

## Genetic therapies for the first molecular disease

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*J Clin Invest.* 2021;131(8):e146394. <https://doi.org/10.1172/JCI146394>.

### Review

Sickle cell disease (SCD) is a monogenic disorder characterized by recurrent episodes of severe bone pain, multi-organ failure, and early mortality. Although medical progress over the past several decades has improved clinical outcomes and offered cures for many affected individuals living in high-income countries, most SCD patients still experience substantial morbidity and premature death. Emerging technologies to manipulate somatic cell genomes and insights into the mechanisms of developmental globin gene regulation are generating potentially transformative approaches to cure SCD by autologous hematopoietic stem cell (HSC) transplantation. Key components of current approaches include ethical informed consent, isolation of patient HSCs, in vitro genetic modification of HSCs to correct the SCD mutation or circumvent its damaging effects, and reinfusion of the modified HSCs following myelotoxic bone marrow conditioning. Successful integration of these components into effective therapies requires interdisciplinary collaborations between laboratory researchers, clinical caregivers, and patients. Here we summarize current knowledge and research challenges for each key component, emphasizing that the best approaches have yet to be developed.

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# Genetic therapies for the first molecular disease

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**Sickle cell disease (SCD) is a monogenic disorder characterized by recurrent episodes of severe bone pain, multi-organ failure, and early mortality. Although medical progress over the past several decades has improved clinical outcomes and offered cures for many affected individuals living in high-income countries, most SCD patients still experience substantial morbidity and premature death. Emerging technologies to manipulate somatic cell genomes and insights into the mechanisms of developmental globin gene regulation are generating potentially transformative approaches to cure SCD by autologous hematopoietic stem cell (HSC) transplantation. Key components of current approaches include ethical informed consent, isolation of patient HSCs, in vitro genetic modification of HSCs to correct the SCD mutation or circumvent its damaging effects, and reinfusion of the modified HSCs following myelotoxic bone marrow conditioning. Successful integration of these components into effective therapies requires interdisciplinary collaborations between laboratory researchers, clinical caregivers, and patients. Here we summarize current knowledge and research challenges for each key component, emphasizing that the best approaches have yet to be developed.**

## Historical perspectives

Studies of sickle cell disease (SCD), a life-threatening, multisystem genetic blood disorder that affects approximately 100,000 Americans and millions worldwide, have driven many advances in science and medicine. In 1910, Herrick described “sickle-shaped cells” in a dental student from the West Indies suffering from pain episodes and anemia (1). In 1949, Pauling and colleagues noted abnormalities in the properties of sickle hemoglobin (HbS), which were shown by Ingram in 1956 to result from altered amino acid composition, distinguishing SCD as “the first molecular disease” (2, 3). Around the same time, Neel and Beet determined SCD inheritance to be autosomal recessive (4, 5). The concept of natural selection for malaria resistance in SCD heterozygotes was proposed by Haldane in 1949 and confirmed by Allison a few years later (6, 7). It wasn't until 1978 that Kan and Dozy reported the diagnosis of SCD by DNA analysis of fetal amniotic fluid cells, heralding a new era in genetic testing (8).

The deadly manifestations of SCD and its genetic features have been recognized for centuries in Africa, where hundreds of

thousands of affected individuals are born each year. The concepts of “ogbanje” (Ibo) and “abiku” (Yoruba), which translate to “a child destined to die and be born repeatedly to the world,” are attributed to SCD (9, 10). Medical advances have improved outcomes of affected individuals in high-income countries, but most patients continue to experience severe morbidities and premature mortality, beginning in adolescence. Now, emerging scientific discoveries are fueling innovative strategies to treat SCD via genetic manipulation of autologous hematopoietic stem cells (HSCs), promising effective cures (11, 12). However, the field has been shaken by recent reports of myeloid malignancies following lentiviral vector-mediated (LV-mediated)  $\beta$ -globin replacement gene therapy. Specifically, of 47 SCD patients treated with LV gene therapy in two related clinical trials (NCT02140554 and NCT04293185) over the past six years, three have been diagnosed with myelodysplastic syndrome or acute myeloid leukemia (13, 14). Here we review autologous genetic therapies for SCD, aiming to provide a balanced view of the risks and benefits of this rapidly evolving field.

## Pathophysiology of SCD

SCD is caused by mutations in the *HBB* gene, which encodes the  $\beta$ -globin subunit of adult hemoglobin (HbA,  $\alpha_2\beta_2$ ) (15, 16). Most affected individuals are homozygous for a p.Glu6Val substitution resulting in the production of  $\beta^S$ -globin, which combines with  $\alpha$ -globin to form HbS ( $\alpha_2\beta^S_2$ ). Another common form of SCD results from compound heterozygosity between HbS and HbC (p.Glu6Lys), resulting in HbSC ( $\alpha_2\beta^S\beta^C$ ) disease. Hemoglobin S and C mutations frequently coexist with  $\alpha$ - or  $\beta$ -thalassemia alleles, which can modify SCD phenotypes (17). Under hypoxic conditions, HbS or HbC form rigid polymers that cause red blood cells (RBCs) to acquire a sickle shape and initiate a complex pathophysiology that includes hemolysis, inflammation, dysregulated nitric oxide metabolism, hypercoagulation, and vasculopathy. Consequently, patients experience severe acute pain episodes, chron-

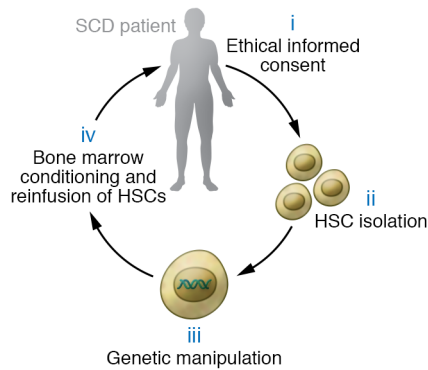
**Authorship note:** PAD and AS contributed equally to this work.

