed where, consequent to ligation of the common carotid artery, a polyethylene cuff was placed close to the ligation site,\(^16\) thereby generating advanced lesions with a SMC-rich fibrous cap and a high prevalence of intraplaque hemorrhages. Moreover, disruptions of fibrous cap integrity with superimposed mural or occlusive thrombosis were observed.\(^16\) Another sophisticated method was developed based on the implantation of a shear modifier device that generates 3 regions of low, high, and oscillatory shear stress.\(^16\) Advanced lesions developed with high macrophage infiltration, increased lipid deposition, reduced SMC and collagen content in the fibrous cap, and increased expression of MMPs.\(^16\) Interestingly, intraplaque hemorrhage was exclusively observed in the low
shear stress region under normotensive or angiotensin-II–mediated hypertensive conditions. A combination of blood flow shear modification and hypertension induced by partial ligation of the renal artery had similar effects. Recently, a model of vulnerable plaque formation based on the induction of a tandem stenosis was proposed. In this model, a high incidence of plaque disruption and intraplaque hemorrhage was observed. As an alternative to surgical approaches, transgenic strategies are used to induce plaque destabilization. The expression of the human diphtheria toxin receptor under the control of the SM22α promoter permitted specific induction of SMC apoptosis after toxin administration. Consequently, marked fibrous cap thinning, reduced matrix deposition, increased apoptotic cell accumulation, and plaque inflammation were observed. However, no events of plaque disruption, intraplaque hemorrhage, or luminal thrombosis were reported.

Because large animal models have similar cardiovascular anatomy and atherosclerosis disease characteristics as in human models, they are important in studying the mechanisms of plaque destabilization as well as translational approaches.

<p>| Table 1. Mouse Models of Induced Plaques With Signs of Vulnerability |</p>
<table>
<thead>
<tr>
<th>Model</th>
<th>Animal Genetic Model</th>
<th>FC</th>
<th>NC</th>
<th>Composition</th>
<th>Endothelium</th>
<th>Plaque Disruption</th>
<th>Intraplaque Hemorrhage</th>
<th>Neovessels</th>
<th>Thrombosis</th>
<th>Intramural Thrombus</th>
<th>Outward Remodeling</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collar Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑ ↑↑</td>
<td>↑↓</td>
<td>↑↓</td>
<td>Macrophage; ↑lipids; ↑cholesterol crystals</td>
<td>↑ICAM-1, VCAM-1; ↓eNOS</td>
<td>− (6 wk)</td>
<td>NA</td>
<td>−</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>von der Thüsen et al&lt;sup&gt;65&lt;/sup&gt;</td>
</tr>
<tr>
<td>Collar: Ad-p53 Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑apoptosis; ↓proliferation</td>
<td>No endothelial erosion</td>
<td>Ad-p53: 18.7%</td>
<td>Ad-p53: 6.2%</td>
<td>Ad-p53+PHE: 35%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Collar Ad-MMP9 Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑apoptosis; ↓collagen; =SMC</td>
<td>NA</td>
<td>53.3%</td>
<td>+</td>
<td>−</td>
<td>13.3%</td>
<td>+</td>
<td>de Nooijer et al&lt;sup&gt;67&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Collar genetically induced hypercoagulability Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑neutrophil; ↑apoptosis; ↓collagen; =SMC</td>
<td>NA</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+ (mural and occlusive)</td>
<td>+</td>
<td>+</td>
<td>Borissoff et al&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cuff Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑</td>
<td>NA</td>
<td>↑</td>
<td>Macrophage; ↑neutrophil; ↑apoptosis; ↓collagen; =MMPs</td>
<td>NA</td>
<td>2 d: 29.4%</td>
<td>2 d: 47.1%</td>
<td>NA</td>
<td>11.8%</td>
<td>(mural); 17.6%</td>
<td>(occlusive)</td>
<td>NA</td>
</tr>
<tr>
<td>Cast Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑lipids; ↓collagen; =MMPs; =SMC</td>
<td>No endothelial erosion</td>
<td>−</td>
<td>28.6% (spontaneous); 75% (AngII)</td>
<td>NA</td>
<td>−</td>
<td>+</td>
<td>NA</td>
<td>Cheng et al&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
<tr>
<td>Partial ligation of carotid and renal arteries Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑apoptosis; ↓collagen; =MMPs; =SMC</td>
<td>NA</td>
<td>+ (buried caps; 80%)</td>
<td>80%</td>
<td>NA</td>
<td>50%</td>
<td>−</td>
<td>NA</td>
<td>Jin et al&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tandem stenosis Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑leukocytes; =lymphocytes; =SMC; =collagen; ↓Lipids</td>
<td>NA</td>
<td>32%</td>
<td>50.6%</td>
<td>Adventitial and intraplaque</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Peter et al&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td>Genetically SMC-specific apoptosis induction SM22α-hDTR Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>Macrophage; ↑apoptosis; ↓collagen; =SMC</td>
<td>No endothelial erosion</td>
<td>−</td>
<td>−</td>
<td>NA</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Clarke et al&lt;sup&gt;73&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

