The NEW ENGLAND JOURNAL of MEDICINE

Perspective

Welcoming the Era of Gene Editing in Medicine

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The recent regulatory approval of exagamglogene autotemcel (exa-cel [Casgevy, Vertex Pharmaceuticals/CRISPR Therapeutics]) in both the United States and United Kingdom for the treatment of sickle cell disease (SCD) and transfusion-dependent β-thalassemia marks the dawn of the era of gene editing in medicine.

This milestone was achieved barely more than a decade after the first scientific report was published describing the conversion of the clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 bacterial immune system into a programmable gene-editing nuclease,[1] and just 3 years after the two senior authors of that article, Jennifer Doudna and Emmanuel Charpentier, shared the Nobel Prize in Chemistry. This rapid translation of genetic medicine exemplifies the virtuous bedside-to-bench-to-bedside cycle that propels academic medicine. But though exa-cel is the first of a promised wave of therapies that directly edit and repair disease-associated genetic mutations, considerable challenges must still be overcome to ensure that even the most severely ill of the millions of people with SCD worldwide will have access to this potentially curative therapy.

Linus Pauling dubbed sickle cell anemia the "first molecular disease" in a 1949 article detailing the difference between normal and sickle cell disease–associated hemoglobin.[2] We now know the most common form of SCD is attributable to a single point mutation (GAG to GTG) resulting in a single amino acid substitution (glutamic acid for valine) at the sixth position of the β subunit of adult hemoglobin, creating hemoglobin S (HbS). People who are homozygous for HbS experience hemoglobin polymerization that deforms red cells and causes sludging in the microcirculation, which deprives tissues of oxygen and causes excruciating episodes of tissue ischemia called vaso-occlusive pain crises. Such events afflict infants starting at roughly 6 months of age, when the γ subunit of fetal hemoglobin (HbF), the dominant oxygen-avid form of hemoglobin expressed during fetal development, switches off and the mature β subunit of HbS turns on, provoking polymerization.

Though a single point mutation among the 3 billion base pairs in the human genome, when inherited from both parents, is sufficient to cause SCD, clinicians have long observed highly variable disease severity, which suggests that mutations in other parts of the genome modify SCD. Most notably, a rare benign condition called hereditary persistence of fetal hemoglobin (HPFH), if coinherited with HbS, reduces pain crises and disease-related morbidity and mortality. To savvy clinician-scientists, these observations suggested a therapeutic hypothesis: reengineering

red cells to reactivate HbF could mimic HPFH and substantially ameliorate SCD symptoms.

In addition to the curiosity-driven studies of bacterial adaptive immunity that led to the discovery of CRISPR-Cas9, the gene-editing triumph of exa-cel also traces its origins to fundamental studies of globin gene regulation. Over the past two decades, Orkin and colleagues at Boston Children's Hospital and Harvard Medical School, alongside many collaborators, have revealed the fundamental molecular mechanisms that control globin switching and identified the genomic target for the CRISPR-Cas9 gene editing that is now approved as exa-cel.