**Conflict of interest:** MJW is a former advisor for and first-tier equity holder in Beam Therapeutics; a second-tier advisory board member for Forma Therapeutics, Cellarity Inc., and Novartis; and a second-tier consultant for Graphite Bio. AS is the St. Jude Children's Research Hospital site principal investigator of clinical trials for genome editing of sickle cell disease sponsored by Vertex Pharmaceuticals Incorporated/CRIS-PR Therapeutics (NCT03745287, titled A Safety and Efficacy Study Evaluating CTX001 in Subjects With Severe Sickle Cell Disease) and by Novartis (NCT04443907, titled Study of Safety and Efficacy of Genome-Edited Hematopoietic Stem and Progenitor Cells in Sickle Cell Disease). The industry sponsors provide funding for the clinical trial, which includes salary support. AS also is a second-tier consultant for Spotlight Therapeutics. JFT is an investigator on the bluebird bio Inc. clinical trial HGB-206 (NCT02140554, titled A Study Evaluating the Safety and Efficacy of bb1111 in Severe Sickle Cell Disease).

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**Reference information:** *J Clin Invest.* 2021;131(8):e146394.

<https://doi.org/10.1172/JCI146394>.



**Figure 1. Four major steps in autologous hematopoietic stem cell (HSC) therapies to treat SCD.** (i) Clinical researchers inform participants about the procedure, associated risks, and alternative treatments, then obtain written consent. (ii) CD34<sup>+</sup> HSPCs are mobilized with plerixafor and isolated from blood by apheresis. (iii) HSPCs are manipulated ex vivo to correct the SCD mutation or induce HbF expression. (iv) The participant receives bone marrow conditioning with myelotoxic/myeloablative agents, followed by infusion of the modified HSPCs.

ic pain, progressive multi-organ damage, and premature death. Common manifestations include stroke, acute lung injury (acute chest syndrome), bone avascular necrosis, and chronic heart, lung, and kidney disease. Loss of splenic function beginning in infancy predisposes patients to sepsis.

## Unmet clinical needs

Medical costs for SCD in the United States exceed 1 billion dollars per year (18). Newborn screening and medical therapies have greatly improved the survival of children with SCD in high-income countries, although most patients continue to experience morbidities and die in early adulthood. In Africa, India, and the Middle East, there are millions of SCD patients who lack access to modern medical care, many of whom die before age 5. Ideally, all children with SCD would receive safe, effective curative therapy early in life to minimize organ damage. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only approved cure, with an overall event-free survival of approximately 90%–95% after transplantation from HLA-matched sibling donors (19–21). Results of HSCT using alternative donor sources are promising, but many patients still experience immunological complications, such as graft rejection and graft-versus-host disease. As the risks of allogeneic HSCT remain substantial, determining which young SCD patients are most likely to benefit from this procedure is complex (22, 23). Hence, safer cures are needed. Genetic correction of autologous HSCs eliminates immune toxicities associated with allogeneic HSCT and expands patient eligibility by allowing affected individuals to be their own HSC donors. The major existing safety concern of these therapies is genotoxicities predisposing to malignant transformation; efforts are underway to understand and avoid this problem.

## Autologous genetic therapies for SCD

All current protocols to treat SCD by autologous HSCT include four major steps (Figure 1): (i) the obtaining of ethical informed consent; (ii) isolation of patient CD34<sup>+</sup> hematopoietic stem and progenitor

cells (HSPCs), a complex mixture of long-term bone marrow-repopulating HSCs and committed progenitors, some of which provide early, short-term hematopoietic reconstitution after HSCT; (iii) ex vivo genetic manipulation of CD34<sup>+</sup> cells targeting the HSC subpopulation to correct the SCD mutation or circumvent its toxicities; and (iv) myelotoxic/myeloablative conditioning to create a receptive bone marrow niche, followed by infusion of the modified HSPCs.

**Ethical informed consent.** Translating scientific advances into SCD patient care is challenging. Participation in high-risk, potentially curative clinical trials can be influenced by patient-perceived disease burden, expected benefits, fear of toxicities, and socio-ecological factors including anticipated stress, religious beliefs, and support systems (24–26). Other barriers include lack of appropriate educational material, therapy-associated costs, and limited access to longitudinal care. A recent report by the National Academies of Science, Engineering, and Medicine (NASEM) noted that “the SCD community has developed a significant lack of trust in the health care system due to the nearly universal stigma and lack of belief in their reports of pain, a lack of trust that has been further reinforced by historical events, such as the Tuskegee experiment” (27). Many patients fear that HIV can be transmitted by lentiviral vectors (LVs), a myth that can be dispelled by better education and a trustful relationship with care providers (28, 29). Some SCD patients may overestimate the potential benefits of an experimental therapy and believe that no alternative care options exist. The participation of individuals with SCD in high-risk/high-reward clinical trials should be explored through a shared decision-making process with health care providers using culturally sensitive disease- and treatment-specific approaches tailored to the knowledge level of study participants. A multilevel decision aid for choosing disease-modifying treatments for SCD incorporates patient features (trust, SCD severity), decision characteristics (risk-benefit, urgency), physician perspectives (patient/family motivation, patient psychosocial characteristics), and environmental factors (institutional practice, insurance coverage) (30). Long-term collaborative relationships between clinical investigators, caregivers, and eligible subjects for SCD clinical trials are essential and require ongoing attention to potential communication barriers, social/cultural issues, and patient values (31). Given the uncertainties of high-risk therapeutic clinical trials, it may be valuable to conduct psychosocial assessments evaluating the emotional function, coping ability, and social support stability of potential participants to facilitate informed consent and better manage outcome expectations. Offering coping strategies and opportunities for clinical trial participants to explore potentially unexpected or less favorable outcomes enhances the shared decision-making model (26).

