

robust, and simplified enough to be acceptable for clinical development.^{5,6} This is where the fact that the academia-industry interface has been present in this work since its inception can prove extremely useful.

Second, this iterative process will benefit from the methodological advances of others, for example, recent use of ZFNs for integration of corrective DNA into the interleukin 2 receptor γ gene in HSCs from an individual with X-linked severe combined immunodeficiency.⁷ Third, gene therapy technology needs to be developed simultaneously with protocols to sustain and maintain autologous HSCs through the collection and transgenesis steps and with a clinical transplantation trial to mediate their significant and permanent engraftment.⁸

Hematopoietic cell transplantation (HCT) was the first gene therapy.⁹ It has been used as a life-saving measure for multiple genetic disorders (in some individuals with SCA, for example, or in mucopolysaccharidosis type I, Fanconi anemia, dyskeratosis congenita, genetic forms of immune deficiencies, adrenoleukodystrophy, epidermolysis bullosa, etc). Viral-mediated gene therapy is already in clinical trials (or in preclinical development) for a majority of them. Gene editing has the potential to be the next conceptual step in gene therapy. Critically, even partial gene correction is likely to be clinically meaningful, as SCA heterozygotes are typically free of disease symptoms, and recipients of HCT with mixed chimerism can derive significant clinical benefits from as little as one-fifth donor hematopoietic cells.

With a quarter of a million new cases each year, SCA is a tremendous health care challenge worldwide. It results in massive human suffering, from pain caused by capricious and sometimes intractable vaso-occlusive or sequestration crises to the chronic stress of dealing with infections, chronic hemolysis, and progressive multiorgan system complications. The current hope is that editing out the one SCA-causing genomic misprint—along with other treatment measures—will relieve those living with it from numerous sources of pain, among them spleen sequestration. Xavier Bichat said that human disease is “a revolt of organs,” and health is “their silence.”¹⁰ If this is true, gene editing may give SCA sufferers symptom-free internal organs, including a “silent spleen,” and, by removing the cause of the

disease, give them fuller, longer, more complete lives.

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

REFERENCES

- Hoban MD, Cost GJ, Mendel MC, et al. Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. *Blood*. 2015;125(17):2597-2604.
- Pauling L, Itano HA, et al. Sickle cell anemia, a molecular disease. *Science*. 1949;109(2835):443.
- Ingram VM. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. *Nature*. 1956;178(4537):792-794.
- Crick FH, Barnett L, Brenner S, Watts-Tobin RJ. General nature of the genetic code for proteins. *Nature*. 1961;192(4809):1227-1232.

5. Osborn MJ, DeFeo AP, Blazar BR, Tolar J. Synthetic zinc finger nuclease design and rapid assembly. *Hum Gene Ther*. 2011;22(9):1155-1165.

6. Gabriel R, Lombardo A, Arens A, et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nat Biotechnol*. 2011;29(9):816-823.

7. Genovese P, Schirotti G, Escobar G, et al. Targeted genome editing in human repopulating haematopoietic stem cells. *Nature*. 2014;510(7504):235-240.

8. Tolar J, McGrath JA. The three-body problem of therapy with induced pluripotent stem cells. *Genome Med*. 2015;7(1):15.

9. Tolar J, Mehta PA, Walters MC. Hematopoietic cell transplantation for nonmalignant disorders. *Biol Blood Marrow Transplant*. 2012;18(1 Suppl):S166-S171.

10. Bichat MF. *Recherches physiologiques sur la vie et la mort*. 3rd ed. Paris: Brosson/Gabon, 1805.

© 2015 by The American Society of Hematology

● ● ● MYELOID NEOPLASIA

Comment on Bowers et al, page 2678

Osteoblast ablation burns out functional stem cells

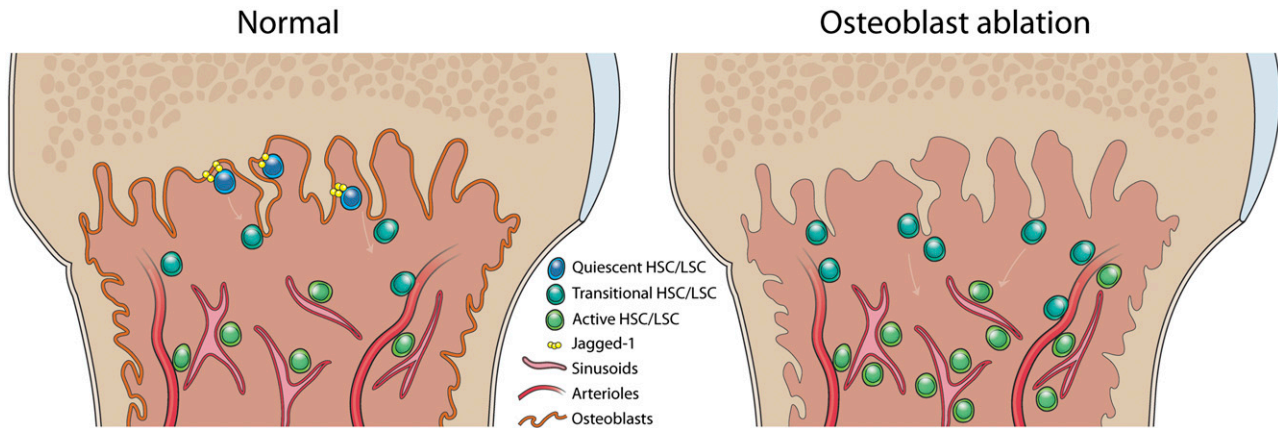
Meng Zhao¹ and Linheng Li^{1,2} ¹STOWERS INSTITUTE FOR MEDICAL RESEARCH; ²UNIVERSITY OF KANSAS MEDICAL CENTER