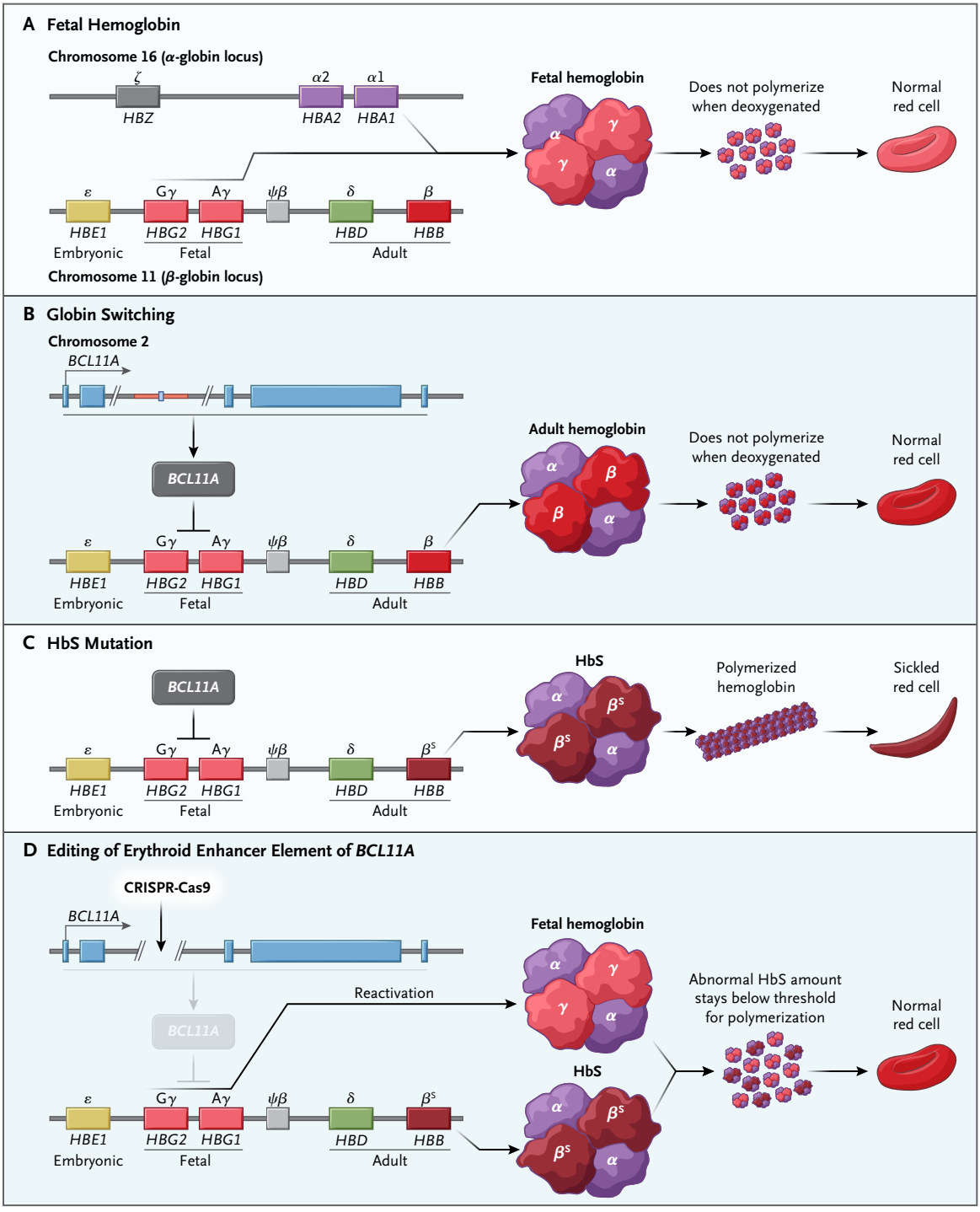
The discovery of the molecular target for reactivating HbF is rooted in modern genomics, in particular the application of genomewide association studies to mapping of the chromosomal locations of genetic variations that dictate HbF levels. In a feat of scientific sleuthing,[3] Thein, Cao, Orkin, and colleagues identified a genomic region of interest around a gene called *BCL11A* that is associated with higher HbF levels and reduced rates of SCD pain crises. Subsequent molecular studies by Sankaran and Orkin established unequivocally that BCL11A was a repressor protein that bound to a regulatory region around the HbF gene, silencing gene transcription in mature adult red cells (see diagram). As proof of principle for a potential therapy, the Orkin group showed that deletion of *BCL11A* in a mouse model of SCD normalized the otherwise pathologic hemolytic anemia, restored red-cell counts and hemoglobin levels, and eliminated red-cell sickling. Then the Bauer and Orkin groups used CRISPR-Cas9 to exhaustively mutagenize the transcriptional control regions of *BCL11A*, in the process identifying the precise locations to target with CRISPR-Cas9 to mimic HPFH and thereby restore HbF expression.

The strategy pursued by CRISPR Therapeutics and Vertex Pharmaceuticals employed a target that these studies identified: the erythroid-specific enhancer controlling *BCL11A* expression. In 2021, clinical data were reported from one patient with SCD and one with transfusion-dependent β-thalassemia, confirming the hypothesis: CRISPR-Cas9 editing to turn off *BCL11A* expression resulted in increased HbF expression and eliminated pain crises in the patient with SCD and transfusion dependence in the patient with β-thalassemia.[4] Over the next 2 years, 29 of 30 treated patients with SCD remained free of vaso-occlusive crises for at least 12 consecutive months (as reported by Frangoul et al. in the *Journal*). Similarly, as Locatelli et al. now report in the *Journal*, transfusion independence has been achieved in 32 of 35 treated patients with β-thalassemia. These impressive results justified the regulatory approval of exa-cel for both conditions. Although analysis of off-target editing has been reassuring (see Yen et al., also published in the *Journal*), long-term follow-up studies are needed to establish the safety of the gene-editing procedures and determine whether this therapy staves off the myriad organ injuries caused by SCD.

