high expression of the IL-13 receptor α1 in megakaryocytes isolated from patients with MF compared with weak expression in megakaryocytes isolated from patients with essential thrombocythemia (ET) and polycythemia vera (PV). It is known that STAT6 signaling is activated downstream of the IL-13/IL-4 receptor and that this leads to the transcription of TGF-β. Concordant with this, they found increased level of phosphorylated STAT6 in megakaryocytes from both healthy donors and patients with MF following IL-13 stimulation (see figure).

To assess the effect of modulating IL-13 signaling in fibrosis progression, the authors investigated both IL-13 overexpression and IL-4ra knockout in MPN mouse models. *Jak2*^{V617F} mice overexpressing IL-13 showed features of early bone marrow fibrosis with increased active TGF-β. Conversely, IL-4ra knockout reduced bone marrow fibrosis, decreased spleen weights in *MPL*^{W515L} mice, and prolonged survival, findings with disease relevance for human MF. Last, Melo-Cardenas et al interrogated single-cell RNA-sequencing data from both prefibrotic and fibrotic bone marrow in both *Jak2*^{V617F} and *MPL*^{W515L} mice and found that mast cells and T cells were increased at the fibrotic stage, with both being known sources of IL-13.[8] Concordant with this, bone marrow mast cells positive for TGF-β and IL-13 have been associated with higher fibrotic grade in human MF.[9] A limitation of the study is that although the authors confirmed mast cells as a key cellular source of IL-13 in their MPN mouse models, they were not able to test if eradicating mast cells reduced the fibrotic phenotype. If that were the case, this would make mast cells an attractive cellular target, as suggested by prior studies in human MF.[9]

The translational potential of the study from Melo-Cardenas and colleagues, through targeting IL-13/IL-4 signaling in MF, is a strength of the article. Lebrikizumab, a monoclonal antibody directed against IL-13, in use for allergic diseases like asthma, is currently in phase 3 clinical trials.[10] Dupilumab is a US Food and Drug Administration–approved monoclonal antibody against IL-4ra used for allergic diseases, like asthma and atopic dermatitis. Another clinically relevant aspect of the study is the fibrosis phenotype observed was TGF-β dependent. Given the historical challenges with therapeutically targeting TGF-β directly in patients with MF, indirect targeting via IL-13/IL-4 inhibition may be an alternative approach. A limitation of the study is that the authors were unable the test the efficacy of blocking IL-13/IL-4 signaling in a mouse model where MF is already established (eg, by using an antibody). Given the profound dysregulation of inflammatory cytokines in patients with MF, it is not clear how clinically impactful blocking a single pathway would be. Although the authors did not formally test this in their study, given the incomplete cytokine blockade achieved by JAK pathway inhibition in MF, a combinatorial clinical trial adding IL-13/IL-4 inhibition to a JAK inhibitor may be beneficial in patients.

In conclusion, in an excellent study with high translational relevance, Melo-Cardenas et al used a combination of mouse models, primary MPN samples, and single-cell approaches to uncover and validate a novel pathway in MF that can be therapeutically targeted. Through identifying and blocking key cytokine nodes in MF, it may be possible to "untangle" pathologic partnerships, such as IL-13–TGF-β, to slow down or even reverse bone marrow fibrosis.

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PLATELETS AND THROMBOPOIESIS

Comment on Ni et al, page 2818

Balancing the Qi in ITP

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In traditional Chinese medicine, 气 (Qi) represents energy or a vital force that is disrupted in disease. Restoring the balance or flow of Qi is a philosophical therapeutic approach. Although there is no Western scientific equivalent to

this concept, in this issue of *Blood*, Ni et al[1] use rigorous scientific methods to describe a rebalancing of energy metabolism using the hypomethylating agent decitabine, which increases platelet counts in immune thrombocytopenia (ITP) in part by restoring energy homeostasis through the liver kinase B1 and AMP-activated protein kinase (LKB1/AMPK) pathway and enhancing myeloid suppressor cell function.