↑ indicates increase; ↓, decrease; =, no change; +, detected; −, not detected; AngII, angiotensin II; eNOS, endothelial nitric oxide synthase; FC, fibrous cap; hDTR, human diphtheria toxin receptor; MMP, matrix metalloproteinase; NA, not analyzed; NC, necrotic core; PHE, phenylephrine; and SMC, smooth muscle cell.
Because of their sensitivity to develop foamy-like atherosclerotic plaques with a cholesterol-rich diet, rabbit models are commonly used. Some rabbit strains, such as the Watanabe heritable hyperlipidemic rabbits, experience familiar hypercholesterolemia, develop advanced atherosclerotic plaques, and experience spontaneous myocardial infarction. However, rabbits fed a hypercholesterolemic diet and subjected to aortic balloon denudation injury generate advanced atherosclerotic plaques. These lesions contain fibrous-rich and lipid-rich components similar to those in humans and undergo plaque disruption and thrombosis. Porcine models of atherosclerosis offer similar advantages. Diabetic hypercholesterolemic swines develop complex lesions with thin fibrous caps, enlarged NC, calcification nodules, and intraplaque hemorrhages. Vulnerable plaque formation can also be accelerated by balloon injury in familiar hypercholesterolemic pigs. Rabbit and porcine models are, therefore, suitable for the development and assessment of novel imaging techniques, stabilizing therapies, and cardiovascular surgical techniques. However, in contrast to mouse models, the limited possibility of genetic modification hampers their use in the understanding of plaque destabilization pathophysiology.

Identification of Plaque Vulnerability

Early detection of vulnerable plaques in asymptomatic patients before leading to associated ACS is a major goal in cardiovascular imaging. Accepted architectural and compositional characteristics that define a vulnerable plaque are mainly inferred from histological analysis of occluded arteries derived from retrospective autopsy studies. Based on this knowledge, vast efforts have been made to detect features of vulnerable plaques in vivo by developing novel techniques of intravascular imaging. Virtual histology intravascular ultrasound, optical coherence tomographic, and near-infrared spectroscopic techniques have been developed to detect unstable traits both in culprit and nonculprit lesions in patients with ACS. Nevertheless, the predictive value of these traits to determine the risk of rupture is unclear, and prospective studies are needed. In this context, the prospective and multicenter Predictors of Response to CRT (PROSPECT) study demonstrated that most of the nonculprit lesions associated with clinical events were characterized by large plaque burden (>70%), small luminal area (<4 mm²), and presence of thin-cap fibroatheroma as assessed by virtual histology intravascular ultrasound. However, the predictive value of thin-cap fibroatheroma for subsequent coronary events was low. Hence, additional features of vulnerability should be considered when identifying high-risk plaques.

Molecular imaging has emerged as a promising complementary tool to identify subclinical lesions by detecting key biological processes of plaque vulnerability. Taking advantage of the phagocytic activity of lesional macrophages, plaque inflammation can be visualized after systemic delivery of radio-labeled or magnetic nanoparticles. In studies using mouse and rabbit models of atherosclerosis, the administration of iodinated or ultrasmall superparamagnetic iron oxide nanoparticles enables detection of macrophage-rich plaques by MRI or X-ray CT. Other multimodality techniques, such as PET-CT or PET/MRI, have been developed to detect lesional macrophages in mice. Macrophage content can also be detected by the uptake and metabolization of the substrate 18F-fluorodeoxiglucose. Successful imaging of high-risk carotid, aortic, or coronary lesions and evaluation of stabilizing therapies in patients with ACS by 18F-fluorodeoxiglucose PET imaging are promising for detection of vulnerable plaques. Moreover, molecular imaging of other destabilizing processes, such as neoimal cell death or protease activity, have been evaluated in preclinical and pilot clinical studies. Similarly, MRI-based quantification of oxidative stress through oxidative-specific epitopes and myeloperoxidase activity or intraplaque neovascularization positively correlates with plaque burden and instability in animal studies. Encouraging results arising from these preclinical studies require further clinical evaluation to assess the feasibility of molecular imaging in early detection of human vulnerable plaques.

