Current treatment strategies have resulted in significant reductions in morbidity and mortality associated with cardiovascular disease; however, significant residual risk remains. As an example, in the PROVE IT–TIMI 22 trial (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22), 22.4% of patients experienced an adverse event despite achieving a median LDL cholesterol concentration of 62 mg/dL (1.6 mmol/L) (1). These and similar findings have led to the search for novel therapeutic modalities. A logical place to start is control of inflammation. Darapladib (GlaxoSmithKline) is a novel oral therapeutic agent with potential antiinflammatory properties that inhibits lipoprotein-associated phospholipase A2 (Lp-PLA2).3

Lp-PLA2 is produced by macrophages and circulates bound to LDL. In experimental models, it appears to be central in the atherosclerotic process. Lp-PLA2 acts on oxidized phospholipids to produce free oxidized fatty acids and lysophosphatidylcholine, a proatherogenic inflammatory mediator that increases expression of adhesion molecules and cytokines, is a chemoattractant for macrophages, and induces vascular smooth muscle migration (2, 3). Immunohistochemical studies have shown that Lp-PLA2 is present in atherosclerotic lesions, and is particularly intense in “rupture-prone lipid laden” lesions with thin-cap fibroatheromas, (4). Unlike many other inflammatory mediators, Lp-PLA2 is not an acute-phase reactant (5); thus issues associated with acute-phase reactants are averted. For these reasons, Lp-PLA2 has emerged recently as an independent marker of cardiovascular risk and events.

Although not all prospective studies have demonstrated an association between high plasma Lp-PLA2 concentrations and poor cardiovascular outcomes, the majority of studies reported are strongly positive. In a recent metaanalysis (6) including more than 20 000 patients from 14 epidemiologic studies, high Lp-PLA2 concentration was an independent risk factor for cardiovascular events. This is especially true in regard to the associations with stroke, which seem particularly strong (7, 8). These findings have persisted despite multiple iterations of the immunologic assay used with very different analytic characteristics and thus widely disparate absolute values (9).

**Inhibition of Lp-PLA2**

The first clinical study performed to assess the effect of Lp-PLA2 reduction with darapladib in humans was recently published (10). It involved 330 patients with angiographically documented coronary disease who were treated with either 160 mg darapladib or placebo for 12 months. Patients were followed to determine the effects of treatment on a primary endpoint of coronary atheroma deformability assessed by intravascular ultrasound (IVUS) palpography and several secondary endpoints. Lp-PLA2 activity was monitored to ensure adequate inhibition of plasma levels.

Plasma Lp-PLA2 activity decreased by 59% in patients receiving darapladib treatment (P < 0.001 vs placebo), whereas there was no significant difference in LDL cholesterol in the placebo vs treatment groups [mean (SD) placebo 88 (34) mg/dL; darapladib 84 (31) mg/dL; P = 0.37] after 12 months. There was no significant difference in plaque deformability (P = 0.22) or high-sensitivity C-reactive protein (hs-CRP) (P = 0.35) between groups. A secondary endpoint, 1 of at least 3 that were hopefully prespecified, was improved. Necrotic core size determined using IVUS-based radiofrequency analysis, increased significantly in the placebo group [4.5 (17.9) mm³; P = 0.009], but did not in the darapladib group [−0.5 (13.9) mm³; P = 0.71]. The authors concluded that since darapladib prevented necrotic core expansion, Lp-PLA2 inhibition seemed
to represent a beneficial therapeutic approach. Although this is interesting and warrants further investigation, the trial failed to demonstrate an effect on the primary outcome measure or other secondary outcomes. Why then was this predominantly negative study published in a positive light? Should the take-home message from this study be different than what was presented in this publication? More importantly, does our current system of peer review and research funding create an environment which increases the likelihood of emphasizing positive results and minimizing negative data?

**Publication Bias**

Publication bias is the tendency of researchers, reviewers, and editors to handle results that are positive (showing an effect) in a different way than negative results or results which do not demonstrate the predicted effect (11). Although journal editors are often blamed, evidence suggests that this accounts for only a small part of the problem (12). Censoring of negative results by study funders is an ominous concern, but it is also not a common etiology. Failure of investigators to submit negative study results because they are not interesting, because they do not lead to promotions or rewards, or because of the perception that editors won’t accept them for publication, is the major reason. This leads to negative trial results not being published, or could lead to reporting bias, as can occur when several outcomes within a trial are measured but are reported selectively depending on the strengths of the associations (13). We have on several occasions spoken out about these issues and lamented the lack of quality control that has allowed some articles to be published in our journals that could be misleading (9, 14).

