capacity of HSCs once they are transplanted in vivo.\(^6\) In this paper, the murine HSCs were transduced directly in vivo without ex vivo manipulation. The study found that 2.45% of bone marrow cells and 7.7% of progenitor LSK cells in hCD46tg mice expressed the transduced gene 4 weeks after transduction; \(~0.5\%\) of huCD45\(^+\) cells and 1.5% of CD34\(^+\) cells in the bone marrow of humanized mice expressed the transduced gene 3 days after transduction, and \(2.5\%\) of huCD45\(^+\) cells in bone marrow expressed the transduced gene 4 week after transduction. The transduction efficiency was not as high as those reported in clinical trials using lentiviral vectors and ex vivo cell processing.\(^7,8\) However, gene-corrected cells may have a selective growth advantage over parental HSCs with defects, and gene therapy can be successful even when only a small fraction of the transplanted HSC has been transduced by the vector.\(^9\) Therefore, this system may prove to be a valuable method of clinical gene therapy.

Several questions must be addressed before translating the findings of this paper to clinical trials. The mobilization regimen in this paper used 4 doses of G-CSF and 1 dose of AMD3100, which resulted in variable numbers of mobilized CD34\(^+\) cells in hCD46tg mice and humanized mice. Because Plerixafor (AMD3100) and G-CSF mobilize different CD34\(^+\) cell populations from bone marrow,\(^9\) it would be interesting to test the transduction efficiency of this system on HSCs that are only mobilized by G-CSF. The increase of CD34\(^+\) HSCs in blood is transient, and the CD34\(^+\) cell counts may fall quickly if a daily dose of G-CSF is not given, so monitoring the white blood cell count and CD34\(^+\) counts daily will be helpful in evaluating the mobilization efficiency and deciding on the best time to conduct gene transduction.

The safety of the newly developed transduction system needs to be comprehensively evaluated. Early-stage gene therapies with retroviral vectors have been associated with side effects, such as leukemia, although lentiviral vectors are more efficient and safer.\(^2\) In this paper, the authors identified 155 distinct SB100x-mediated integration sites and no integration within or near a proto-oncogene, but it appears that chromosome 13 had more integration sites than the others, so it would be beneficial to further investigate the genotoxicity of this system. In addition, the toxicities of the adenoviral vectors must be carefully assessed. Although the Ad5/35\(^+\) vector was not detected in the liver, the genome for some adenoviral vectors can be found in the lung, liver, heart, kidney, and spleen. Off-target toxicities are especially worrisome for adenoviral vectors due to the death of a patient in a gene therapy protocol using adenoviral vectors in 1999.\(^{10}\)

In summary, the work of Richter provides promising results in the development of in vivo gene transduction system, which may simplify gene therapy by eliminating autologous cell collection and ex vivo cell manipulation. After further characterization and proof of the method’s safety, it may prove to be useful for clinical gene therapy.

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Comment on Rauch et al, page 2253

Down for the count in acute myeloid leukemia

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In this issue of Blood, Rauch et al provide evidence for a novel mechanism to explain a fundamental yet enigmatic observation that has plagued hematologists for decades: the decline in nonleukemic hematopoiesis in the bone marrow of patients with acute myeloid leukemia (AML). The authors found that high expression of the thrombopoietin (TPO) receptor MPL on AML blasts predicts neutropenia and thrombocytopenia, and that AML blasts expressing high levels of MPL deplete TPO in cell culture and in mouse models. Rather than crowding out normal bone marrow hematopoietic stem cells (HSCs), might MPL-expressing AML blasts impair hematopoiesis by stealing the cytokine TPO?\(^1\)

Most patients with AML present with cytopenias in 1 or more cell lineages. However, the mechanisms by which AML impairs normal hematopoiesis have remained elusive. Is it simple competition for nutrients or space? Why do some patients with a bone marrow full of blasts have relatively preserved blood counts, whereas others experience life-threatening cytopenias with far less bone marrow involvement?