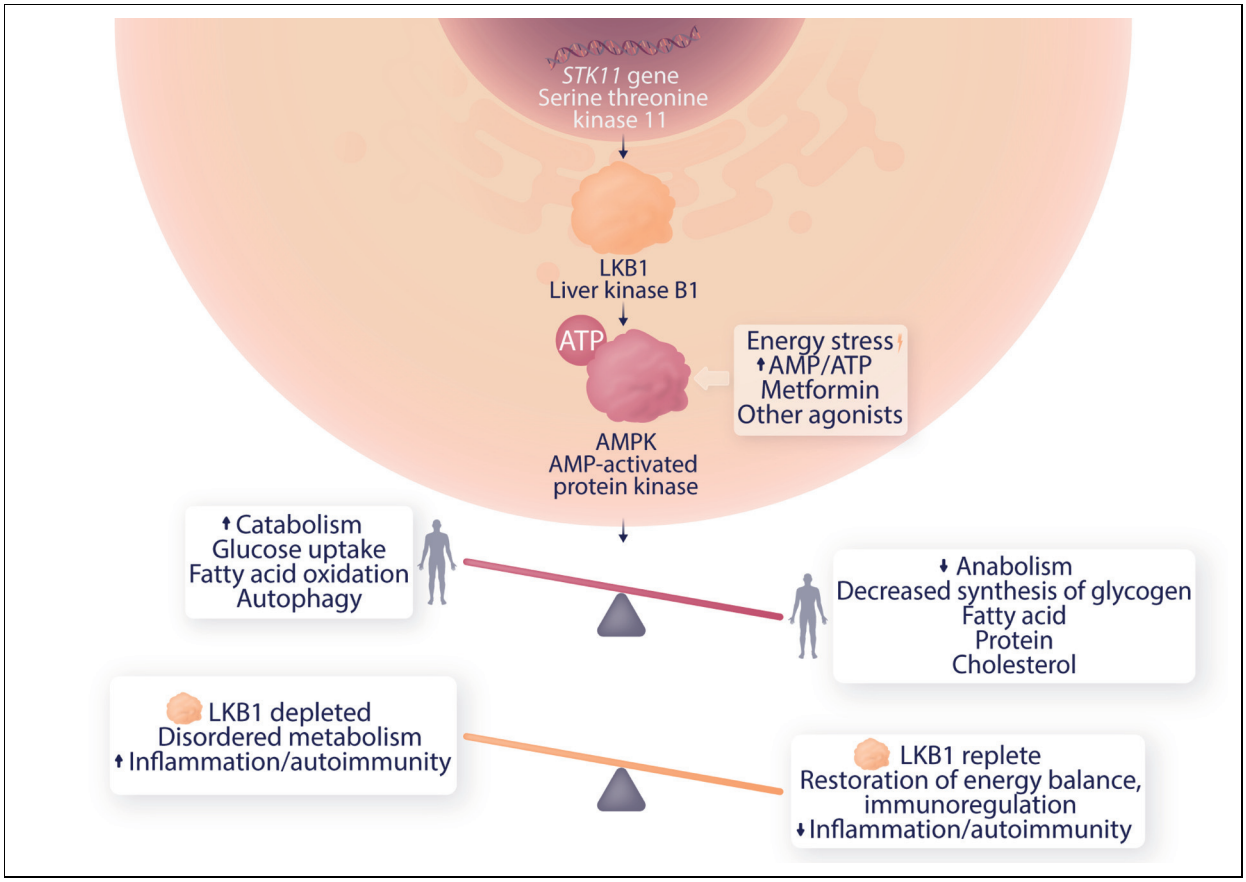
ITP is an autoimmune disease resulting in low platelet counts and bleeding. The pathophysiology of ITP is complex and incompletely elucidated with increased platelet destruction as well as regulatory T cell dysfunction and a proinflammatory environment that disrupts regulation of thrombopoiesis. Treatment of ITP has revolved around immunosuppression with steroids remaining first line therapy with multiple other modalities including anti-CD20 antibodies, thrombopoietin agonists, kinase inhibitors and other immune modulators. Chronic ITP is common in adult patients, resulting in multiple treatment regimens, drug toxicity and diminished quality of life. Thus, a better understanding of the underlying pathophysiology of ITP, leading to targeted, effective and less toxic therapies is needed.

5-aza-2′-deoxycytidine (decitabine) is a hypomethylating agent typically used in the treatment of myelodysplastic syndrome. When used in low doses, decitabine promotes cellular differentiation and can ameliorate cytopenias, including thrombocytopenia, in MDS. Wang et al[2] studied the effect of low dose decitabine in mouse models demonstrating an enhancement of megakaryocyte maturation and platelet release. Zhou et al[3] extended these findings to in vitro studies of ITP patients, demonstrating a similar beneficial effect of decitabine. This group then extended their in vitro work with a multicenter safety and efficacy trial of low dose decitabine in patients with refractory ITP, with sustained responses in 44% of patients (20 of 45) at 6 months, and improved quality of life in responders.[4]

Given the complex dysfunctional immune environment of ITP, Han et al[5] postulated that the effects of decitabine in ITP were likely to include beneficial immunoregulatory modulation in addition to simply increasing megakaryocyte maturation and platelet release. Using ITP patient samples in vitro and in a mouse model of ITP, they then demonstrated that low-dose decitabine restored immune tolerance in ITP with improved regulatory T -cell function, a decrease in proinflammatory cytokines and decreased STAT3 activation.

