

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Hemoglobinopathies in the Fetal Position**

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The hemoglobinopathies, disorders that are caused by mutations in genes that encode the “adult” globins, exert a devastating toll on patients and their families. Worldwide, approximately 300,000 babies are born with sickle cell disease¹ and 60,000 are born with β -thalassemia each year. However, if patients’ functional fetal globin genes could be used to compensate for the mutated adult globin genes, clinical outcomes could be greatly improved. A recent study by Grevet et al.² provides a basis for developing such a therapy.

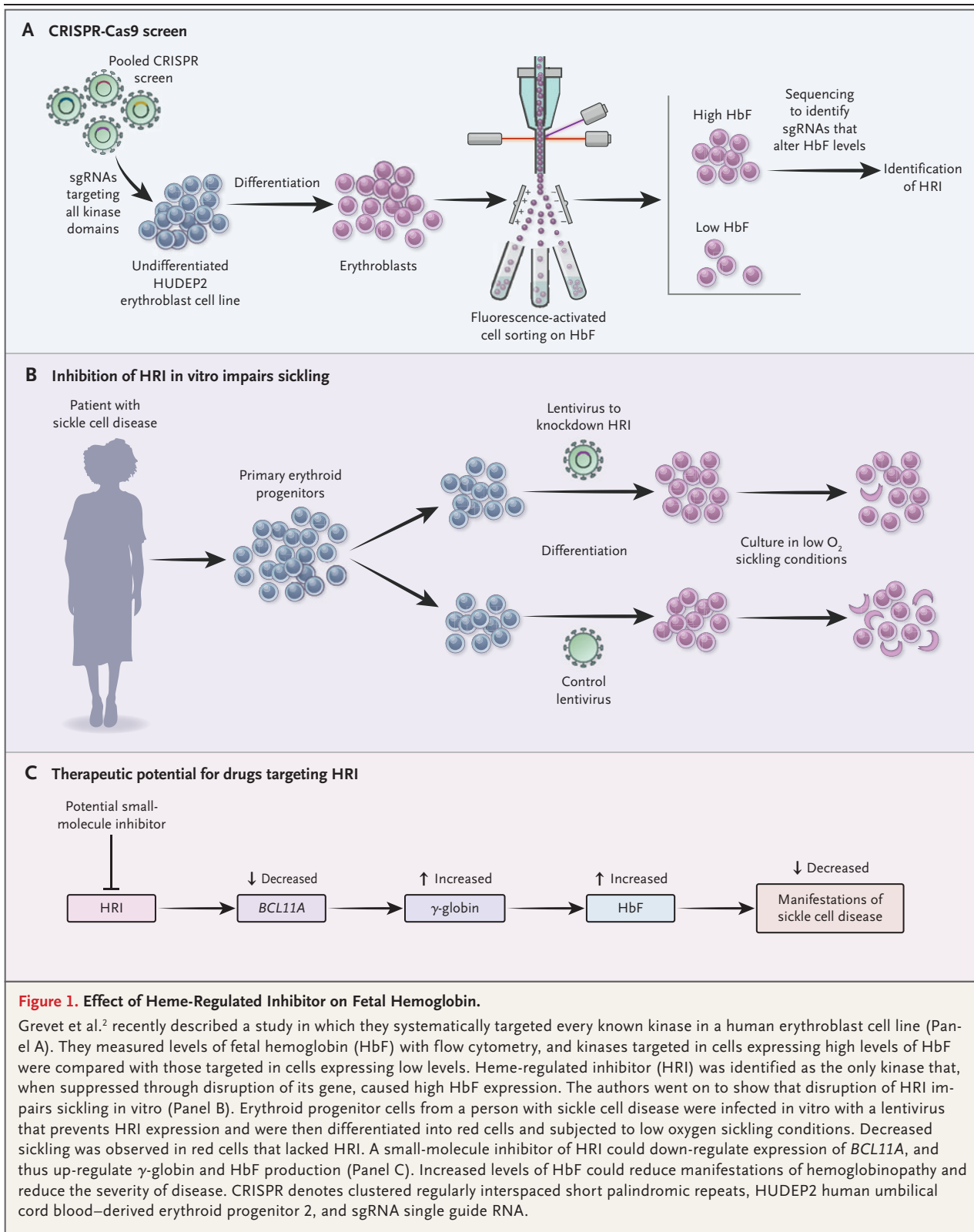
Adult hemoglobin (HbA) consists of two α -globin and two β -globin chains ($\alpha_2\beta_2$). Sickle hemoglobin (HbS) is caused by a single mutation (Glu6Val) of the β -globin gene ($\alpha_2\beta^S_2$). Deoxygenated HbS polymerizes and alters erythrocytes in such a way that they become elongated, adopting a sickle shape that is fragile and inflexible. Patients with sickle cell disease have a range of acute and chronic complications including hemolysis and anemia, painful crises, and infarction of major organs. Transfusion-dependent patients with thalassemia inherit mutations in their β -globin genes that reduce or abolish β -globin expression, resulting in globin-chain imbalance and in ineffective erythropoiesis, which leads to life-threatening anemia.