Previous studies reveal multiple mechanisms by which AML blasts might induce cytopenias, including inhibition of normal HSC proliferation and differentiation.\(^2,3\) Leukemic cells also alter the stromal microenvironment, creating abnormal malignant niches that sequester normal
At the level of clearance, platelet and megakaryocyte expression of the TPO receptor MPL lead to MPL-mediated TPO endocytosis and TPO removal. The balance of these mechanisms enables TPO and platelet homeostasis in normal individuals.

Rauh et al showed no significant correlation between TPO serum concentration and platelet count in AML patients. This lack of correlation was driven by AML patients with severe thrombocytopenia who had lower than expected TPO serum levels. Given the models of TPO homeostasis described previously, severe thrombocytopenia should result in increased circulating TPO. However, consistent with prior studies, the authors found that selected AML patients with severe thrombocytopenia and low TPO levels had high expression of the TPO receptor MPL on AML blasts. Might high MPL levels on AML blasts clear TPO and thereby lead to cytopenia?

From a mechanistic perspective, the authors provided evidence that AML blasts with high expression of MPL (MPL\textsuperscript{hi}) could deplete TPO levels in tissue culture. Xenotransplantation of MPL\textsuperscript{hi} AML cells vs MPL\textsuperscript{lo} AML cells also demonstrated in vivo depletion of TPO by MPL\textsuperscript{hi} AML cells in a humanized TPO mouse model.

In microarray analysis of bone marrow from AML patients, expression of MPL and TPO pathway genes were enriched in patients with severe thrombocytopenia (0–20) at diagnosis compared with patients with platelet counts of 50 to 100. Notably, patients with MPL\textsuperscript{hi} AML blasts had significantly lower platelet counts and ANC (see figure). Consistent with previous observations, mean MPL expression was also higher in t(8;21) than in other AML cytogenetic and molecular subgroups. Accordingly, platelet counts and ANC were lower in t(8;21) AML. However, no difference in hemoglobin concentration was seen between patients with MPL\textsuperscript{lo} vs MPL\textsuperscript{hi} AML, or between t(8;21) and other AML subgroups, suggesting that anemia in AML patients is driven by mechanisms independent of AML MPL expression.

This provocative work raises multiple questions. Given the in vivo depletion of TPO by MPL\textsuperscript{hi} blasts in a mouse model, do AML patients with MPL\textsuperscript{hi} t(8;21) blasts have lower serum levels of TPO? Why is high MPL expression on AML blasts associated with thrombocytopenia and neutropenia, but not with anemia? As adult erythropoiesis is significantly regulated by erythropoietin production in the kidneys, this distinct homeostatic mechanism might account for the independence of anemia from MPL expression in AML patients. Finally, how can we reconcile the TPO-scavenging model of AML cytopenias with existing data showing that AML cells inhibit normal HSC proliferation and differentiation and alter the bone marrow microenvironment?

The biology of TPO and MPL is complex, and TPO and its receptor MPL play pleiotropic and far more important roles in regulating hematopoiesis than simply triggering thrombopoiesis. TPO can both induce expansion of HSCs but also paradoxically maintain HSC quiescence, possibly by operating at different signaling levels. Clinically, the TPO mimic eltrombopag promotes trilineage hematopoiesis in patients with aplastic anemia. It is therefore possible that loss of TPO in AML patients might partially explain the inhibition of normal HSC and progenitor cell proliferation and differentiation. If MPL-mediated TPO scavenging is indeed a major driver of anemia and neutropenia in AML patients, could additional TPO rescue normal HSCs? Might TPO itself inhibit expression of the HSC quiescence-inducing transcription factor Egr3? These concepts provide fertile ground for laboratory experiments. However, given the potentially growth-promoting properties of TPO for AML blasts, caution is warranted when considering the use of TPO in AML patients with cytopenias. As an alternative, blast eradication with induction chemotherapy serves as an effective method for increasing TPO levels in AML patients with MPL-expressing blasts.

Although it is unlikely that TPO scavenging by MPL is the only determinant of cytopenias in AML patients, the work by Rauh et al supports an intriguing and novel model to explain the impairment of normal hematopoiesis in patients with AML. By stealing TPO, high levels of MPL on AML blasts might leave AML patients down for the count.

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