clinical questions such as which patients are best selected for ibrutinib treatment and who would benefit from prophylaxis. The overall incidence of these infections is low enough that routine prophylaxis for all patients with CLL who are taking single-agent ibrutinib would be excessive, especially given the cost and drug interactions with ibrutinib. However, prophylaxis is reasonable in select patients with additional risk factors. More work such as the 5-year follow-up by O'Brien et al[2] will need to be done to better identify who might benefit from prophylaxis and to define the secondary factors that add to risk.

Clinicians need to stay vigilant for signs of aspergillosis and other fungal infections in their ibrutinib-treated patients so that these serious infections are rapidly diagnosed and treated. Although it is important to understand the risks of any therapy, ibrutinib remains the best option for treating the malignancies of many patients and, in most cases, risk for invasive fungal infections should not deter its use.

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

REFERENCES

1. Ghez D, Calleja A, Protin C, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. *Blood.* 2018;131(17):1955-1959.
2. O'Brien S, Furman RR, Coutre S, et al. Single-agent ibrutinib in treatment-naïve and relapsed/refractory chronic lymphocytic leukemia: a 5-year experience. *Blood.* 2018; 131(17):1910-1919.
3. Brown JR. Ibrutinib: coming of age? *Blood.* 2018;131(17):1880-1882.
4. Ahn IE, Jerussi T, Farooqui M, Tian X, Wiestner A, Gea-Banacloche J. Atypical Pneumocystis jirovecii pneumonia in previously untreated patients with CLL on single-agent ibrutinib. *Blood.* 2016;128(15):1940-1943.
5. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with ibrutinib and other small molecule kinase inhibitors targeting immune signaling pathways. *Clin Infect Dis.* 2018;66(1):140-148.
6. Lionakis MS, Dunleavy K, Roschewski M, et al. Inhibition of B cell receptor signaling by ibrutinib in primary CNS lymphoma. *Cancer Cell.* 2017;31(6):833-843.e5.
7. Rogers KA, Luay M, Zhao Q, et al. Incidence and type of opportunistic infections during ibrutinib treatment at a single academic center [abstract]. *Blood.* 2017;130(Suppl 1). Abstract 830.
8. Sun C, Tian X, Lee YS, et al. Partial reconstitution of humoral immunity and fewer infections in patients with chronic lymphocytic leukemia treated with ibrutinib. *Blood.* 2015;126(19):2213-2219.
9. Honigberg LA, Smith AM, Sirisawad M, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci USA.* 2010; 107(29):13075-13080.
10. Dubovsky JA, Beckwith KA, Natarajan G, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood.* 2013;122(15): 2539-2549.

DOI 10.1182/blood-2018-02-832154

RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Antoniani et al, page 1960

A chance to cut (the genome) is a chance to cure

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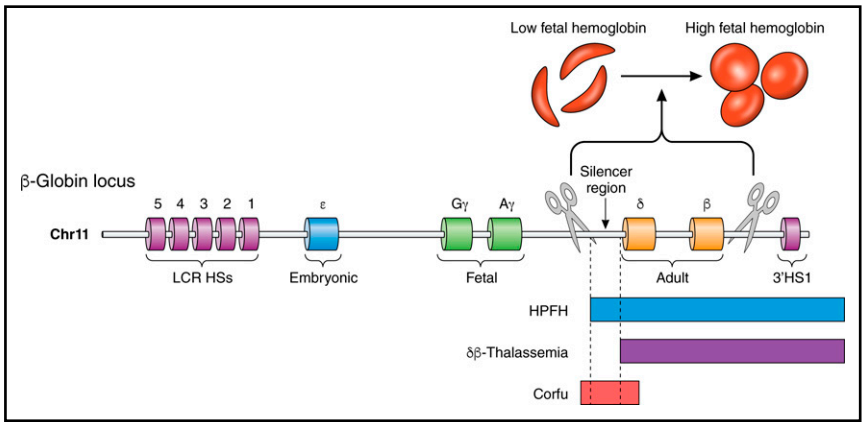
In this issue of *Blood*, Antoniani et al identify an innovative genome editing approach to induce fetal hemoglobin (HbF), which may eventually lead to therapeutic strategies for ameliorating or curing sickle-cell disease (SCD) and β-thalassemia.[1]

Significant advances have been made in deciphering the molecular underpinnings of hemoglobin switching. These findings hold substantial promise for being able to identify improved approaches for HbF induction to treat SCD and β-thalassemia.[2] However, for patients with these diseases, treatment remains predominantly palliative, with allogeneic hematopoietic stem cell (HSC) transplantation being the only curative therapy available. Experimental gene therapy has shown promise, but these approaches have a number of limitations, including concerns about the inability to produce sufficient hemoglobin by randomly integrating lentiviral transgenes. With the recent explosion of genome editing tools, including clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) (CRISPR/Cas9), there is potential for endogenous correction of pathogenic mutations, which could overcome the challenges present with HSC transplantation or gene therapy.