Massive sequencing of common genetic polymorphisms in genome-wide association studies provides new insights into plaque destabilization pathophysiology by identifying new potential biomarkers of cardiovascular risk. Coronary Artery Disease Genome-Wide Replication and Meta Analysis (CARDIoGRAM), an international consortium, presents the largest genome-wide association studies of CAD. Their studies identified novel genetic risk variants and corroborated others previously identified (ie, 9p21 locus, PCSK9, or SORT1). Moreover, network analysis revealed 4 pathways linked to lipid metabolism and inflammation that mapped 85% of the identified genes with potential implication in CAD. Interestingly, some of these genetic variants were not associated with previously known CAD risk factors. However, despite the importance of genome-wide association studies in identifying novel potential biomarkers of CAD, their predictive value is still unclear. The analysis of the predictive ability of genetic risk scores for CAD using several of these genetic variants showed no improvement of prediction compared with prediction based on traditional risk factors. Hence, the use of a combination of imaging and biomarkers (genetic or plasma proteins) may represent an improvement in early prediction of future cardiovascular events.

Novel Treatment Strategies

Randomized clinical trials have demonstrated the beneficial effect of statins, antiplatelet drugs, or antihypertensive compounds in diminishing ACS prevalence and, accordingly, current clinical guidelines recommend their administration in patients with atherosclerotic vascular disease. The plaque-stabilizing properties of statins, antiplatelet drugs, and antihypertensive drugs are likely due to their ability to reduce thrombus formation, inflammation, and EC dysfunction. Of note, the importance of lipid-lowering effects on cardiovascular risk reduction remains controversial. Although some preclinical studies suggest the importance of lipid-independent pleiotropic mechanisms, other animal and human studies support the relevance of cholesterol reduction. However, even in patients treated with statins, a considerable residual burden of cardiovascular risk remains. Hence, increasing knowledge of processes underlying vulnerable plaque formation has emerged in novel therapies targeting specific molecules involved in plaque destabilization (Table 2).
Given the importance of inflammation during plaque destabilization, tremendous efforts are being made to develop novel immunomodulatory strategies. Results from preclinical studies show encouraging results when targeting or instructing cytokines. Particularly, the use of blocking antibodies against inflammatory interferon-γ or administration of recombinant anti-inflammatory interleukin (IL)-13 induces plaque stability. However, the beneficial effect of cytokine-targeting therapies in patients with ACS is not clear. IL-12/23-targeted or tumor necrosis factor-α-targeted anti-inflammatory therapies used in psoriasis or rheumatoid arthritis treatment have not shown satisfactory results in reducing secondary cardiovascular events. However, a recent clinical trial showed a decrease in C-reactive protein, IL-6, and fibrinogen levels without affecting circulating lipids after IL-1β neutralization. However, recent evidence from animal studies suggesting a stabilizing function of IL-1 signaling should be taken into account when IL-1β inhibition therapies are administered in patients with established vulnerable plaques.

The disruption of chemokine receptor–ligand interactions to reduce leukocyte recruitment is a promising alternative in the treatment of plaque vulnerability. The administration of a specific CCL5 antagonist reduces mouse plaque progression and enhances plaque stability. Similarly, the use of Maraviroc, the Food and Drug Administration–approved CCR5 antagonist for treatment of HIV infection, reduces plaque instability in mouse studies. The proven efficacy and safety of this compound may facilitate its approval for cardiovascular disease treatment in humans. Interestingly, CCR2 inhibition through a systemic delivery of lipid nanoparticle carrying siRNA inhibits inflammatory monocyte recruitment, progression of atherosclerosis, and myocardial infarction. Likewise, pharmacological blockade of CCR2 in patients with ACS showed a significant reduction in circulating C-reactive protein levels and may have beneficial effects on reducing cardiovascular events.

