Could improving the design of preclinical murine atherosclerosis studies help increase the success rate of cardiovascular clinical trials? In this Viewpoint, the authors advocate for a change from prevention to intervention study designs and rigorous lesion analyses that they argue will enhance the translational potential of murine atherosclerosis studies.

Clinical management of patients with coronary artery disease (CAD) has made great progress by reducing risk factors like low-density lipoprotein cholesterol level, hypertension, and diabetes mellitus. However, late-stage events associated with coronary atherosclerosis including myocardial infarction still account for $\pm 16\%$ of worldwide mortality and are forecasted to increase in prevalence. $^{1}$ These events arise from 3 main processes: plaque rupture, plaque erosion, and shedding of calcific nodules. Plaque rupture accounts for the majority of CAD and typically occurs in vulnerable atherosclerotic lesions termed thin-capped fibroatheromas, $^{2}$ characterized by thin fibrous caps (<65 $\mu$m) with high numbers of CD68$^{+}$ macrophages relative to Acta2$^{+}$ smooth muscle cells (SMC), large lipid-rich necrotic cores, and often evidence of intraplaque hemorrhage. Much of the difficulty in therapeutically targeting atherosclerosis can be attributed to (1) the nearly ubiquitous prevalence of atherosclerosis development; (2) the slow, clinically-silent progression but acute and often catastrophic presentation of symptoms; and (3) most importantly, the difficulty in identifying patients at high risk for plaque rupture or erosion and subsequent cardiovascular events. Therefore, clinical trials seeking to investigate novel CAD therapies are forced to enroll thousands of patients who already have manifestations of advanced CAD and spend hundreds of millions of dollars to conduct adequately powered phase III clinical trials to rigorously test therapeutic efficacy. And yet, cardiovascular disease trials have one of the lowest success rates of dollars to conduct adequately powered phase III clinical trials to rigorously test therapeutic efficacy. Therefore, clinical trials seeking to investigate novel CAD therapies are forced to enroll thousands of patients who already have manifestations of advanced CAD and spend hundreds of millions of dollars to conduct adequately powered phase III clinical trials to rigorously test therapeutic efficacy. And yet, cardiovascular disease trials have one of the lowest success rates of events. To overcome this challenge, several groups have attempted to induce plaque rupture in mice. These models use extraordinary measures like mechanical disruption, $^{6}$ vascular casting, $^{7}$ and adenoviral delivery of p53 $^{8}$ to induce rupture at a rate that allows for the use of a reasonable number of mice but are unlikely to reflect mechanisms that cause spontaneous plaque rupture in humans. Importantly, despite the lack of a reliable rupture model in mice, many of the cellular processes that are thought to control the transition from stable fibroatheroma to thin-capped fibroatheromas in humans (eg, the accumulation of macrophages and T cells, the production of matrix-remodeling proteinases, the investment of contractile protein-expressing SMC in the fibrous cap, changes in the lipid and collagen content, crosslinking of extracellular matrix [ECM] components) do occur in murine atherosclerosis.

However, many preclinical studies limit their analysis to plaque burden as determined by the cross sectional area of isolated lesions in the aortic root or brachiocephalic artery and/or en face analysis of lipid deposition, which is unable to distinguish fatty streaks from more advanced lesions. Furthermore, it has become increasingly clear that lesion size is not a suitable surrogate for lesion stability, which is more accurately the integral of the stabilizing and destabilizing processes at work within the lesion. Therefore, novel therapeutics should seek to modulate lesion stability by either inhibiting processes involved in destabilization (eg, lipid lowering with statins or PCSK9 inhibitors) or inducing those that augment stability (eg, enhancing the production of protective extracellular...
matrix components within the fibrous cap). Although the extracellular matrix–producing cells within the fibrous cap have been assumed to be primarily SMC derived, only recently have rigorous lineage-tracing studies from our laboratory and others provided compelling evidence that SMC play a critical role in lesion pathogenesis. However, contrary to dogma, they may not always perform beneficial roles. By using SMC lineage-tracing Myh11 ER2Cre eYFP ApoE−/− mice fed 18 weeks of Western diet to induce advanced atherosclerotic lesions, we have shown that (1) >80% of SMC-derived cells lack detectable Acta2 expression and (2) >30% of Lgals3+ cells are SMC derived in both mouse and human lesions. By combining lineage tracing with simultaneous SMC-specific gene knockout of pluripotency factors Klf4 or Oct4, we have shown that SMC can play a critical role in the pathogenesis of late-stage lesions, which can be either atheroprotective or atheropromoting depending on the nature of their phenotypic transitions. For example, Klf4-dependent transitions, including formation of SMC-derived macrophage-marker foam cells, exacerbated lesion pathogenesis, whereas Oct4-dependent transitions were atheroprotective, being required for migration and stable investment of SMC into the fibrous cap. Taken together, these results highlight the critical importance of identifying factors and mechanisms that promote plaque stabilizing atheroprotective changes within SMC and other major cell types within lesions and therapeutic approaches that can induce such changes. The findings also highlight the importance of lineage tracing in atherosclerosis. Because of significant plasticity in multiple lesion cell types, there is substantial ambiguity in cell identification when using traditional markers (eg, Acta2, CD68, and CD31), which can only be overcome by the use of rigorous lineage-tracing techniques.

