Previously identified mechanisms of TKI-resistance in Ph⁺ ALL involve BCR-ABL1 mutations that prevent biochemical kinase inhibition and TKI-mediated survival signaling through activation of B-cell lymphoma.² The study by Mallampati et al demonstrates an unexpected side-effect of TKI treatment on MSCs that enables TKI resistance through the reinstatement of normal pre-B-cell cytokine signaling in Ph⁺ ALL cells. In genetic experiments based on inducible ablation of IL-7 signaling in Il7r⁻/⁻ BCR-ABL1-transformed ALL cells, the authors demonstrate the critical importance of this pathway. Indeed, IL-7-IL-7R signaling is a critical mediator of survival signaling in mouse pre-B cells. However, this is not the case in human B-cell development, and the MSC-secreted cytokine(s) that enable TKI resistance in human Ph⁺ ALL remain to be identified. Like IL-7 in mouse pre-B ALL cells, MSC-derived cytokines that enable TKI resistance in human Ph⁺ ALL likely signal through JAK activation. Importantly, the authors demonstrate that inhibition of JAK activity in combination with BCR-ABL1 inhibition can effectively short-circuit MSC-mediated TKI resistance (see figure). This concept is timely and important because it provides a mechanistic rationale for recent preclinical studies suggesting that combinations of dasatinib (BCR-ABL1-TKI) and ruxolitinib (a JAK inhibitor) may be effective in preventing drug resistance in Ph⁺ ALL.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
normal hematopoiesis during development, if at all, is currently unknown. Shi et al use the zebrafish as an easy genetic model to address this question.

Analysis of expression patterns and genomic locations of zebrafish (z)zidh1/2 shows conservation of these enzymes between zebrafish and mammalian IDH1/2. Using genetic approaches, the authors demonstrated that morpholino-injected embryos knocked down for zidh1/2 and the genetic mutant of zidh1 disrupt primitive myelopoiesis. Although the myeloid marker pu.1 was upregulated, myeloid markers of more differentiated cells were decreased, providing a hint for the involvement of zidh1/2 in myeloid differentiation (see figure).

Interestingly, restoring the expression of zidh1 could reverse this phenotype, but the mutated zidh1 that mimics mutations found in AML patients could not rescue the phenotype. Additionally, zidh1, but not zidh2, modulated the emergence of definitive hematopoietic stem cells, although the molecular mechanisms underlying this phenomenon remain unknown. Together, these data clearly implicate IDH enzymes in hematopoietic formation and differentiation during development in zebrafish.

Because these enzymes are involved in AML, recreating the disease in zebrafish can provide useful insight into leukemic conservation between species. To this end, we know from other studies that mutations in IDH1 and IDH2 are present in approximately 15% of cytogenetically normal AML samples. In almost all cases, IDH mutations are heterozygous, result in a gain-of-function phenotype, and occur mostly in 3 conserved arginine residues: IDH1-R132, IDH2-R140, and IDH2-R172. Normally, these enzymes catalyze the oxidative decarboxylation of isocitrate and produce α-KG in a manner dependent on reduced NAD phosphate. The mutations observed in AML patients confer a neomorphic enzymatic activity that leads to the reduction of α-KG to 2-HG. 2-HG competes with α-KG as cofactor for various enzymes, including ten-eleven translocases and JmjC histone demethylases, and abrogates their function. This results in epigenetic deregulation that contributes to AML pathogenesis, at least in part through impaired hematopoietic differentiation. The impact of IDH mutations in patients’ survival is not really clear, but it has been proposed that 2-HG levels can be used for screening and prognostic purposes.

