investment in others is needed. Third, because Usp24 silencing could kill cells as well as Mcl-1 silencing but did not regulate Mcl-1 as efficiently, inhibition of Usp24 likely affects other pathways that influence myeloma cell survival. Finally, targeting multiple DUBs may overcome compensatory changes in expression, thus explaining why WP1130 is more effective than silencing.

Because both Usp9x and Usp24 can play a role in the survival of malignant B cells, one would conclude that a drug like WP1130 would be a promising candidate to move forward toward the clinic. Unfortunately, this compound has solubility issues that limit its effectiveness in vivo. Previously, a structure–activity relationship was performed to identify related compounds that would be more active against Usp9x and Usp24 as well as have better drug-like properties. In the current report, the activity of one of these compounds, G9, is characterized. G9 is a more potent inhibitor of Usp9x and has better solubility that WP1130. It induces apoptosis in myeloma and lymphoma cell lines in vitro and prevents xenograft tumor growth with little overt toxicity. These are promising findings; however, its ability to reduce tumor burden was not directly tested in these studies. One of the more interesting aspects of G9 activity is that, in addition to inhibiting Usp9x and Usp24, it inhibits Usp5. Inhibition of Usp5 results in an upregulation of p53 and because p53 is not commonly mutated in B-cell malignancies, this may also contribute to the therapeutic potential of this or related agents. Thus, indirectly targeting Mcl-1 via inhibition of DUBs may prove to be more efficacious than direct Mcl-1 inhibition (see figure 10). However, targeting Mcl-1 degradation in this manner may come at a cost. The data demonstrate that proteasome inhibition reverses the effect of DUB inhibition on Mcl-1 expression. If Mcl-1 that is decorated with ubiquitin remains functional, then combinations with proteasome inhibitors, which are backbone drugs in myeloma treatment, may be antagonistic. This would not be the case with a direct Mcl-1 inhibitor. Additional studies using G9 and future analogs with proteasome inhibitors are needed to see how this promising approach will complement current active agents.

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PLATELETS AND THROMBOPOIESIS

Comment on Wang et al, page 3627

A paradigm shift in platelet transfusion therapy

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In this issue of Blood, Wang et al report that ex vivo–derived human megakaryocytes infused into mice are trapped in the pulmonary vasculature and release functional platelets into the circulation. Because of the difficulty in scalable generation of ex vivo–derived functional platelets, this strategy may be a substitute for platelet transfusion therapy.1

A nucleated blood platelets play a crucial role in hemostasis and thrombosis at the site of vascular injury. Since they have a short life span (7–10 days), platelets are constantly generated from megakaryocytes to maintain normal levels in the blood (150–400 × 10⁹ platelets per microliter). One mature megakaryocyte in the bone marrow produces 2000 to 10 000 platelets in response to thrombopoietin and other cytokines.5 Previous studies also showed that a recombinant protein containing the receptor-binding N-terminal domain of thrombopoietin infused into mice stimulates platelet production from megakaryocytes in the lungs, suggesting that platelet production occurs outside the bone marrow.3

Despite efficient platelet production in the bone marrow and lungs, patients who undergo radiation treatment, chemotherapy, or organ transplant surgery often suffer from bleeding as a result of life-threatening thrombocytopenia (<10-20 × 10⁹ platelets per microliter). Although transfusions using donor-derived platelets remain the most effective way to treat thrombocytopenic patients, numerous concerns have been raised, including the limited shelf life and storage-related deterioration of donor-derived platelets, development of alloantibodies in recipients, and transmission of infectious disease. Thus, many efforts have been put forward to generate functional platelets using human embryonic stem cells and induced pluripotent stem cells (iPSCs) as a potential source for platelet transfusion (see figure panel A). However, the low yield and poor functionality of stem cell (SC)–derived platelets remain a considerable challenge in this strategy.

A previous study showed that infused ex vivo–derived mouse megakaryocytes are trapped in the pulmonary vasculature, where they shed platelets with a normal size that have appropriate surface markers and a 1-day
half-life. In this study, Wang and colleagues further tested human megakaryocytes in mice (see figure panel B). After infusion of ex vivo–derived human megakaryocytes into macrophage-depleted NOD/SCID/interferon-γ–deficient mice, two different pools of human platelets were generated independent of the starting source of megakaryocytes (CD34+ cells derived from human bone marrow, hematopoietic mononuclear cells derived from fetal liver, and iPSCs): in vivo–generated platelets shed from megakaryocytes trapped in the pulmonary vasculature and ex vivo–derived platelets (or platelet-like particles) released from megakaryocytes during culture and/or processing. Intriguingly, in vivo–generated platelets showed characteristics similar to those of human blood platelets in terms of their size, surface markers (CD42a and CD42b), and in vitro and in vivo functionality. However, the authors did not test whether in vivo–generated platelets were able to restore hemostatic function in thrombocytopenic mice or whether CD42a and CD42b make a functional complex. They found that compared with donor platelets, ex vivo–derived platelets exhibited numerous functional defects, including a wide range of sizes and a shorter half-life. In addition, CD42b was shed from ex vivo–derived platelets, and annexin V was expressed on the platelet surface, probably as a result of the enhanced basal activation state. Consistent with previous studies describing functional deficiencies of platelets generated from SC-derived megakaryocytes, these results suggest that ex vivo–generated platelets may not act like blood platelets.