Alternatively, several studies aimed at ameliorating atherosclerosis by induction of immune tolerance against specific antigens. In the context of plaque vulnerability, subcutaneous or oral administration of apolipoprotein B100 peptides, heat shock protein 60, or oxidized low-density lipoprotein reduces plaque destabilization and induces plaque regression in mice. Other strategies are based on an intravenous injection of dendritic cells pulsed with apolipoprotein B100 peptides. As a result, an immunosuppressive effect by tolerogenic dendritic cells is initiated, thereby limiting atherosclerosis burden.

The inhibition of extracellular protease activity or specific targeting of lesional neoangiogenesis seems to be a potential strategy to improve plaque stability. The destabilizing activity of MMPs suggests that their inhibition could have beneficial effects in plaque stability. However, evidence from animal studies supports the idea of functional diversity of various MMP family members. Unspecific blockade of MMP activity results in variable effects in preclinical studies or negative results in patients treated with the MMP inhibitor doxycycline. Nevertheless, specific pharmacological inhibition of MMP-12 and MMP-13 reduces plaque destabilization in established mouse atherosclerosis and represents a more plausible strategy to vulnerable plaque treatment. Hence, clinical trials are needed to assess the potential efficacy of these inhibitors. The administration of antiangiogenic compounds to reduce intimal neoangiogenesis may improve plaque stability. Antiangiogenic agents based on vascular endothelial growth factor inhibition used in tumor or age-related macular degeneration treatment may have potential benefits in plaque stabilization. However, systemic administration of these compounds has been associated with increased risk of thromboembolic events. Local delivery approaches are required for safe use of these drugs. In this regard, efficient stenting of vulnerable plaques opens the possibility for the use of novel drug-eluting stents to treat high-risk plaques through local drug delivery. Hence, local delivery of antiangiogenic agents using stent devices may result in potential effective stabilizing therapies.