**Isolation of HSPCs from individuals with SCD.** Most genetic therapies for SCD require collecting  $4 \times 10^6$  to  $15 \times 10^6$  autologous peripheral blood CD34<sup>+</sup> HSPCs per kilogram (32, 33). Mobilization with granulocyte CSF is contraindicated because of potentially life-threatening immune cell activation (34). Until recently, bone marrow aspiration was the standard method for collecting CD34<sup>+</sup> HSPCs from SCD patients. This strategy usually requires two to four separate harvests under general anesthesia, which can trigger serious complications (34). However, four recent studies indicate that CD34<sup>+</sup> HSPCs can be mobilized safely in adults with SCD

**Table 1. Plerixafor-mediated HSC mobilization in patients with SCD**

Study	Plerixafor dose ( $\mu\text{g}/\text{kg}$ )	No. of participants	Patient age	Peak CD34 <sup>+</sup> cell count ( $/\mu\text{L}$ )	Product Hct (%)	CD34 <sup>+</sup> cell recovery (%)	CD34 <sup>+</sup> cell yield ( $\times 10^6/\text{kg}$ )
Uchida et al., 2020 (38)	240	15	29 (20–50)	52 (9–183) <sup>A</sup>	4.5 (2.7–7.5)	46.8 (26–96)	6.3 (2.2–12.0)
Lagresle-Peyrou et al., 2018 (37)	240	3	20 (19–21)	>80 <sup>A</sup>	5.8 (4.8–8.2)	82 (31–92)	4.6 (4.5–5.8)
Esrick et al., 2018 (36)	180	3	26 (19–30)	36 (31–65) <sup>A</sup>	5.6 (3.7–17)	54 (20–81)	0.616 (0.069–1.2)
	240	3	25 (25–38)	156 (27–290) <sup>A</sup>	11.7 (10.5–16.4)	59 (41–61)	16.38 (2.94–24.53)
Boulad et al., 2018 (35)	80	6	30.5 (21–34)	27.5 (7–132) <sup>B</sup>	NA	NA	NA
	160	3	32 (25–37)	43 (7–251) <sup>B</sup>	NA	NA	NA
	240	6	34.5 (23–46)	30.5 (10–95) <sup>B</sup>	NA	NA	NA

Median (range) values are shown. Hydroxyurea was discontinued at least 2 weeks before HSC mobilization in the studies, except Boulad et al. 2018, in which hydroxyurea was not discontinued. <sup>A</sup>Measured 2 hours after plerixafor. <sup>B</sup>Measured 12 hours after plerixafor. Hct, hematocrit.

using plerixafor, a small molecule that inhibits interaction of the HSC chemokine receptor CXCR4 with its ligand, stromal-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), on bone marrow niche cells (Table 1), with fewer severe adverse events in comparison with bone marrow harvesting in one study comparing both methods (35–38). Several conclusions derive from these studies. First, plerixafor mobilization of HSPCs, followed by apheresis harvesting, is generally safe and effective in SCD. The major toxicity noted was vaso-occlusive pain crisis that resolved with medical therapy. It is unclear whether this toxicity was caused by the drug, apheresis collection, or both. Second, the fraction of long-term repopulating HSCs and their suitability for genetic manipulation may be superior in plerixafor-mobilized HSPCs compared with those obtained by bone marrow aspiration (39, 40). Third, peripheral blood CD34<sup>+</sup> cell counts peak as early as 3–6 hours after plerixafor administration, and apheresis should be initiated within this time frame. Fourth, the safest and most effective plerixafor dose appears to be 240  $\mu\text{g}/\text{kg}$ , although higher doses are worth studying. Fifth, the CD34<sup>+</sup> cell yield varies greatly between subjects. Hydroxyurea therapy was associated with reduced CD34<sup>+</sup> cell numbers and therefore should be discontinued for at least 2–4 weeks prior to CD34<sup>+</sup> cell mobilization and collection, and longer periods may be better. To minimize adverse events associated with plerixafor mobilization and apheresis, RBC transfusion (simple or exchange) should be initiated to maintain blood hemoglobin level of 10 g/dL with HbS fraction less than 30% after discontinuing of hydroxyurea.

The CD34<sup>+</sup> cell yield after a single plerixafor dose and apheresis cycle is usually insufficient for successful autologous therapy. Several strategies to maximize the HSPC yield per collection cycle are under investigation. Inflammation may alter the properties of SCD HSPCs and impair collection and purification. Adjustment of apheresis parameters to compensate for these features can improve CD34<sup>+</sup> cell yields, but may also interfere with subsequent purification steps by increasing RBC contamination (37, 38). New CXCR4 antagonists are under study (41–44). In non-SCD individuals, the CXCR2 agonist GRO $\beta$ , given alone or with plerixafor, can efficiently mobilize a unique population of HSPCs with superior long-term repopulating capabilities (45). These new agents may improve HSPC collection from individuals with SCD.