In addition to studying more relevant pathological parameters, scientists should consider implementing more relevant study designs. Currently, the overwhelming majority of atherosclerosis studies implement models of prevention. Namely, the researcher deletes gene x or provides drug y before—or in the early stages of—lesion formation, and therefore, the therapeutic agent or genetic manipulation is given to young, healthy mice and is present throughout lesion development (Figure). With these prevention models, we have learned a great deal about the steps of atherosclerosis development—determining the key cellular players and identifying thousands of genes that can alter lesion accumulation—but unfortunately, this has translated to few novel therapies. Therefore, although prevention models may be suitable for assessing therapeutic feasibility, we advocate for additional interventional studies before initiation of clinical trials. Here, mice are treated with the therapy or gene knockout only after they have established advanced atherosclerosis. By providing the therapy at this stage and then assessing for changes in the indices of lesion stability as described above, we obtain a better prediction of how the intervention will impact the processes regulating late-stage lesion vulnerability and, hopefully, gain better insight into its impact on advanced human lesions. Clearly, different processes are at work during lesion development versus late-stage disease. For example, elegant studies from Filip Swirski’s group have shown that monocyte recruitment drives early lesion macrophage accumulation but that as the lesion matures the accumulation becomes a function of local macrophage proliferation. These fundamental biological differences between disease stages may indeed provide one possible explanation for the poor translation of prevention studies to patients with advanced disease. It should be noted that the idea of implementing interventional models to the study of atherosclerosis is not novel. Indeed, there are many excellent examples in the literature using both pharmacological (eg, delivering collagen IV-targeted

Figure. Proposed modifications to preclinical pipeline for experimental atherosclerosis studies. Prevention studies (A) may represent a good proof-of-principle for a novel therapeutic or gene knockout but in the setting of atherosclerosis may not translate to older patients with established disease. Instead, we should implement intervention studies (B), which we argue will better predict the effect of a therapeutic strategy for treating humans with advanced atherosclerotic lesions. Both of these approaches should analyze parameters that not only provide information about lesion size but also investigate multiple key cellular processes implicated in lesion vulnerability in humans. In addition, it is critical to identify innovative ways for validating results in preclinical animal studies to the extent possible including use of classic immunohistological analyses of human autopsy specimens and large-scale genomic approaches (C). Of course, the final validation of new cardiovascular disease therapies will require well-designed clinical trials (D).
nanoparticles containing proresolving peptide, Ac2-26\textsuperscript{13} and genetic (eg, SMC-specific knockout of Akt1 after 16 weeks of Western diet)\textsuperscript{14} interventions validating its importance to the field—but we feel that it remains heavily underutilized. Another study design that has been used to study the processes critical in late-stage atherosclerosis is the regression model. These are variations of the intervention model in which atherosclerosis is induced by chronic hypercholesterolemia but then regressed by either normalizing specific lipid components or transplanting diseased vessels into healthy organisms. These models have been helpful to identify the processes that may be reversible in advanced atherosclerosis like CCR7-mediated macrophage egress\textsuperscript{15} or reverse cholesterol transport and may prove valuable when investigating strategies to encourage these processes.

In summary, we hope to motivate investigators to re-evaluate the way we apply mouse models to the study of atherosclerosis. With the residual risk for atherosclerotic complications that remains despite current standard of care, there exists a critical need for therapies that not only focus on limiting destabilization but also seek to promote better inflammatory resolution, healing, and overall plaque stabilization. We think that the first step toward achieving this goal is to begin studying potential therapeutics using intervention models in mice and to rigorously characterize their effect on processes critical in maintaining lesion stability. Although no animal model completely recapitulates human disease, we can and need to do a better job of matching our experimental animal model designs to the unmet clinical needs.

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References
Shifting the Focus of Preclinical, Murine Atherosclerosis Studies From Prevention to Late-Stage Intervention

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