Current knowledge about lipid-driven lesional cell death and defective clearance of apoptotic cells has led to various therapeutic approaches to enhance plaque stability. For instance, partial inhibition of acyl-CoA:cholesterol acyltransferase (enzyme responsible of free cholesterol sterrification) reduces the macrophage content and apoptosis in animals. Because complete ablation of acyl-CoA:cholesterol acyltransferase activity results in accelerated atherosclerosis, the variable degree of acyl-CoA:cholesterol acyltransferase inhibition may explain why its application in clinical trials has failed. ER stress alleviation strategies may also be important to reduce macrophage apoptosis and consequent plaque instability. The administration of chemical chaperones reduces ER stress by increasing its folding capacity, and their use in atherosclerosis-prone mice protects against macrophage apoptosis. Interestingly, macrophage removal through autophagy is stimulated under low ER stress conditions. However, during advanced atherosclerosis, prolonged ER stress inhibits autophagy and promotes cell apoptosis and plaque necrosis. Pharmacological induction of macrophage autophagy through mammalian target of rapamycin inhibition (Everolimus) reduces murine atherosclerosis progression and plaque instability. Similarly, implantation of Everolimus-eluting stents in atherosclerotic arteries of high-fat diet–fed rabbits reduces the macrophage content by induction of autophagy. Second-generation everolimus-eluting stents led to increased lesion area but reduced NC 1 year after implantation and induced plaque regression at 2-year follow-up. Pharmacological correction of impaired efferocytosis and stimulation of inflammation resolution during advanced atherosclerosis may emerge as a novel therapeutic strategy. In this context, several nuclear receptors, such as peroxisome proliferator-activated receptor and liver X receptor, are linked to macrophage polarization toward an M2 phenotype and, consequently, enhanced efferocytosis capacity. The activation of nuclear receptors through specific agonists is effective in experimental inflammatory models, including atherosclerosis. However, the association with several adverse side effects limited their use. In contrast, R211945, the novel liver X receptor agonist, led to plaque regression in a rabbit model of atherosclerosis without secondary adverse side effects. However, the oral administration of sphingosine-1-phosphate mimetic, FTY720, dampens NC formation and plaque size. This effect could be attributed to its anti-inflammatory and
Table 2. Novel Therapeutic Strategies in Plaque Stabilization in Animal and Human Studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>Disease</th>
<th>Compound</th>
<th>Disease</th>
<th>Animal</th>
<th>Clinical Trial</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ receptor mutant</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (12-wk and 16-wk HFD)</td>
<td>↓Lesion; ↑plaque stability</td>
<td>—</td>
<td>Koga et al&lt;sup&gt;97&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>IL-13 recombinant protein</td>
<td>Atherosclerosis</td>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (16-wk HFD)</td>
<td>↑Plaque stability, M2 macrophage polarization</td>
<td>—</td>
<td>Cardillo-Reis et al&lt;sup&gt;98&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Anti-IL-12/23 (ustekinumab/ briakinumab)</td>
<td>Chronic psoriasis</td>
<td>—</td>
<td>—</td>
<td>Various</td>
<td>No MACE reduction</td>
<td>Ryan et al&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-TNF-α (adalimumab/ etanercept/infliximab)</td>
<td>Chronic psoriasis</td>
<td>—</td>
<td>—</td>
<td>Various</td>
<td>No MACE reduction</td>
<td>Ryan et al&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anti-IL1β (canakinumab)</td>
<td>CAD</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (46-wk CD)</td>
<td>↓Lesion; ↑plaque stability, ↑Treg response</td>
<td>—</td>
<td>Ridker et al&lt;sup&gt;100&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CCL5 antagonist ([44AANA47]-RANTES)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (22-wk HFD)</td>
<td>↓Lesion; ↑plaque stability</td>
<td>—</td>
<td>Braunersreuther et al&lt;sup&gt;101&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CCR2 inhibition (siRNA; MLN1202) and MI</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (16-wk HFD)</td>
<td>↓Lesion; ↑macrophage content</td>
<td>MLN1202 Study Group</td>
<td>↓C-reactive protein</td>
<td>Leuschner et al; Gilbert et al&lt;sup&gt;102,103,104&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCR5 antagonist (maraviroc)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (39-wk CD)</td>
<td>↓Lesion; ↑macrophage content</td>
<td>—</td>
<td>Cipriani et al&lt;sup&gt;105&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ApoB-derived peptides</td>
<td>Atherosclerosis</td>
<td>ApoB&lt;sup&gt;−/−&lt;/sup&gt;Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (24-wk HFD+3 wk CD)</td>
<td>↓Lesion; ↓macrophage content, ↑Treg response</td>
<td>GLACIER Study (phase II)</td>
<td>No results published</td>
<td>Schioju et al&lt;sup&gt;106&lt;/sup&gt;</td>
</tr>
<tr>
<td>ApoB-HSP60-derived peptides</td>
<td>Atherosclerosis</td>
<td>ApoB&lt;sup&gt;−/−&lt;/sup&gt;Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (26-wk CD)</td>
<td>↓Lesion; ↑plaque regression; ↑macrophage content</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anti-oxLDL</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (24-wk HFD+3 wk CD)</td>
<td>↓Lesion; ↑plaque regression; ↑macrophage content</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Apo-B100-loaded dendritic cells</td>
<td>Atherosclerosis</td>
<td>huB&lt;sup&gt;−/−&lt;/sup&gt;Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (10-wk HFD)</td>
<td>↓Lesion; ↓plaque inflammation</td>
<td>—</td>
<td>Hermansson et al&lt;sup&gt;107&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>CAD</td>
<td>—</td>
<td>—</td>
<td>Various</td>
<td>No MACE reduction</td>
<td>Newby&lt;sup&gt;108&lt;/sup&gt;</td>
</tr>
<tr>
<td>MMP-12 inhibitor (RXP470.1)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (8-wk HFD)</td>
<td>↓Lesion; ↑plaquelstability</td>
<td>—</td>
<td>Johnson et al&lt;sup&gt;109&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MMP-13 inhibitor (MMP13i-A)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (10-wk HFD/inh or 10-wk HFD+10-wk inh)</td>
<td>↓Lesion; ↑plaquelstability</td>
<td>—</td>
<td>Quillard et al&lt;sup&gt;110&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Anti-VEGF (bevacizumab)</td>
<td>Tumor/age-related macular degeneration</td>
<td>—</td>
<td>—</td>
<td>Various</td>
<td>↑Thromboembolic events</td>
<td>Ferroni et al&lt;sup&gt;111&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cell death and clearance</td>
<td>ACAT inhibitor (F1394)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (14-wk HFD+14-wk HFD/inh)</td>
<td>↓Lesion; ↑macrophage content</td>
<td>—</td>
<td>Rong et al&lt;sup&gt;112&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACAT inhibitor (pactimibe)</td>
<td>CAD</td>
<td>—</td>
<td>—</td>
<td>ACTIVATE study</td>
<td>=Atheroma volume; ↑MACE</td>
<td>Nissen et al&lt;sup&gt;113&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chemical chaperone (4-PBA)</td>
<td>Atherosclerosis</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt; (8-wk HFD)</td>
<td>↓Lesion; ↓macrophage apoptosis</td>
<td>—</td>
<td>Erbay et al&lt;sup&gt;114&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Everolimus</td>
<td>Atherosclerosis</td>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (14-wk HFD)</td>
<td>↓Lesion; ↑plaquelstability</td>
<td>—</td>
<td>Mueller et al&lt;sup&gt;115&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Everolimus</td>
<td>Atherosclerosis</td>
<td>New Zealand White rabbits (40-wk HFD)</td>
<td>↓Macrophage content</td>
<td>—</td>
<td>Verheyen et al&lt;sup&gt;116&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PPAR-γ agonist (rosiglitazone)</td>
<td>CAD</td>
<td>—</td>
<td>—</td>
<td>Various</td>
<td>↑MI and death from cardiovascular cause</td>
<td>Nissen and Wolski&lt;sup&gt;117&lt;/sup&gt;</td>
</tr>
<tr>
<td>LXR agonist (r211945)</td>
<td>Atherosclerosis</td>
<td>New Zealand White rabbits (21-wk HFD)</td>
<td>↑Plaque regression</td>
<td>—</td>
<td>Vucic et al&lt;sup&gt;118&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
efferocytosis-stimulating properties. Despite the use of oral FY720 in the treatment of multiple sclerosis, no clinical studies have evaluated its possible effect in patients with ACS. Moreover, dietary administration of omega-3 fatty acids has been shown to reverse defective efferocytosis in murine atherosclerotic lesions.\(^{32}\) The meta-analyses of the implications of omega-3 fatty acids in reducing cardiovascular outcome, however, gave inconclusive\(^ {122}\) or negative\(^ {123}\) results. An alternative strategy to repair the engulfment synapse is based on the improved opsonization of phosphatidylserine on apoptotic cells.\(^ {124}\) Herein, a fusion protein of Annexin V (which binds to phosphatidylserine) and a Arg-Gly-Asp motif (which binds to vitronectin receptor on efferocytes) enhances phagocytic resolution of inflammation\(^ {125}\) Finally, a nanoparticle-based delivery approach using a fragment of the resolving protein Annexin A1 reduced inflammation and provided functional improvement in a mouse model of hindlimb ischemia.\(^ {125}\)