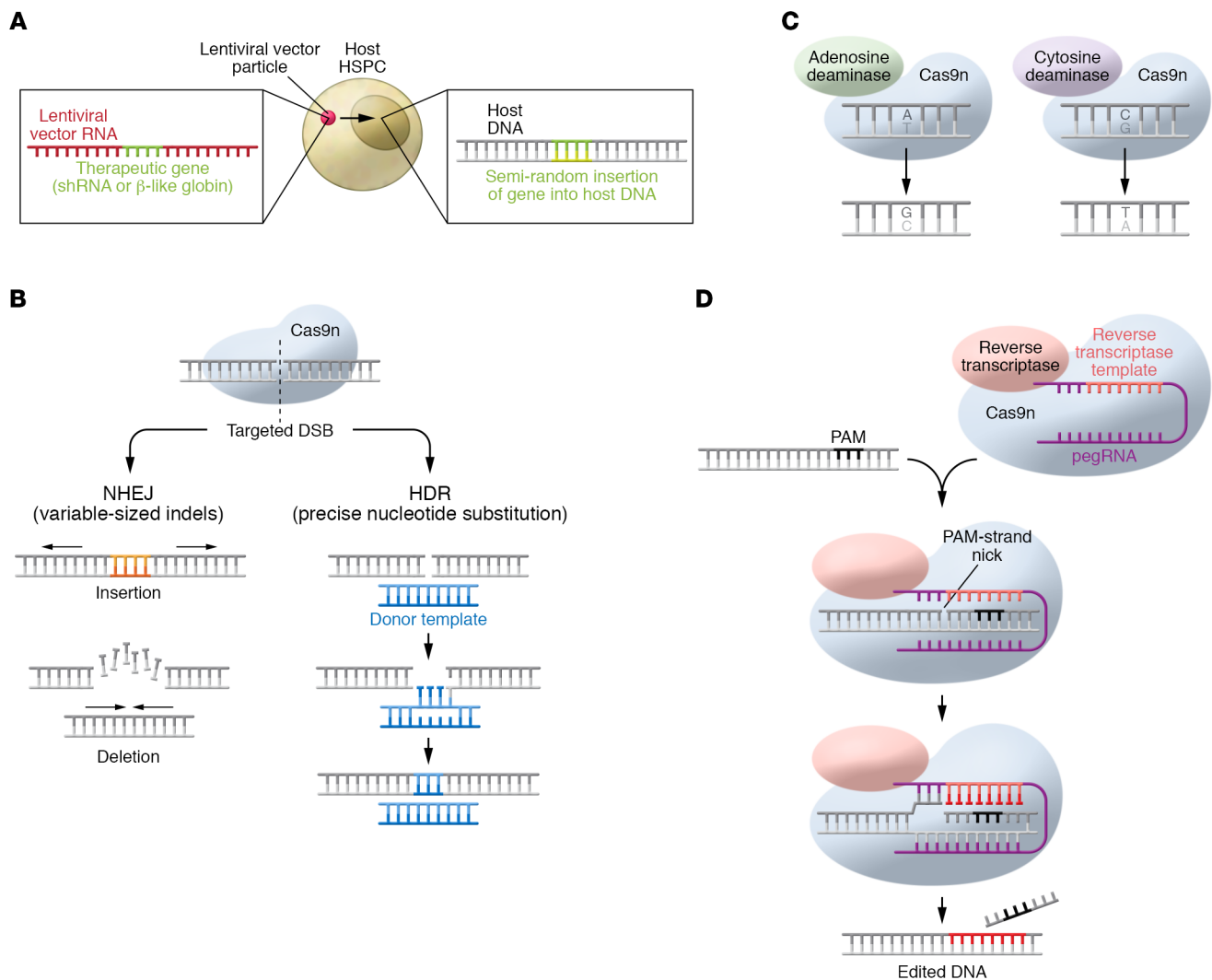
The CD34<sup>+</sup> population contains only a small, variable proportion of the key target cells for SCD gene therapy, which include

multipotent progenitors and HSCs that provide short- and long-term hematopoietic reconstitution, respectively (46). Both the CD90<sup>+</sup>CD45RA<sup>-</sup> and CD38<sup>-</sup> subfractions of CD34<sup>+</sup> cells are enriched for HSCs (47, 48). Enumerating these subpopulations in autologous donor CD34<sup>+</sup> cells may estimate more accurately the number of biologically relevant target cells available for gene therapy, correlate better with engraftment levels, and provide a refined target population for genetic modification in order to simplify manufacturing and reduce associated costs.

*In vitro genetic correction of HSCs.* Multiple tools and strategies exist for modifying HSCs to circumvent SCD pathologies (Figures 2 and 3): (a) gene therapy via transduction with an LV encoding an antisickling  $\beta$ -like globin gene driven by erythroid-specific regulatory elements; (b) creation of genetic alterations that induce RBC fetal hemoglobin (HbF) expression; or (c) direct repair of the mutant SCD codon (valine), either to normal (glutamic acid) or to a benign, nonsickling variant, such as hemoglobin G-Makassar (alanine). Tools for genome editing include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), CRISPR/Cas9, base editors, and prime editors (49, 50). Early clinical studies to treat SCD using LVs and genome editing are under way (Table 2).

*$\beta$ -Globin gene addition via lentiviral vectors.* Because HSC self-renewal and differentiation require multiple rounds of cell division, therapeutic transgenes for hematopoietic disorders must be integrated into chromosomal DNA. The use of self-inactivating, HIV-based LVs for this purpose has been developed and refined extensively over more than 25 years and is now producing promising results for treating multiple blood disorders, including  $\beta$ -hemoglobinopathies (SCD and  $\beta$ -thalassemia) (11, 12, 51, 52). Importantly, LVs can accommodate the complex regulatory elements required to support high-level, erythroid-specific expression of a  $\beta$ -like globin transgene while maintaining the capacity to transduce HSCs efficiently (Figure 2A).