Many questions clearly arise from the evidence above. Can IDH mutations confer to a leukemogenic phenotype in zebrafish? Is there conservation of leukemogenic potential between species? The authors readily answer these questions by providing evidence that expression of a mutant human IDH1 or its zebrafish orthologue recapitulates the phenotypic characteristics of human AML patients, such as increased levels of 2-HG, reduced 5-hydroxymethylcytosine, and expansion of progenitor hematopoietic cells. IDH-specific inhibitors like AGI-5198 could reverse the phenotype caused by the human mutation, indicating that drugs can be easily tested in a zebrafish model (see figure). Similar results were obtained with a mouse IDH1-R132H hematopoietic-specific knockin. Although this mutant displayed all the phenotypic features of AML patients, it failed to develop leukemia.

Even though this study is a clear step toward our understanding of the role of IDH enzymes in normal and malignant hematopoiesis, new challenges emerge. The authors provide ample evidence for the role of IDH enzymes in developmental hematopoiesis, but it would be interesting to study adult zidh knockout and mutant knockin animals. What kind of developmental pathways are perturbed in zidh knockouts? Recently, IDH enzymes have been implicated in deregulation of mitochondrial function involving BCL-2 as a potential therapeutic target. Is this phenotype recapitulated in zebrafish? The answers to these questions will shed additional light on the physiological roles of IDH enzymes and provide added evidence for the validity of modeling human diseases in zebrafish. Such evidence will open avenues to use the large-scale in vivo chemical screening capabilities of zebrafish for discovering novel therapeutic strategies. In addition, the ease of genetic manipulation of the zebrafish makes it an ideal model to study the cooperative effects of IDH enzymes with other genetic aberrations in a large-scale manner. This model can be used to perform studies on the role of IDH mutations in AML, initiation, maintenance, and progression, as was done for IDH2-R140Q in mice. In summary, the work from Shi et al offers the basis for a genetic model that can shed light on various aspects of AML.

Conflict-of-interest disclosure: The author declares no competing financial interests.
In conclusion, data by Sanders and coworkers highlight the utility of the assay of VWF:Ag ratio in the diagnosis of type 1, 2, and 3 VWD patients. Sanders and coworkers assessed VWF:Ag levels in type 3 VWD patients (VWF:Ag < 5 IU/dL). Although the majority of patients had undetectable VWF:Ag levels, VWF:Ag was detectable in 41% of patients, indicating that VWD in these patients was a result of extremely rapid clearance of VWF (type 1C VWD). Type 3 VWD patients lack VWF in plasma and also lack a regulated VWF storage pool (Weibel-Palade bodies). In contrast, type 1C VWD patients are expected to have normal regulated storage of VWF. The true type 3 VWD patients in this study were found to have a more severe bleeding phenotype than the VWD patients with increased VWF clearance, which may reflect the absence of a VWF storage pool.

The assay of VWF:Ag is clinically significant because identification of patients with reduced VWF survival affects treatment of these patients. Desmopressin is the most common treatment of type 1 VWD, releasing VWF from Weibel-Palade bodies to increase plasma VWF levels. Although type 1C VWD patients may release stored VWF, the released VWF is rapidly cleared from circulation. The VWF released in these patients may be insufficient to achieve hemostasis in minor bleeding situations but may not be adequate for surgery or major bleeding. Type 3 VWD patients require VWF replacement therapy rather than desmopressin administration. The routine assay of VWF:Ag in patients with VWF levels consistent with type 3 VWD (< 5 IU/dL) is likely to lead to reclassification of many as having type 1C VWD, which may expand the treatment options.

In conclusion, data by Sanders and coworkers highlight the utility of the assay of VWF:Ag ratio, and VWF:Ag ratio in suspected VWD patients. Sanders and coworkers, together with other previous publications, suggest this assay would be a valuable addition to the diagnostic panel, particularly in patients with low VWF:Ag levels. Although there may be limited utility in the diagnosis of type 2 VWD, the ability to identify true type 3, type 1C, and other type 1 VWD patients is clinically valuable.