The hematopoietic system, in particular, is uniquely poised to host major advances in genome editing. Pursuing this strategy in HSCs circumvents many current obstacles



Induction of HbF by a genome editing-based deletion can ameliorate SCD. This illustration depicts the human β-globin locus on chromosome 11 (chr11) with a 3.5-kb silencer region upstream of the δ-globin gene. Typical deletions implicated in HPFH and δβ-thalassemia, as well as the Corfu thalassemia deletion, are illustrated below the locus. The schematic shows that disruption of the silencer region, in addition to the δ- and β-globin genes, using genome editing tools (depicted as scissors) can lead to a robust elevation in HbF production and ameliorate the SCD phenotype. HSs, hypersensitivity sites; 3'HS1, downstream hypersensitivity site; LCR, locus control region. Professional illustration by Patrick Lane, ScEYEnce Studios.

facing genome editing in other tissue types. HSCs can be manipulated ex vivo and then delivered effectively to patients.[2] Groundbreaking studies have illustrated how disruption of endogenous genes in human HSCs using genome editing can be therapeutically valuable, and genetic studies have been critical in guiding these approaches. One important example is the inactivation of the HIV coreceptor CCR5 to treat individuals with acquired immunodeficiency syndrome, a strategy that was motivated by identifying individuals resistant to HIV infection who had natural deletions in CCR5.[2]

In the case of SCD and β-thalassemia, correction of the causal mutations could be achieved using genome editing. However, such approaches are challenging to perform in bulk HSC populations, where the majority of genome editing events result in gene disruption.[3,4] This has motivated the search for alternative strategies that take advantage of the high frequency of genomic disruption events. For example, an erythroid enhancer of the key regulator of HbF, BCL11A,[5] has been a target of potential genome editing-based approaches.[6,7] There have also been efforts to recapitulate naturally occurring hereditary persistence of fetal hemoglobin (HPFH) deletions found in rare individuals. CRISPR/Cas9-mediated recreation of a 12.9-kb Sicilian HPFH deletion or a short 13-bp deletion in the γ-globin promoters in human hematopoietic progenitors has enabled effective HbF induction.[8,9]

Although these studies are promising, the actual effectiveness of such approaches remains uncertain. These deletions have only been reported in 1 or a few patients, and therefore, the extent of HbF induction that could be achieved by recreating the deletions in vivo is unclear. Several years ago, we had analyzed a large group of HPFH and δβ-thalassemia deletions, with the former having higher HbF levels than the latter, which are insufficient to compensate for globin chain imbalance.[10] These mapping studies had allowed us to identify a 3.5-kb region upstream of the δ-globin gene that was proposed as a potential silencer region (see figure). This region was intact in individuals with lower HbF levels because of δβ-thalassemia deletions but was absent in those with higher HbF levels because of HPFH deletions. Interestingly, this region appears to be bound by BCL11A and its cofactors,[5,10] suggesting a potential mechanism of action. However, the functionality of these regions has remained untested.

In the present study, Antoniani et al directly address this question of functionality by examining how specific genomic modifications in the β-globin locus can result in HbF induction. Specifically, the authors use a combination of deletional mapping and epigenomic data to select 3 target regions for disruption with CRISPR/Cas9 genome editing: (1) the 3.5-kb silencer region itself; (2) a 7.2-kb deletion (the known "Corfu" deletion), which includes the silencer region and the δ-globin gene; and (3) a 13.6-kb deletion that removes the silencer region, as well as the δ- and β-globin genes (see figure). Interestingly, the authors observe that the 3.5-kb and 7.2-kb deletions caused little or no HbF induction, whereas deletion of the 13.6-kb region led to a robust elevation of HbF, suggesting that an element of gene competition between the fetal and adult globin genes may limit the degree of HbF induction that can be achieved. Moreover, the authors demonstrate that the creation of the 13.6-kb deletion alters chromatin conformation patterns and can ameliorate the sickling phenotype in SCD patient–derived primary erythroid cells. A fascinating observation is that inversion of this 13.6-kb region appears to be as effective for HbF induction as deletion of this region, suggesting that conformation of this region has a critical role in its activity.

This work is exciting and raises a number of important questions for future studies. The reason why removal of the silencer region with or without the adult globin genes can have such a variable impact on HbF levels will require further study. In addition, the exact necessity of specific elements within the targeted 13.6-kb region will require further mechanistic dissection. Finally, the reason why inversion of this region is as effective as deletion is unclear with current models of globin gene regulation, emphasizing the critical need for further studies. In addition to the mechanistic implications, the findings reported here suggest a highly effective and potentially valuable genome editing strategy for treatment of SCD and β-thalassemia. Further preclinical studies will be needed, such as through the use of improved immunodeficient xenograft models, but the work reported here is promising and may even be more effective than other proposed editing strategies that are under clinical consideration.[6,9] This paper nicely illustrates how by utilizing experiments of nature, we can better understand the molecular basis for a wide range of biological processes and define effective therapeutic opportunities.

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

REFERENCES

1. Antoniani C, Meneghini V, Lattanzi A, et al. Induction of fetal hemoglobin synthesis by CRISPR/Cas9-mediated editing of the human β-globin locus. *Blood*. 2018; 131(17):1960-1973.
2. Sankaran VG, Weiss MJ. Anemia: progress in molecular mechanisms and therapies. *Nat Med*. 2015;21(3):221-230.
3. DeWitt MA, Magis W, Bray NL, et al. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Sci Transl Med*. 2016;8(360):360ra134.
4. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*. 2016; 539(7629):384-389.
5. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. *Science*. 2008;322(5909): 1839-1842.
6. Chang KH, Smith SE, Sullivan T, et al. Long-term engraftment and fetal globin induction upon *BCL11A* gene editing in bone-marrow-derived CD34$^+$ hematopoietic stem and progenitor cells. *Mol Ther Methods Clin Dev*. 2017;4:137-148.
7. Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*. 2015; 527(7577):192-197.
8. Ye L, Wang J, Tan Y, et al. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: an approach for treating sickle cell disease and β-thalassemia. *Proc Natl Acad Sci USA*. 2016;113(38): 10661-10665.
9. Traxler EA, Yao Y, Wang YD, et al. A genome-editing strategy to treat β-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. *Nat Med*. 2016; 22(9):987-990.
10. Sankaran VG, Xu J, Byron R, et al. A functional element necessary for fetal hemoglobin silencing. *N Engl J Med*. 2011;365(9):807-814.

DOI 10.1182/blood-2018-03-839787