Although ibrutinib is an example of the class of “targeted therapy,” there is increasing evidence that ibrutinib also mediates alterations in T-cell function and is, at least in part, able to reverse the T-cell exhaustion phenotype typical of CLL.  

Notably, CAR-T cells exhibit increased efficacy when the T cells are obtained after ibrutinib treatment. It is therefore highly likely that the combined effects seen here result from increased effector and cytotoxic function of the ibrutinib-treated T cells brought together in effective synapse interactions with the target CLL cells by the bispecific antibody. 

The work here is novel but, of course, is all preclinical, with the data presented being either in vitro or in xenograft mouse models. We are therefore still far from translating this work to patients. Blinatumomab is licensed for and highly effective for the treatment of acute lymphoblastic leukemia. There is no convincing data presented to explain why there should be such difference in activity in vivo between blinatumomab and the novel bispecific antibody construct when they work similarly in vitro. The experiments using samples from patients treated long term with ibrutinib remain difficult to interpret, and it is not clear if this is attributable to an effect of the ibrutinib on T cells or simply a reflection of taking samples when the CLi burden in the patients was much less. There is also no clear reason why ibrutinib-resistant cells should have been less sensitive to the bispecific antibody treatment as there is no suggestion that B-cell receptor-mediated signaling is in any way associated with its activity. We await with great interest the data that await with great interest the data that investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014;371(3):213-223.


**PLATELETS AND THROMBOPOIESIS**

**Comment on Peng et al, page e1**

**Platelet lipidomics and function: joining the dots**

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In this issue of Blood, Peng et al use a quantitative lipidomics platform, comprising mass spectrometry, network analysis, and in vitro studies to provide a comprehensive quantitative analysis of the platelet lipidome. This study sheds new light on how the platelet lipidome is altered during platelet activation and, importantly, how changes in the platelet lipidome may modulate platelet function. 

Lipids have fundamental structural, signaling, and metabolic roles. Specific lipid species are well known as important actors in mediating hemostasis and thrombosis. For example, thromboxane A2 (TXA2) represents one of the major platelet agonists and plays a central role in regulating thrombus formation. Aspirin, which inhibits TXA2 generation, remains one of the most widely used and most successful drugs globally based on its antiplatelet effects. Moreover, lipids generated during platelet activation such as diacylglycerol, inositol 1,4,5-trisphosphate, and the phosphatidylinositols play key roles as signaling molecules governing protein kinase C activation, calcium mobilization, and integrin activation: all fundamental processes regulating platelet function. Moreover, phospholipids located within the platelet plasma membrane, such as phosphatidylserine, become exposed in highly activated platelets and thus provide the requisite negatively charged platform required for coagulation complex assembly. Lastly, activated platelets and shed microparticles both express abundant phosphatidylincholine in the cell membrane, which facilitates the dissociation of pentameric C-reactive protein into its prothrombotic and proinflammatory isoforms. 

More recently, the aperture through which we view the role of lipids in platelet biology has been significantly widened with the application of lipidomic approaches. Highlighting the vast array of lipid species contained within platelets, analysis of the platelet lipidome by Slatter et al has demonstrated that platelets from healthy volunteers contain up to 8000 different lipids. Significantly, in thrombin-stimulated platelets, cytosolic phospholipase A2 generates multiple lipid species, which serve as substrates for mitochondrial energy production and thus play an important role in platelet activation and procoagulant function. Interestingly, aspirin also significantly alters the thrombin-activated platelet lipidome. Although aspirin is a putative selective COX-1 inhibitor, it has become apparent using novel lipidomic approaches that multiple...
lilipid species generated by phospholipases upstream of arachidonic acid are inhibited by aspirin, suggesting that the antiplatelet effects of aspirin may be more complex than first anticipated.

The application of lipidomic approaches is beginning to extend into the clinical arena, with data emerging demonstrating that platelets from patients with coronary artery disease display altered lipidomic profiles. However, how these “pathological” platelet lipidomic profiles may influence platelet function is yet to be established. By providing the first quantitative analysis of the platelet lipidome, the findings presented by Peng et al provide an important basis for understanding how the lipidome affects platelet function and, conversely, how platelet activation influences the lipidomic profile. In accordance with previous reports, the studies by Peng et al highlight the vast array of lipids present within the platelet lipidome, which covers ~400 lipid species over a concentration range of 7 orders of magnitude. However, surprisingly, the analysis of the resting platelet lipidome revealed that only 15 lipids comprise 70% of the entire lipid mass of platelets. Likewise, studies analyzing the platelet lipidome after activation with the potent agonists thrombin and/or collagen-related peptide reveal the lipidome to be remarkably stable in the context of platelet activation. Here, <20% of the lipidome was altered upon activation. Significantly, of the 15 most abundant platelet lipids, only phosphatidylinositolos change significantly after activation, consistent with the known role of phosphatidylinositol signaling in platelet activation. In contrast, the majority of lipids altered during platelet activation are lipids of low-medium abundance.

To validate the ability of their lipidomic approach to monitor disease-related lipidomic changes in platelets, sphingomyelin phosphodiesterase 1 (SMPD1)-deficient mouse platelets were analyzed. These studies revealed that although SMPD1 deficiency is associated with a relatively stable lipidomic profile, lysosphingomyelin (SPC) concentrations are increased 10-fold in SMPD1-/- platelets as compared with controls.

Strikingly, complementary studies demonstrated that SPC is a potent platelet inhibitor since the exogenous treatment of platelets with SPC was demonstrated to inhibit αβ activation, platelet aggregation, platelet degranulation, and platelet thrombus formation under shear. However, importantly, only platelet degranulation and thrombus formation under hemodynamic shear are inhibited by SPC in an SMPD1-dependent fashion. How SPC can inhibit these other facets of platelet activation remains to be established and will no doubt be the basis for future investigation.

These studies were performed in mouse platelets, and therefore, it will be important to investigate whether these changes in the platelet lipidome, and platelet inhibitory effects of SPC, are translatable to human platelets, particularly patients with a deficiency of SMPD1, such as those with Niemann-Pick disease.

The findings of Peng et al add another piece to the platelet lipidome puzzle, however, a key outstanding issue, which these studies have started to decipher, is the functional consequence of how an altered platelet lipidome may regulate platelet function. Unraveling the functional consequences of different platelet lipidomes raises the prospect of uncovering novel therapeutic targets in the quest for new antithrombotics that spare hemostasis. Moreover, understanding how the platelet lipidome may regulate platelet function will yield significant new insights into disease biology. In this regard, it is well accepted that platelets promote atherosclerosis, and hyperlipidemia potentiates platelet function. Thus, given the ability of platelets and megakaryocytes to take up cholesterol, it is highly probable that having an altered platelet lipidome is intertwined with platelet hyperactivity. These questions are particularly pertinent given the consistent observation that reductions in low-density lipoprotein cholesterol, either by statins or anti-PCSK9 antibodies, are linked to reduced rates of atherothrombotic events. Lastly, it is tempting to speculate that certain platelet lipidomic profiles may serve as a disease signature and therefore represent a “biomarker,” helping to predict clinical risk or treatment response. To this end, given the multiple roles of platelets in cardiovascular disease and the emerging roles of platelets in immune, inflammatory, and malignant diseases, the story of the platelet lipidome is only beginning to be told: with the potential to be a bestseller.

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