The first apparent gene therapy cure for SCD, reported in a 13-year-old boy in France in 2017, used an LV encoding the antisickling  $\beta$ -globin variant  $\beta^{\text{A-T87Q}}$  (HGB-206, NCT02140554; ref. 53). At 15 months after therapy, the blood hemoglobin level was 11.8 g/dL with 48% HbA<sup>T87Q</sup> and 49% HbS, and the participant was symptom free. In a parallel trial conducted in the United States (HGB-206, NCT02140554), an evolution over three successive



**Figure 2. Tools for genetic manipulation of patient CD34<sup>+</sup> HSPCs to treat SCD.** (A) LV gene therapy: An antisickling  $\beta$ -like globin gene or *BCL11A* shRNA flanked by erythroid regulatory elements is inserted into a replication-deficient LV that is packaged into vector particles. The LV integrates semi-randomly into the host HSPC genome and is expressed in erythroid progeny. The  $\beta$ -like globin forms functional hemoglobin, while the *BCL11A* shRNA induces  $\gamma$ -globin expression to raise HbF levels. (B) Genome editing: The RNA-guided Cas9 nuclease binds the DNA target site via its associated guide RNA (gRNA) and creates a precise DSB that is repaired either by NHEJ, generating insertion-deletion mutations that induce HbF; or by HDR, which utilizes a donor DNA repair template to correct the SCD codon. (C) Base editing: Catalytically impaired Cas9n fused to either a cytosine or adenosine deaminase introduces precise base pair alterations. Adenosine (A) base editors convert A:T to G:C; cytosine base editors convert C:G to T:A. Base editors are used to induce HbF or convert the SCD codon to a benign variant. (D) Prime editing: Cas9n fused to a reverse transcriptase binds the target site via base pairing with the guide portion of the associated prime editing guide RNA (pegRNA) and creates a single-stranded DNA nick. The reverse transcriptase domain uses the pegRNA template to synthesize the desired edit following the nick. Cellular DNA repair machinery removes the endogenous DNA “flap” and repairs the nick to generate a heteroduplex intermediate that is converted to the edited product by DNA repair.

patient groups has been reported in a series of abstracts (54–58). Initially, efficacy was suboptimal owing to inefficient HSPC collection and transduction (HGB-206/NCT02140554, group A;  $n = 7$ ). These problems have been gradually overcome by clinical and technical modifications in group B ( $n = 2$ ), including pretherapy RBC transfusions, shifting from bone marrow–derived HSCs to plerixafor-mobilized cells, and cell manufacturing protocol refinements, including LentiBOOST (Sirion Biotech), an amphipathic membrane-modifying molecule that improves LV transduction (59–61). Seventeen individuals treated under the most recent version of the protocol (group C) with a median follow-up of 11 months became RBC transfusion free and experienced a

greater than 99% reduction in severe pain events (55). The median vector copy number (VCN) was 3.8 (2.3–5.7) copies per diploid genome, resulting in a blood hemoglobin level of 11.2 (10.5–16.2) g/dL, approximately half of which was derived from the  $\beta^{\text{A-T87Q}}$  transgene. Additional LV gene therapy trials for SCD have been opened recently (Table 2), and new strategies being developed to improve the efficacy of LVs are likely to improve this approach (62–65). Overall, current evidence indicates that LV gene therapy for SCD is effective, although recently raised safety concerns must be addressed (13, 14).

*Genetic modifications to induce HbF expression.* Normally around birth, transcription of  $\gamma$ -globin switches gradually to that

**Table 2. Autologous genetic therapies for SCD and  $\beta$ -thalassemia in clinical trials**

Strategy	Modality	Lead group	Status	Clinical trial number	No. of participants	Results	References
$\beta$ -Like globin gene replacement	$\beta^{A1870}$ LV	bluebird bio	Phase III, marketing approval (Europe)	NCT02140554, NCT02906202	40 SCD, 23 $\beta$ -thalassemia	25 SCD (group C) participants followed for 3–25 months. Most had near-pancellular expression of HbA <sup>1870</sup> $\geq$ 6 months after therapy with 99.5% mean reduction in the annualized VOC+ACS rate overall; 89% of evaluable participants with $\beta$ -thalassemia are transfusion independent at a median of 19 months follow-up.	55, 210
	Modified $\gamma^{G16D}$ -globin LV	Cincinnati Children's Hospital Medical Center	Phase I/II	NCT02186418	3	3 SCD participants, follow-up 6–30 months, with clinical improvement (decreased VOs)	154
	Modified $\gamma^{G16D}$ -globin LV	CSL Behring	Phase I	NCT04091737	3	No results posted	
	LV expressing the $\beta$ A53-globin gene	Assistance Publique–Hôpitaux de Paris	Phase I/II	NCT03964792	10	No results posted	
	$\beta$ A53-FB LV	University of California, Los Angeles	Phase I/II	NCT02247843	6	No results posted	
HbF induction	<i>BCL11A</i> shRNA LV	Dana-Farber Cancer Institute	Phase I/II	NCT03282656	6	6 SCD participants infused, follow-up 7–29 months. No patient has had a VOC, ACS, or stroke since the gene therapy infusion.	33
	Cas9 NHEJ; disruption of erythroid enhancer in <i>BCL11A</i> gene	CRISPR Therapeutics/Vertex Pharmaceuticals	Phase I/II	NCT03745287	2 SCD, 5 $\beta$ -thalassemia	2 SCD participants with follow-up of 3 and 12 months have had no SCD-related VOs since infusion of gene-modified cells; 5 $\beta$ -thalassemia participants with follow-up of 3–15 months are transfusion independent.	32, 83
	Cas9 NHEJ	Novartis/Intellia	Phase I/II	NCT04443907	Not reported	No results posted	
	ZFN NHEJ; disruption of erythroid enhancer in <i>BCL11A</i> gene	Sangamo/Bioverativ/Sanofi	Phase I/II	NCT03432364	2	2 participants with $\beta$ -thalassemia infused, follow-up 3–6 months. Both require intermittent transfusions, though requirements are decreased.	211