Though this achievement is cause for celebration, challenges remain. Exa-cel requires gene editing in the context of autologous bone marrow transplantation, which entails stem-cell collection, ex vivo gene transduction, and a specialized intensive-care–level transplantation procedure associated with considerable morbidity and which not all U.S. hospitals can manage. Few transplant centers exist in under-resourced regions, including sub-Saharan Africa, where millions of people have SCD. Moreover, exa-cel is extremely costly, with pricing set at $2.2 million per treatment. Though a single treatment may yield a functional cure, it carries the risk of infertility, a further barrier to wide acceptance. The statement of the organizing committee of the Third International Summit on Human Genome Editing (held in March 2023) highlighted the imperative for equitable access to these potentially curative genetic therapies and for ongoing research on approaches to democratizing treatment.

Drugs are more scalable than gene therapy. The recently approved compound voxelotor (Oxbryta, Pfizer) was approved for SCD as an antisickling therapy, though not a cure. Development of a pill that can induce HbF expression remains a high priority. Further innovation in CRISPR-based gene-editing techniques include base editing and prime editing, which can rewrite the HbS mutation. Prime editing may be able to replace the mutant base with the base carried by healthy people.

Finally, a scalable, cost-efficient therapy would deliver the CRISPR gene-repair machinery in intravenous or intramuscular injections to effect gene editing of target hematopoietic stem cells in vivo, obviating the need for autologous marrow harvest, ex vivo gene editing, and bone marrow transplantation. A simple "one and done" strategy, akin to a vaccination, remains the holy grail of genetic medicine and would ad-



Schema for Globin Gene Switching and Exa-cel Reactivation of Fetal Hemoglobin Expression in Red Cells.

Hemoglobin is a tetramer of two alpha (α) subunits expressed from a gene cluster on chromosome 16, and two subunits from a β-globin gene cluster on chromosome 11, which consists of embryonic (ε), fetal (γ), and adult (β) genes. During most of fetal development, γ is the predominant gene expressed (Panel A). Globin switching (Panel B) entails expression of the *BCL11A* gene (located on chromosome 2), which dampens expression of the two γ-globin genes, leading to up-regulation of β-globin expression. If the β subunit carries the hemoglobin S (HbS) mutation, the abnormal hemoglobin is prone to polymerization, which causes pathologic sickling of the red cells (Panel C). Exa-cel exploits a guide RNA-directed CRISPR-Cas9 complex, akin to molecular scissors that cleave and disrupt an enhancer within *BCL11A*. This enhancer is critical to the expression of *BCL11A* within the erythroid lineage. The relief of *BCL11A* repression reactivates γ-globin gene expression, which substitutes for and dilutes the abnormal HbS to below the threshold for polymerization in red cells, eliminating sickling.

vance global health equity. As described in an editorial by McCune and Kiem, now published in the *Journal*, this goal is a priority of the Cure Sickle Cell Initiative launched in 2018 by the National Heart, Lung, and Blood Institute, which has secured partnership funding from the Bill and Melinda Gates Foundation. Proof of principle for intravenous delivery of a prime-editing cargo targeted to bone marrow hematopoietic stem cells has been achieved in a mouse model of SCD,[5] but delivery-method refinements are needed before clinical studies can proceed. Given the speed of innovation and momentum from recent success, I believe efficient systemic delivery of gene-editing cargo will inevitably make its way from bench to bedside, ultimately making gene editing an equitable, accessible treatment method.

As the first approved medicine based on gene editing, exa-cel will profoundly affect patients fortunate enough to receive treatment. Exa-cel is but one of several potentially curative therapies currently available for SCD. Bone marrow transplantation from an HLA-matched sibling remains the standard of care, and new protocols for transplantation using haploidentical donors are showing impressive results. Lentiviral vector replacement of β-globin (lovotibeglogene autotemcel [Lyfgenia, Bluebird Bio]) was approved on the same day as exa-cel. Choosing among these therapies will be challenging, since the safest and most effective approach is not yet fully defined. The hard work of both basic and clinical translational research must continue.

Disclosure forms provided by the author are available at NEJM.org.

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This article was published on April 24, 2024, at NEJM.org.

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DOI: 10.1056/NEJMp2314279