During fetal life, the γ -globin genes rather than the β -globin genes are active. They produce HbF (fetal hemoglobin [$\alpha_2\gamma_2$]) rather than HbA. During the first year of postnatal life, the γ -gene is down-regulated, and the β -gene is up-regulated. In adults, the level of HbF is usually about 1% of total hemoglobin. However, the level of HbF in adults varies, and some adults with sickle cell disease and thalassemia who have comparatively high levels of HbF have better clinical outcomes than those who have low levels of HbF. Hydroxy-

urea, a drug that is often used to reduce the severity of symptoms of hemoglobinopathies, may act, in part, by elevating HbF levels. A drug that more effectively increases HbF is a holy grail of hemoglobinopathy research.

The transcription factor BCL11A is a critical repressor of γ -globin in adults,³ and BCL11A polymorphisms influence HbF levels and clinical severity in patients with hemoglobinopathies.⁴ However, directly targeting such factors is challenging. A more promising approach may be to modify enzymes that control transcription factors. Grevet et al. used genetic screening to search for protein kinases critical for HbF regulation in an immortalized human cell line that can be differentiated into erythroid cells.² But before differentiating the cell line, Grevet and colleagues first genetically disrupted every known human protein kinase domain: 496 kinase domains in 482 kinase genes were disrupted across a pool of cells. The edited cells in this pool were then differentiated into erythroblasts and screened for high levels of HbF protein by flow cytometry. High levels of HbF were found in cells in which heme-regulated inhibitor (HRI [also known as EIF2AK1]), a kinase highly expressed in erythroblasts, was disrupted (Fig. 1). This result suggested that HRI, which has already been implicated in the coordination of globin and heme synthesis,⁵ represses the expression of HbF in human erythroid cells.

Using RNA sequencing and mass spectrometry (“-omics”), the authors found that impairing HRI exerted specific effects on γ -globin messenger RNA expression; it is crucial to note that erythroid differentiation and regulation of other critical components, such as other globin genes, membrane proteins, and enzymes critical for heme synthesis, were unperturbed. HRI defi-



ciency in primary human erythroblasts also up-regulated γ -globin transcription and increased HbF production, and again the effects of HRI depletion were relatively specific to γ -globin. An important finding is that in erythroblasts generated from the stem cells of patients with sickle cell disease, HRI depletion up-regulated γ -globin expression and HbF protein and protected cells from sickling when they were cultured in low-oxygen conditions (Fig. 1), which suggests that the HbF induction achieved through this approach would be clinically beneficial. Finally, HRI knock-down depleted *BCL11A* expression by inhibiting transcription. When *BCL11A* protein levels were forcibly increased by overexpression of the gene, the effects of HRI depletion on HbF were largely reversed. Therefore, HRI probably acts by controlling the expression of *BCL11A*.

HRI inhibition is a promising strategy for raising HbF levels in patients, perhaps in combination with other known HbF inducers. However, effective and specific pharmacologic inhibitors of HRI are currently lacking. Furthermore, data so far are derived only from cell models in the laboratory. Extensive further characterization will be needed before an HRI inhibitor can be tested in clinical trials in humans. Notwithstanding these issues, the study by Grevet and colleagues highlights the value of rational genetic-screening approaches in defining potential drug targets

and the value of -omics approaches in validating the specificity of these pathways before pharmaceutical development. HRI is now an exciting molecule for further study by researchers who are aiming to find a way to lessen the burden of hemoglobinopathies in patients worldwide.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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