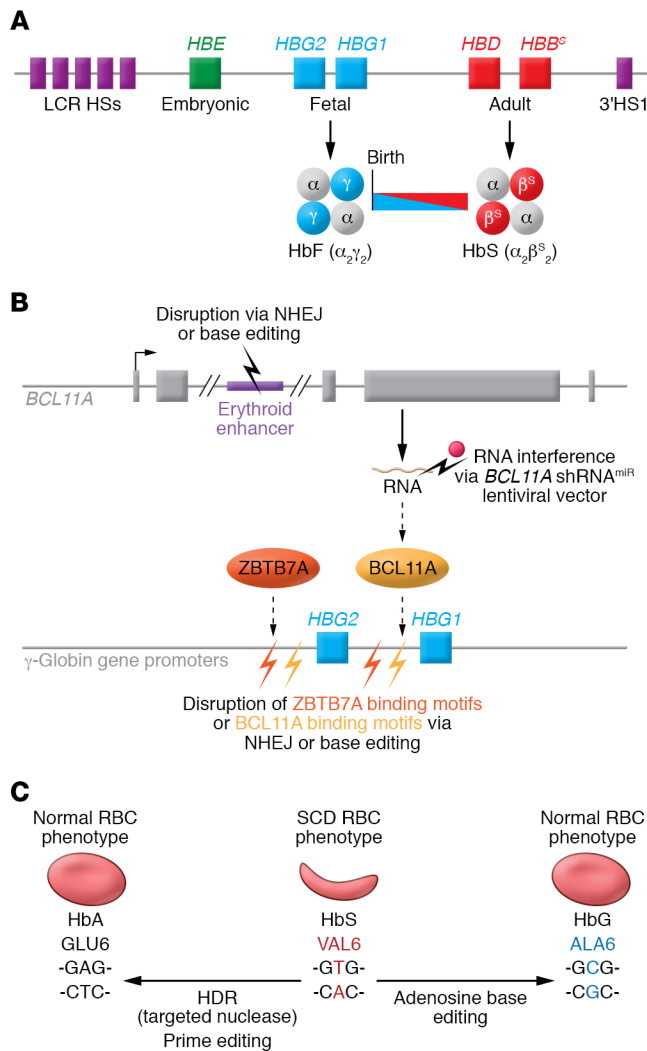
All clinical trials for SCD listed on ClinicalTrials.gov as of January 2021 are shown. Some trials also include patients with  $\beta$ -thalassemia. ACS, acute chest syndrome; NHEJ, non-homologous end joining; VOC, vaso-occlusive crisis; VOE, vaso-occlusive pain event; ZFN, zinc finger nuclease.

of  $\beta$ -globin, thereby shifting the production of HbF to HbA. The onset of SCD symptoms coincides with this switch, usually around 6 months of age (Figure 3A). Methods to induce HbF therapeutically for  $\beta$ -hemoglobinopathies have been sought for decades (66–68). This HbF therapy premise derives from a naturally occurring benign genetic condition termed hereditary persistence of fetal hemoglobin (HPFH), which results in persistently elevated HbF levels (more than 30%) in all RBCs (69, 70). Individuals who coinherit HPFH and SCD exhibit few or no SCD effects because HbF inhibits HbS polymerization (71) and  $\gamma$ -globin gene (*HBG1* and *HBG2*) induction competes for the locus control region, a powerful upstream enhancer, to suppress mutant *HBB<sup>S</sup>* gene expression (72, 73). Two transcriptional repressors, *ZBTB7A* and *BCL11A*, participate in the developmental silencing of *HBG1* and *HBG2* by binding to their respective promoter *cis*-regulatory elements (74–76). Some HPFH variants disrupt these *cis* elements to inhibit repressor binding, while other variants create nearby de novo binding sites for transcriptional activators (70). Numerous approaches to induce RBC HbF therapeutically for SCD via genetic manipulation of HSCs are under investigation (Table 2 and Figure 3B).

**RNA interference to silence *BCL11A* expression.** Transduction of normal or SCD donor CD34<sup>+</sup> cells with a microRNA-adapted short hairpin RNA (shRNA<sup>miR</sup>) that suppresses *BCL11A* expression induced high-level HbF in RBC progeny generated in vitro

or in vivo after xenotransplantation into immunodeficient mice (77–79). Six subjects have been treated in a clinical trial using this approach (NCT03282656). Preliminary results with 7–29 months follow-up include RBC HbF levels of 20%–41% and hemoglobin levels of 9.3–11.4 g/dL (33). No post-gene therapy acute vaso-occlusive pain events, acute chest syndrome, or stroke were reported in any participant, although one subject continued to experience priapism for up to 8 months after gene therapy and another remains transfusion dependent for cerebral vasculopathy, albeit at a decreased transfusion frequency.