The current article by Ni et al extends these observations to a defect in metabolic regulation. LKB1 is a tumor suppressor and product of the serine-threonine kinase 11 (*STK11*) gene.

Germ line mutations in *STK11* are associated with Peutz-Jeghers syndrome.



Role of LKB1 in AMPK regulation of metabolism and possible mechanism of modulation of autoimmunity in ITP. Professional illustration by Somersault18:24.

These patients have gastrointestinal polyps in a setting of chronic inflammation, STAT3 activation, and increased expression of inflammatory factors associated with cancer progression. Somatic variants in *STK11* are seen in skin, lung and other cancers. Deficiency of LKBP1 results in downregulation of MAPK, dysregulating metabolism toward an anabolic, proinflammatory state (see figure), and is implicated in the Warburg effect observed in cancer where anaerobic glycolysis is activated in normoxic conditions.[6] Ni et al studied myeloid-derived suppressor cells (MDSC) which down-regulate immune responses in ITP patients and controls in vitro and in an animal model of severe ITP. This group has previously shown that MDSC were deficient in ITP patients and improved by dexamethasone therapy. They now present evidence for a role of LKB1 deficiency in MDSC in ITP patients and demonstrate that low dose decitabine therapy restores LKB1 levels, energy balance and MDSC function.

The body of work by these investigators demonstrates that low dose decitabine has multiple salutary effects on restoration of immune regulation and energy balance in ITP. In addition to broadening our understanding of the pathophysiology of ITP and illuminating additional therapeutic choices (eg, AMPK agonists) this work provides a compelling rationale for further clinical trials to define the use case for low dose decitabine therapy for ITP.

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THROMBOSIS AND HEMOSTASIS

Comment on van Moorsel et al, page 2844

A novel VWF-associated thrombolytic agent

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In this issue of *Blood*, van Moorsel et al report the first results on Microlyse, a novel agent with treatment potential that uses a new target to lyse pathologic thrombi in acute ischemic stroke.[1] In a brief report, they present their mouse studies of experimentally induced stroke treated with either Microlyse, recombinant human tissue-plasminogen activator (rtPA), or vehicle. They show that Microlyse degraded thrombin-induced fibrin-rich thrombi in the middle cerebral artery (MCA), with results comparable to those obtained by rtPA infusion.

For decades, thrombolysis via rtPA has been the mainstay of treatment of acute ischemic stroke in humans.[2] Timely infusion of rtPA leads to a better functional outcome and less morbidity in ischemic stroke in humans.[3] However, a significant number of rtPA-treated patients have thrombi that are resistant to treatment. Another important downside of rtPA-induced thrombolysis is the induction of bleeding or hemorrhagic transformation. Therefore, new treatment options for patients suffering from acute ischemic stroke are still needed. Others have already shown that ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) has potential as a therapeutic agent for ischemic stroke, degrading von Willebrand Factor (VWF) within the VWF-containing thrombi in cerebral arteries in a mouse model.[4]

Microlyse is a VWF-targeting plasminogen activator that was recently developed by fusing anti-VWF VhHs (single variable domain on a heavy chain; nanobodies) directed to the C-terminal cystine knot (CT/CK) domain of VWF and the protease domain of human urokinase plasminogen activator (mini-uPA).[5] Upon binding to VWF, which is abundantly present in a thrombus, Microlyse activates plasminogen to plasmin. Plasmin can degrade, among other coagulation factors, both fibrin and VWF. In a previous study, the same research group showed that Microlyse can be used to degrade microthrombi in mouse thrombotic thrombocytopenic purpura (TTP) models by plasmin destruction of platelet VWF complexes.[5]

In the Van Moorsel et al study, the authors examined the use of VWF-targeted thrombolysis to overcome rtPA resistance in a mouse model of thrombotic stroke. The authors compared results with the use of the new thrombolytic agent Microlyse to those with rtPA and vehicle (as control) in experimental stroke, by inducing the formation of fibrin-rich and platelet-rich thrombi in the MCA. In the fibrin-rich thrombi stroke model, both rtPA and Microlyse were superior to vehicle in obtaining cortical reperfusion and reducing the volume of cerebral lesions. In the platelet-rich thrombi, neither rtPA nor Microlyse was better than vehicle in obtaining cortical reperfusion. However, a significant reduction of cerebral lesion volume was seen in Microlyse-treated animals, compared to those treated with vehicle or rtPA. Although this study was carried out in only a limited number of mice, these results are interesting and may