### Conclusions

Vulnerable plaques are defined as atherosclerotic lesions susceptible to undergoing rapid progression and giving rise to superimposed thrombosis, ultimately leading to acute cardiovascular events. The mechanisms that control plaque destabilization remain largely unknown. Despite the emergence of novel animal models, we still do not fully understand the complex molecular orchestra leading to the accumulation of necrotic cells, intraplaque neoangiogenesis, or alterations in ECM composition. The identification of molecular targets using genetic approaches and mechanistic studies in mouse models of plaque destabilization may allow for the development of lead compounds to be first tested in small animal models. After optimization and validation in large animal models, such compounds may potentially access clinical studies. Finally, in combination with molecular imaging techniques and novel biomarkers, individualized therapies may be adopted with higher beneficial effects in reducing the risk of ACS.

### Sources of Funding

The research was supported by the De Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) (VIDI project 91712303), the Deutsche Forschungsgemeinschaft (DFG) (SO876/3-1, SO876/6-1, FOR809, SFB914-B08), the LMU excellent program, and the Else Kröner Fresenius Stiftung.

### Disclosures

None.

### References


to an oxidized low-density lipoprotein epitope induce rapid regression of atherosclerosis in apoE-(-/-)/low-density lipoprotein receptor(+/-) mice. J Am Coll Cardiol. 2007;50:2313–2318.


Atherosclerotic Plaque Destabilization: Mechanisms, Models, and Therapeutic Strategies
Carlos Silvestre-Roig, Menno P. de Winther, Christian Weber, Mat J. Daemen, Esther Lutgens and Oliver Soehnlein

doi: 10.1161/CIRCRESAHA.114.302355

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/114/1/214

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/