**Genome editing to activate  $\gamma$ -globin gene expression.** Genome-editing nucleases, such as ZFNs, TALENs, and CRISPR/Cas9, introduce precisely targeted double-stranded DNA breaks (DSBs) that are subsequently resolved by endogenous repair pathways, either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Figure 2B and refs. 49, 50). The Cas9 nuclease is easiest to use because its sequence specificity is programmed by an associated guide RNA (gRNA) that binds the DNA target site via Watson-Crick base pairing, in contrast to other genome-editing nucleases that are programmed by more complicated protein engineering. In HSCs, most DSBs are resolved by NHEJ, which typically introduces base pair insertions or deletions (indels) that can disrupt DNA regulatory motifs. Using this strategy, several groups have induced RBC  $\gamma$ -globin transcription and HbF expres-



**Figure 3. Genetic manipulations to treat SCD. (A)** The developmentally regulated  $\beta$ -like globin gene cluster is shown. Noncoding transcriptional regulatory regions are shown as DNase I-hypersensitive sites (HSs) at the locus control region (LCR) and 3' to the *HBB* gene (3'HS1). The fetal  $\gamma$ -globin genes (*HBG1* and *HBG2*) are expressed during late gestation, resulting in the production of RBC HbF. Around birth,  $\gamma$ -globin expression declines and is replaced by  $\beta$ -globin, resulting in a shift from HbF to HbA ( $\alpha_2\beta_2$ ) normally, or HbS ( $\alpha_2\beta^S_2$ ) in the case of SCD. Inhibiting the  $\gamma$ - to  $\beta$ -globin switch has been a historical Holy Grail for treating SCD and  $\beta$ -thalassemia. **(B)** Induction of HbF by interfering with the expression or function of *HBG1*/*HBG2* transcriptional repressors *BCL11A* or *ZBTB7A*. Strategies for manipulation of autologous SCD patient HSCs include disruption of an erythroid-specific *BCL11A* gene enhancer via genome-editing nuclease-mediated NHEJ or base editing, transduction with an LV that drives erythroid-specific expression of a *BCL11A* shRNA, and disruption of *BCL11A* or *ZBTB7A* binding motifs in the *HBG1* and *HBG2* promoters. **(C)** Conversion of the mutant SCD codon (valine) to normal glutamic acid can be engineered by genome-editing nuclease-mediated HDR or by prime editing. Alternatively, the SCD mutant valine codon can be converted to alanine by adenosine base editing to generate the nonsickling benign variant Hb G-Makassar.

sion by creating indels that disable an erythroid-specific *BCL11A* gene enhancer (80–82). Preliminary results from a clinical study (CLIMB SCD-121, NCT03745287) are promising (Table 2 and refs. 32, 83). Two SCD participants (follow-up 3 and 12 months) maintained a hemoglobin level of around 10 g/dL with 46.8% and 42.4% HbF. Two participants (one with SCD and one with  $\beta$ -thalassemia) exhibited sustained high-level editing of the targeted alleles (78%–81%) in bone marrow cells at 6 and 12 months. Although longer studies in more individuals are required, these findings indicate efficient modification of long-term repopulating HSCs.

An alternative strategy to induce RBC HbF uses genome editing-mediated NHEJ to disrupt *HBG1* and *HBG2* promoter motifs that recruit the *BCL11A* or *ZBTB7A* repressor proteins (Figure 3B and refs. 84–86). Another clinical study (NCT04443907) is examining the safety and efficacy of Cas9-mediated disruption of a *BCL11A* binding site in the  $\gamma$ -globin gene promoters (Table 2).

It is also possible to disrupt DNA regulatory elements using base editors, engineered Cas9-directed DNA modification enzymes that introduce precise, targeted nucleotide alterations (Figure 2C and ref. 49). Base editors contain modified versions of Cas9, termed Cas9 nickase (Cas9n), fused to a nucleotide deaminase. Adenosine base editors contain Cas9n fused to a laboratory-

evolved adenosine deaminase that converts targeted A:T base pairs to G:C pairs. Cytosine base editors fuse Cas9n with a cytosine deaminase to convert targeted C:G base pairs to T:A pairs. Base editors are now being used to disrupt DNA elements that silence  $\gamma$ -globin expression, including the *BCL11A* erythroid enhancer (87) and binding sites for *BCL11A* or *ZBTB7A* in the *HBG1* and *HBG2* genes (88, 89).

*Direct repair of the mutant SCD codon.* The most desirable strategy for genetic correction of SCD is to convert the mutant codon (valine, GTG) to the normal one (glutamic acid, GAG). Codon conversion can be achieved by genome editing-mediated HDR, or prime editing (Figure 2C). Achieving high-level SCD correction by genome editing-mediated HDR is complicated by low rates of HSC correction, concomitant formation of indels that disrupt the *HBB* reading frame, and the requirement for an exogenous DNA repair template, which can be challenging to deliver and is potentially cytotoxic (90–94). In most preclinical studies of genome editing, human HSCs are approximated by their capacity to repopulate the bone marrow of immunodeficient mice at 16 weeks following transplantation. An early study attained  $11.8\% \pm 3.7\%$  allele correction by HDR and  $55\% \pm 19\%$  indels in bulk HbSS CD34<sup>+</sup> HSPCs; following xenotransplantation, allele correction measured in HSCs was 2.3%

$\pm 1.8\%$ , with  $46\% \pm 6\%$  indels (95). Another early study achieved approximately 50% SCD allele correction in bulk HbSS CD34<sup>+</sup> cells (92). Although xenotransplantation analysis of this population was not reported, the investigators achieved 3.5% HDR at the same target site in bone marrow-repopulating HSCs using a GFP-encoding DNA repair template. A more recent study reported HDR correction of 33.6% SCD alleles in bulk CD34<sup>+</sup> cells. Four months after xenotransplantation, the correction rate declined to 23%, with approximately 30% having at least one corrected allele and 57% containing biallelic gene-disrupting indels (96). In a humanized mouse model for HbSS SCD, investigators have achieved an average of 14.8% (1%–35.4%,  $n = 9$ ) allele correction in repopulating HSCs, with improvement of hemolytic anemia (97). Direct correction of the SCD codon will likely improve with protocols and technologies designed to enhance the rates of HDR in HSCs and/or select for those that are genetically corrected (98–104).

