The molecular pathogenesis of MDS and the mechanism of its transformation to AML are not well understood. Although several mouse models of MDS have been developed recently, most fail to recapitulate key features of the human disease. In one of the best genetically defined systems, transplantation of bone marrow cells retrovirally transduced with Evil results in bone marrow failure, erythroid dysplasia, and evidence of increased apoptosis. Despite reproducing these hallmarks of MDS, the mice do not develop AML, suggesting that additional genetic events are required for full transformation.

The AML1 gene is a frequent target of mutations and rearrangements in human leukemia. Although point mutations of AML1 are rare in most AML French–American–British (FAB) subtypes, they are relatively common in M0 AML (12%–33%), MDS (23%), and in therapy–related and radiation–associated MDS/AML (38%–46%). In this issue of Blood, Watanabe-Okochi and colleagues use a murine retroviral transduction/bone marrow transplantation model to characterize 2 mutant AML1 alleles previously identified by their group: a D171N missense mutation in the latent domain that reduces DNA binding and transcriptional activity. Interestingly, although reconstitution of lethally irradiated mice with cells transduced by either allele die with similar kinetics (latency of ~150 days and penetrance of 70%–80%), the disease phenotypes are strikingly different. The AML1D171N mice develop dysplasia and pancytopenia with infrequent progression to AML. The AML1D171N mice have a more proliferative phenotype marked by leukocytosis, hepatosplenomegaly, dysplasia, and more frequent progression to AML. In nearly half the AML1D171N cases, the authors mapped retroviral integration sites to the 5’ flanking region of Evil and were able to demonstrate that this was associated with Evil overexpression. They went on to show that coinfection of cells with AML1D171N and Evil retroviruses shortened disease latency by 50 days and increased penetrance to 100%, providing additional evidence that Evil and mutant AML1 can cooperate to induce MDS/AML in mice.

Although the mechanism of Evil activation is artificial in this model (retrovirus-mediated insertional mutagenesis), the observations by Watanabe-Okochi and colleagues have important clinical relevance. Evil is deregulated by the t(3;3)(q21;q26) and inv(3)(q21;q26) rearrangements in MDS and AML. Several lines of evidence have also directly linked AML1 and Evil in human leukemia biology. First, the genes are fused by the t(3;21)(q26;q22) rearrangement in blast-crisis CML. In addition, the proteins physically interact, leading to reduced AML1 DNA binding and transcriptional activity. This leads to the prediction, not tested in the current study, that Evil and the AML1D171N allele cooperate in vivo because they conspire to reduce AML1 activity. If further studies support this model, there would be a rational basis for developing therapies that target this protein–protein interaction.

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

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time. In this issue of Blood, Dominici and colleagues use a murine model for conducting a comprehensive set of serial transplantations to determine whether this outcome is related to the transplantability of cells with limited osteopoietic potential, or rather due to the engraftment of cells with robust potential that are nevertheless subject to regulatory influences, which prevent sustained therapeutic levels of donor osteopoiesis. This elegant study demonstrates that normal bone marrow donor stem cells with osteopoietic potential home and engraft after transplantation, but that these cells offer only limited regenerative contribution to host osteopoiesis. Although the precise mechanism for this lack of sustained contribution remains unclear, the authors use serial transplantation to suggest that it is not due to a limited cell potential, but rather results from either intrinsic cell or extrinsic microenvironmental regulation. Together with other recent studies, this work demonstrates that MSCs are transplantable intravenously, do engraft within the bone marrow, and do contribute to osteopoiesis. However, this work highlights the lack of sustained contribution in any therapeutic use of these cells as a critical issue for the future long-term treatment of bone disorders. Further studies will be required to determine what causes the lack of durable donor-derived osteopoiesis, and will hopefully lead to treatments that will allow the widespread use of marrow transplantation to treat bone disorders.

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CLINICAL OBSERVATIONS
Comment on Ataga et al, page 3991

Gardos pathway to sickle cell therapies?

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In this issue of Blood, Ataga and colleagues report that treatment of sickle cell disease patients with senicapoc, a Gardos channel inhibitor, reduces the number of dehydrated cells, increases hemoglobin levels, and diminishes hemolysis.

The journey from laboratory bench to this clinical study began half a century ago with 2 independent observations. In 1958, the Hungarian physiologist Gyorgy Gárdos described calcium-dependent potassium loss from red cells.1 The “Gardos pathway” is now known to be mediated by a calcium-activated K channel. D. C. Tosteson’s seminal studies of abnormal cation content and permeability in sickle cells2 led to the characterization of dehydrated cells with high hemoglobin concentration. The importance of these dehydrated cells was reinforced by subsequent discoveries that polymerization is exquisitely sensitive to Hb S concentration, and that dehydrated sickle cells are very short-lived, selectively trapped in the microcirculation, and removed during vaso-occlusive episodes.

Sickle cell dehydration is thought to result from a complex interplay of Hb S polymerization and several cation transport systems in sickle cells. A transport pathway that normally regulates volume in reticulocytes, the potassium-chloride cotransporter (KCC) appears to function pathologically in sickle cells, overshooting its target hemoglobin concentration1 and priming the reticulocyte to sickle. Hb S polymerization activates a nonselective cation leak pathway in a fraction of sickle cells upon deoxygenation. Calcium entry via this sickling-induced pathway triggers activation of the Gardos channel, which mediates rapid KCl and water loss.4 Abnormal KCC activity in the sickle reticulocyte may thus facilitate a vicious spiral in which sickling and Gardos channel activation reinforce each other to dehydrate the cell. In vitro and animal studies have been insufficient, however, to elucidate how these pathways interact in vivo. Brugnara’s pioneering clinical investigation of another Gardos channel blocker, clotrimazole,5 laid the foundation for the development of senicapoc. The demonstration in the current study that senicapoc reduces the number of dense sickle cells establishes conclusively that the Gardos pathway is active in vivo and contributes to sickle cell dehydration.

Ataga and colleagues show that senicapoc treatment was well-tolerated, resulted in increased hemoglobin, and reduced markers of hemolysis—reticulocyte count, bilirubin, LDH levels—strongly suggesting that sickle cell survival was improved. Thus, the study demonstrates that prevention of dehydration in a clinical setting is feasible and decreases in vivo hemolysis in sickle disease.

Recently, a phase 3 trial of senicapoc was terminated early because of low probability of achieving a reduction in crisis rate, the primary
Osteopoietic stem cells: transplantable, but regeneratively limited

Susie Nilsson