In this issue of *Blood*, Bowers et al report that osteoblasts maintain a subset of quiescent stem cells and that osteoblast ablation converts bone marrow into a proliferation-promoting environment for both normal and malignant stem cells.¹

Hematopoietic stem cells (HSCs) are thought to localize in a discrete bone marrow microenvironment, or niche, which is critical for their maintenance and regulation. In the long-standing search for HSC supporting stromal cells, osteoblasts and bone-lining cells in the endosteal zone of trabecular bone were initially identified through functional genetic studies.^{2,3} Subsequent studies have shown that large numbers of HSCs often localize near endothelial and/or perivascular cells in the central marrow, which together constitute the perivascular niche.⁴⁻⁶ Functionally, deleting critical HSC regulation factors from the perivascular niche dramatically reduces HSC abundance in the bone marrow; however, significant HSC function is still maintained, as determined by a bone marrow transplantation assay.⁷ Other studies have reported that a rare HSC subpopulation with deep quiescence and long-term self-renewal potential is located in the trabecular bone area and has a robust

capacity for recovering hematopoiesis.^{8,9} This indicates the possible existence of a reserve HSC population, which is maintained in the endosteal zone instead of the perivascular niche.

By using a collagen $\alpha 1$ type 1 promoter-mediated long-term ablation system, Bowers et al investigated the role of osteoblasts from the endosteal zone in HSC maintenance and in leukemia development. Osteoblast ablation did not lead to a significant reduction of the HSC (defined by CD150⁺FLk2⁻CD48⁻LSK) pool size. Instead, there was a slight increase in HSC and progenitor numbers. Although this is largely consistent with a previous study which found that the bulk of the HSC pool localizes in the perivascular niche, deleting HSC regulation factors from osteoblasts may not have significant effects on overall HSC number.⁷ The increase in HSCs and progenitors can be explained by osteoblast ablation causing a subset of HSCs to lose quiescence and enter into cell cycle for



Different stem cells are maintained in different niche zones in bone marrow. After osteoblast ablation, the quiescent HSC subset is lost, and bone marrow is converted into a proliferation-promoting microenvironment.

proliferation and differentiation. Indeed, through careful and detailed characterization of HSC subpopulations, Bowers et al observed that a rare CD49b⁻CD229⁻-marked quiescent HSC subpopulation was significantly reduced after osteoblast ablation. Losing this subset of HSCs resulted in reduced stem cell engraftment, particularly over the long term, which is consistent with the concept that a reserve HSC subpopulation is critical for long-term hematopoiesis maintenance.¹⁰ Overall, this study sheds light on a controversy in HSC niche studies: whether and how the osteoblastic niche regulates HSCs. Feasibly, the osteoblasts in the endosteal zone either directly or indirectly maintain a reserve HSC subpopulation, which is low in number but significantly contributes to HSC long-term function.

Bowers et al further investigated the consequences of osteoblast ablation in the context of leukemogenesis. In the tested chronic myelogenous leukemia model, osteoblast ablation resulted in increased malignant proliferation and accelerated leukemia development, suggesting a conversion in bone marrow into a proliferation-promoting microenvironment. However, leukemic cells from this converted microenvironment had impaired leukemogenesis capacity in the secondary transplantation recipients, which suggests the osteoblastic niche may also contribute to preserving leukemia stem cells (LSCs). In the future, both whether and how the endosteal zone directly contributes to LSC maintenance and drug resistance will need to be studied. Consistent with a previous study,⁹ Bowers et al also show that Jagged-1, which is highly

expressed in the endosteal zone of trabecular bone area, facilitates the maintenance of both normal and malignant stem cells in vitro.

In conclusion, Bowers et al provide strong evidence to verify the role of the osteoblastic niche in the endosteal zone in maintaining the quiescence and long-term self-renewal potential of normal HSCs and preserving LSCs in the leukemia model. This study also supports the concept that different HSC subpopulations are maintained in different niches in bone marrow (see figure).

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Bowers M, Zhang B, Ho Y, Agarwal P, Chen C-C, Bhatia R. Osteoblast ablation reduces normal long-term hematopoietic stem cell self-renewal but accelerates leukemia development. *Blood*. 2015;125(17):2678-2688.
2. Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003;425(6960):836-841.
3. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003;425(6960):841-846.
4. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*. 2005;121(7):1109-1121.
5. Kunisaki Y, Bruns I, Scheiermann C, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*. 2013;502(7473):637-643.
6. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity*. 2006;25(6):977-988.
7. Ding L, Saunders TL, Enikolopov G, Morrison SJ. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. 2012;481(7382):457-462.
8. Wilson A, Laurenti E, Oser G, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell*. 2008;135(6):1118-1129.
9. Guezguez B, Campbell CJ, Boyd AL, et al. Regional localization within the bone marrow influences the functional capacity of human HSCs. *Cell Stem Cell*. 2013;13(2):175-189.
10. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. *Science*. 2010;327(5965):542-545.

© 2015 by The American Society of Hematology

● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Magwenzi et al, page 2693

Disabling the platelet's brakes to promote thrombosis

Roy L. Silverstein MEDICAL COLLEGE OF WISCONSIN

In this issue of *Blood*, Magwenzi et al from the University of Hull report a novel mechanistic connection between oxidized low-density lipoprotein (oxLDL)-induced prothrombotic platelet signaling and the inhibition of endogenous platelet anti-activating signaling mediated by the nitric oxide (NO)/guanosine 3',5'-cyclic monophosphate (cGMP)/protein kinase G (PKG) pathway.¹