Although these findings provide evidence that human megakaryocytes infused into mice release functional platelets into the circulation, there might be several hurdles to clear before this strategy can be used in a clinical setting. The critical issue is whether cultured megakaryocytes can be directly infused into humans because large-size megakaryocytes and their aggregates may occlude small vessels after transfusion. Although the concern about SC-derived teratoma formation may be eliminated by transfusing ex vivo–derived megakaryocytes after irradiation, it remains to be determined whether irradiation of the megakaryocytes affects platelet production and function in vivo. Thus, future studies are required to assess the safety of infused ex vivo–derived megakaryocytes into animals. Second, because of the difficulty in obtaining bone marrow–derived megakaryocytes, this strategy still requires megakaryocytes obtained from the differentiation of SCs or megakaryocyte progenitor cells derived from the bone marrow or fetal liver. Because in vitro or ex vivo culture systems do not necessarily mimic the bone marrow environment, ex vivo–derived megakaryocytes are unlikely to act like bone marrow–derived megakaryocytes. Indeed, this speculation is also supported by the results of Wang et al showing a low ploidy of ex vivo–derived megakaryocytes and a limited number of in vivo–generated platelets in mice. Third, although the Wang et al study showed the incorporation of in vivo–generated human platelets into the growing mouse platelet thrombus, the number of platelets produced from infused megakaryocytes in a different stage of maturation still needs to be investigated, along with whether in vivo–generated platelets have normal hemostatic function during bleeding.

Because the need for platelet transfusions has been increasing during the past decade, scalable generation of ex vivo–derived platelets is a promising strategy. Recent reports indicated that a large number of platelets can be generated ex vivo by using a bioreactor or a 3-dimensional tissue model that mimics the bone marrow niche. The long-standing question is whether we can find the optimal conditions for generating a sufficient number of functional platelets that have characteristics similar to those of blood platelets. Because in vivo–generated platelets, unlike ex vivo–derived platelets, are similar to donor platelets in several aspects, this strategy may be a breakthrough in platelet transfusion therapy.

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Atypical HUS may become a diagnosis of inclusion

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In this issue of Blood, Gavriilaki and colleagues1 describe an assay that could convert atypical hemolytic uremic syndrome (aHUS) from a diagnosis of exclusion into a direct pathophysiologic diagnosis.

Patients with aHUS usually have microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. However, these features occur in many diseases, including thrombotic thrombocytopenic purpura (TTP), and the more common form of Shiga toxin–expressing Escherichia coli hemolytic uremic syndrome. Making the correct diagnosis is critical because these disorders require different treatment. For example, treatment of aHUS with plasma exchange is associated with up to 8% mortality during the first episode and progression to end-stage renal failure in most survivors. However, treatment with the complement inhibitor eculizumab can halt the thrombotic microangiopathy, prevent or reverse renal failure, and forestall damage to the brain, heart, and other organs,2 which makes sense because aHUS is caused by defects in the regulation of the alternative complement pathway.

Unfortunately, no method has been described that reliably detects the hyperactive alternative complement pathway in aHUS. Relatively few patients have low serum C3 levels, and complement consumption is not specific for aHUS. Complement mutations in aHUS are heterozygous, and the corresponding protein concentrations in blood are not consistently abnormal. At least for now, genetic testing is slow and uninformative in up to 50% of cases. Instead, aHUS is diagnosed by ruling out ADAMTS13 deficiency, Shiga toxin–expressing E. coli, and other confounding causes of thrombotic microangiopathy. The patients remaining are said to have aHUS, but for most of them, we have no way to rapidly verify the underlying disease mechanism.

To address this problem, complement activation products have been measured, expecting that impaired complement regulation should increase their levels in blood. On average, levels of anaphylatoxin C3a and the terminal complement complex C5b-9 are higher in aHUS compared with acquired TTP but cannot accurately classify many patients because the ranges overlap.3 These and other biomarkers of complement activation may4 or may not5 be increased for aHUS patients in remission.

A direct assessment of complement dysregulation might be more successful. For example, confocal microscopy showed that more C5b-9 was deposited onto HMEC-1 microvascular endothelial cells by serum from patients with aHUS compared with healthy controls. Interestingly, abnormalities were present during active disease, during remission, and in unaffected carriers of complement mutations.6 This approach appears promising, but plasma from patients with TTP also induces complement deposition on HMEC-1 cells, raising questions about specificity.6

To overcome this roadblock, Gavriilaki et al1 took advantage of their experience with paroxysmal nocturnal hemoglobinuria (PNH) to draw a useful analogy between PNH and aHUS (see figure). Because PNH cells are sensitized to complement damage, PNH-like cells might be sensitive to complement dysregulation in aHUS. This elegant lateral move paid off.

First of all, Gavriilaki et al confirmed that aHUS plasma promotes C5b–9 deposition on Ea.hy926 cells, another human endothelial cell line, and confirmed that the method does not clearly distinguish between aHUS and TTP. However, treating Ea.hy926 cells with a phospholipase to remove cell-surface complement regulatory proteins sensitized the resultant PNH-like cells to the deposition of C5b–9 and improved the discrimination between aHUS and other conditions by confocal immunofluorescence microscopy. Flow cytometry for C5b–9 was also effective and less subjective, but it was challenging to automate. This obstacle was addressed by replacing immunofluorescence detection with a cell-viability end point. Cells were treated with a compound that living cells convert into formazan, a highly colored product. Exposure to aHUS plasma killed the cells and reduced the production of formazan. With this modification, the assay could be performed with a microplate reader.

However, the removal of complement regulators with phospholipase is cumbersome, incomplete, and transient because cells can resynthesize them. This last problem was overcome by using human myeloid TF–1 cells that lack PIGA, which is required to make the phosphatidylinositol anchors of complement inhibitors CD55 and CD59 on cells. With this optimized method, patients with aHUS were easily distinguished from various control groups during active disease and also during remission. Furthermore, with appropriate dilution, defects in complement-dependent cell killing were detected in patients with aHUS.
A paradigm shift in platelet transfusion therapy

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