The molecular pathogenesis of MDS and 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 Evi1 results in bone marrow failure, erythroid dysplasia, and evidence of increased apoptosis.1 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 (12%-33%), MDS (23%), and in subtypes, they are relatively common in M0. Although point mutations of AML1 are rare in most AML French-American-British (FAB) subtypes, they are relatively common in M0 (12%-33%), MDS (23%), and in subtypes, they are relatively common in M0. Although point mutations of AML1 and Evi1 retroviruses shortened disease latency by 50 days and increased penetrance to 100%, providing additional evidence that AML1 and mutant AML1 can cooperate to induce MDS/AML in mice. Although the mechanism of Evi1 activation is artificial in this model (retrovirus-mediated insertional mutagenesis), the observations by Watanabe-Okochi and colleagues have important clinical relevance. Evi1 is deregulated by the t(3;3)(q21; q26) and inv(3)(q21;q26) rearrangements in MDS and AML.3,4 Several lines of evidence have also directly linked AML1 and Evi1 in human leukemia biology. First, the genes are fused by the t(3;21)(q26;q22) rearrangement in blast-crisis CML.5 In addition, the proteins physically interact, leading to reduced AML1 DNA binding and transcriptional activity.6

This leads to the prediction, not tested in the current study, that Evi1 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.

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Osteopoietic stem cells: transplantable, but regeneratively limited

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Questions about the transplantability of mesenchymal stem cells, their ability to engraft within the bone marrow of recipients, and hence their clinical usefulness have been hotly debated for several decades. In this issue of Blood, Dominci and colleagues demonstrate robust serial osteopoietic engraftment, but highlight that osteopoietic chimerism declines to negligible levels after 6 months.

Due to the presence of hematopoietic stem cells in the bone marrow, bone marrow transplantation has been successfully used to treat hematological diseases for many decades. Based on the concept that bone marrow also contains microenvironmental or mesenchymal stem cells (MSCs) with osteopoietic potential, the clinical use of bone marrow transplantation to treat bone disorders is also very appealing. Although initial studies suggested that MSCs cannot be transplanted intravenously, more recently documented animal models and preclinical studies have demonstrated that donor MSCs engraft in the bone marrow and give rise to bone and muscle.1,4 Furthermore, a series of clinical trials performed by this group,5,6 involving children with severe osteogenesis imperfecta, demonstrated that transplanting unmanipulated bone marrow resulted in a marked improvement in total body mineral content, accelerated linear growth, and decreased fracture rates. Similar results were evident following transplantation of purified MSCs. In all of these studies, however, the observed accelerated growth diminished over...
time. In this issue of Blood, Dominici and colleagues use a murine model for conducting a comprehensive set of serial transplantsations 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.

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

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The journey from laboratory bench to this clinical study began half a century ago with 2 independent observations. In 1958, the Hungarian physiologist Gyorgy Gardos described calcium-dependent potassium loss from red cells. 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 cells led to the characterization of dehydrated cells with high hemoglobin concentration. The importance of these dehydrated cells was reinforced by subsequent discoveries that polymericization 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 concentration 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. 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, 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

**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.
Osteopoietic stem cells: transplantable, but regeneratively limited

Susie Nilsson