In fairness to the authors of the darapladib study, they clearly pointed out that primary endpoints for the study were not significantly different between the placebo and treatment groups. However, among the secondary endpoints measured, the potential beneficial effect on necrotic core volume was the primary focus of the discussion.

**IVUS**

We are not experts in the use or interpretation of IVUS, but an understanding of that measurement technology is integral to interpreting the findings of this study. IVUS has been proposed as a means of detecting vulnerable atherosclerotic plaques. Pathologic studies demonstrate that rupture-prone plaques are characterized by the presence of a large necrotic core and a thin fibrous cap infiltrated by macrophages. Recent studies using spectral analysis of radiofrequency ultrasound signals have allowed more detailed assessment of plaque types, which can be identified as fibrous, fibrofatty, dense calcium, or having a necrotic core (15). There are good correlations between the derived parameters and corresponding histology (15); however, data linking any of these parameters to outcomes are not available. Thus, predicting coronary plaque rupture with IVUS is unproven. In addition, some have argued that the present techniques are imprecise. Thus, issues such as the degree of variability of measurements and the optimal timing of such measurements, to say nothing about which of a large number of potential measurements should be used, are unclear. It could be the case that rather than 1 solitary parameter, a combination of measures including arterial remodeling index, cap thickness, and necrotic core area or thickness may be necessary for prediction (16). Thus, to pick a given parameter, especially one that is a secondary endpoint, and to make claims for a positive study seems questionable.

**Darapladib Inhibition**

In the trial, 175 patients received darapladib, whereas 155 received placebo. Eighty-six percent (n = 130) in the placebo group completed treatment compared with 88% (n = 152) in the darapladib group. Darapladib therapy decreased plasma Lp-PLA2 activity by 59%. Although the authors used what appear to be reasonable estimates of variability for the IVUS and CRP measures to determine adequate sample size to identify a treatment effect, the incomplete characterization of the multiple IVUS measurements, and the fact that the effect of an Lp-PLA2 inhibitor on imaging parameters is unknown, puts into question whether this study was adequately powered. Furthermore, because CRP concentration reflects systemic inflammatory processes while the action of Lp-PLA2 is more localized to the vessel wall, one could question whether changes in CRP concentration should be affected by Lp-PLA2 inhibition, particularly since plasma concentrations of Lp-PLA2 and CRP are not correlated (17, 18). Given this information, one could question whether any of the outcome measures used in this study were appropriate.

The most important aspect of this study may be the investigation of the safety profile. Lp-PLA2 is also known as platelet-activating factor acetylhydrolase; thus Lp-PLA2 reduction could result in increased platelet activity and associated adverse outcomes. The authors monitored for effects on markers of platelet activation including P-selectin, soluble CD40 ligand, and urinary 11-dehydro thromboxane B2, as well as for clinical events related to platelet activity. There were no significant differences, except for higher levels of soluble CD40 ligand at the 12-month time point. Impor-
tantly, they found no differences in reported clinical outcomes despite higher mean systolic blood pressure values in the darapladib group. The authors point out that this issue will require careful attention in future studies.

Where Do We Go From Here?

Lp-PLA2 is a promising new risk marker for coronary heart disease and stroke. Interest in its inhibition is based on reasonable basic science correlates and multiple epidemiologic studies demonstrating an independent association between Lp-PLA2 concentrations and adverse cardiovascular endpoints. These associations persist despite the difficulties associated with measurement of this protein at low plasma concentrations (µg/L) and the assay problems mentioned above. One negative trial should not end the investigations of a potentially novel therapeutic target in our view. The safety profile suggests that such investigations can continue to be conducted safely while monitoring the toxicity of treatment and the effects of treatment on hard clinical outcomes.

Of equal importance from our perspective is the need for us to remediate our publication systems so that valuable negative articles like this one can be published in a more straightforward manner so the field is not misled by spin and by a literature bias toward positive articles.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.
Consultant or Advisory Role: A.S. Jaffe, Siemens, Beckman, Ortho Critical, Inverness Medical, Hoffman-La Roche, Singulex, and Nanosphere.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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