REFERENCES

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Comment on Sanders et al, page 3006

VWF propeptide in defining VWD subtypes

Sandra L. Haberichter  BLOODCENTER OF WISCONSIN

In this issue of Blood, Sanders and coworkers define the pathophysiology of types 1, 2, and 3 von Willebrand disease (VWD) in the Willebrand Institute in the Netherlands (WiN) study by using the ratios of von Willebrand factor propeptide (VWFpp) or factor VIII activity to VWF antigen.

Von Willebrand factor (VWF) is synthesized in endothelial cells and megakaryocytes as pre-pro-VWF consisting of a 22–amino acid (aa) signal peptide, 741-aa VWFpp, and 2050-aa mature VWF molecule. After extensive intracellular modifications, VWF and VWFpp are packaged in endothelial cell Weibel-Palade bodies or platelet α-granules for regulated secretion. In plasma, VWFpp and VWF circulate independently of one another, with half-lives of 2 to 3 hours and 8 to 12 hours, respectively. The ratio of VWFpp to VWF antigen (VWF:Ag) can be used to assess the rates of synthesis, secretion, and clearance of VWF.

The ratio of FVIII coagulant activity (FVIII:C) to VWF:Ag has been used in a similar manner. An increased ratio of VWFpp/VWF:Ag defines reduced survival of VWF, and an increased ratio of FVIII:C/VWF:Ag suggests reduced VWF synthesis. The pathophysiology within VWD subtypes is diverse, including primarily increased VWF clearance, reduced VWF synthesis, a combination of increased clearance/reduced synthesis, and other undefined mechanisms (see figure, panel A). The relative contribution of each mechanism to phenotype is variable among subtypes (see figure, panel B). However, to definitively define the mechanism behind a particular phenotype associated with a specific VWF mutation would require in vitro expression studies, which is not feasible given the large number of VWF mutations that continue to be identified.

Perhaps the most striking use for the VWFpp assay is to identify true type 3 VWD patients. Sanders and coworkers assessed VWFpp levels in type 3 VWD patients (VWF:Ag < 5 IU/dL). Although the majority of patients had undetectable VWFpp levels, VWFpp was detectable in 41% of patients, indicating that VWD in these patients was a result of extremely rapid clearance of VWF (type 1C VWD). Type 3 VWD patients lack VWF in plasma and also lack a regulated VWF storage pool (Weibel-Palade bodies). In contrast, type 1C VWD patients are expected to have normal regulated storage of VWF. The true type 3 VWD patients in this study were found to have a more severe bleeding phenotype than the VWD patients with increased VWF clearance, which may reflect the absence of a VWF storage pool.

The assay of VWFpp is clinically significant because identification of patients with reduced VWF survival affects treatment of these patients. Desmopressin is the most common treatment of type 1 VWD, releasing VWF from Weibel-Palade bodies to increase plasma VWF levels. Although type 1C VWD patients may release stored VWF, the released VWF is rapidly cleared from circulation. The VWF released in these patients may be insufficient to achieve hemostasis in minor bleeding situations but may not be adequate for surgery or major bleeding. Type 3 VWD patients require VWF replacement therapy rather than desmopressin administration. The routine assay of VWFpp in patients with VWF levels consistent with type 3 VWD (<5 IU/dL) is likely to lead to reclassification of many as having type 1C VWD, which may expand the treatment options.

In conclusion, data by Sanders and coworkers highlight the utility of the assay of VWFpp level, VWFpp/VWF:Ag ratio, and FVIII:C/VWF:Ag ratio in suspected VWD patients. The assay of VWFpp is not routinely included in the diagnostic workup for VWD; however, the data presented by Sanders and coworkers, together with other previous publications, suggest this assay would be a valuable addition to the diagnostic panel, particularly in patients with low VWF:Ag levels. Although there may be limited utility in the diagnosis of type 2 VWD, the ability to identify true type 3, type 1C, and other type 1 VWD patients is clinically
Fish provide ID(H)eas on targeting leukemia

Eirini Trompouki