Prime editors contain Cas9n fused to an engineered reverse transcriptase that directly copies edited sequence information from a prime editing guide RNA (pegRNA) into a target DNA locus, then causes the cell to replace the original DNA sequence on both strands with the newly synthesized DNA flap (Figure 2D and refs. 49, 105). Prime editing can convert the SCD mutation to the wild-type allele at relatively high efficiencies in HEK293 cells (26%–52%; ref. 105) but requires further optimization for high-frequency targeted modification of human HSCs.

Base editors cannot create the T-to-A transversion required to revert the mutant SCD codon (valine, GTG) to wild-type (glutamic acid, GAG). However, adenine base editors can convert the mutant valine to alanine (GCG) to generate hemoglobin G-Makassar (Hb G-Makassar), a rare naturally occurring, nonsickling variant discovered in Southeast Asia (Figure 3C). Hb G-Makassar heterozygotes and one reported homozygote exhibit normal RBC indices, indicating that the variant is benign (106–109). Protein evolution strategies have developed an A base editor that can convert HbS alleles to Hb G-Makassar efficiently in HEK293 cells (110) and in mouse repopulating HSCs from subjects with SCD (111).

*Genotoxicities associated with genetic modification of HSCs.* All methods to manipulate the HSC genome carry the potential for genotoxicity, the major concern being inadvertent clonal malignant transformation. Leukemia caused by  $\gamma$ -retroviral vector-mediated insertional activation of the *LMO2* proto-oncogene was a major setback for early clinical studies of gene therapy for immunodeficiency (11, 12, 112). While the use of modified LVs markedly reduced this problem, Espinoza et al. reported dysplastic clonal hematopoiesis following LV transduction of HSPCs in a rhesus macaque model (113). This study identified two factors that increased the risk for malignancy: a high VCN (nine) and a potent murine stem cell virus (MSCV) constitutively active promoter-enhancer in the LV long terminal repeat. The LV used in the study was a hybrid vector that likely affected the insertional profile, contributing to the malignancy. None of the LVs used in clinical trials for  $\beta$ -hemoglobinopathies use the MSCV element (11). In the HGB-207 (NCT02906202) clinical trial for  $\beta$ -thalassemia, one participant treated with LV gene therapy developed a dominant HSC clone with insertional activation of *HMGA2*, a DNA-binding protein associated with benign and malignant tumor formation (114). Hematopoiesis remained stable over

15 months, and there have been no subsequent reports on this patient. It remains to be determined whether the myeloid malignancies recently reported in two individuals with SCD gene therapy are related to LV insertion (13, 14).

To our knowledge, no clinically relevant adverse event attributable to LV integration has been reported in any clinical trial for any indication (12). Nonetheless, preclinical safety studies of all LVs must determine integration sites after transduction in vitro, and assess for clonal dominance after transplantation of transduced cells in animal models (112). In human LV gene therapy trials, long-term (15 years) clinical evaluations and longitudinal determinations of vector integration sites in purified hematopoietic lineages must be included to assess clonal dominance as part of safety monitoring. These studies may also offer insights into the biology of hematopoietic differentiation and clonal succession (115, 116). It should be possible to enhance such studies by analyzing single cells, which is now being done to map clonal trajectories in malignant hematopoiesis (117).

Genome-editing nucleases may induce several genotoxicities, although it is too early to know the clinical impact (118–120). First, genome-editing proteins can create unintended off-target DSBs followed by formation of small indels, usually in DNA regions with homology to the targeted site. Second, on- and off-target DSBs can create kilobase-scale local DNA rearrangements or deletions, including loss of the entire chromosomal arm telomeric to the DSB. Moreover, DSBs can activate the TP53 tumor suppressor protein, leading to cell cycle delay, apoptosis, and selective pressure for *TP53* gene loss, which can promote malignant transformation (121–125). Methods to investigate potential genotoxicities include computational algorithms based on homology to the on-target nucleotide sequence, whole genome sequencing, and molecular cloning approaches that enrich for genomic segments with genome editing-induced DSBs, followed by next-generation sequencing (118–120). Standard karyotyping approaches and/or more sensitive high-throughput sequencing-based methods, such as Uni-Directional Targeted Sequencing (UDiTaS), may detect translocations and chromosomal rearrangements caused by genome editing (126). Current methods can detect genome-editing off-target mutations at sensitivities from 0.01% to 1%. Theoretically, oncogenic mutations could occur at lower rates. Moreover, the current regulatory standard for tumorigenicity is to evaluate a patient-sized dose of genome-edited HSPCs in immunodeficient mice. As the natural life span of these mice is less than 1 year, this assay may fail to detect oncogenic mutations that require longer time frames for clonal expansion. Hence, a major challenge for therapeutic genome editing is to develop more sensitive detection methods for genotoxicities, including cell-based approaches to assess oncogenic risks associated with specific gene targeting protocols.

Approaches to reduce off-target DSBs by enhancing the specificity of genome-editing nucleases have focused mainly on CRISPR/Cas9 systems, which are more versatile and adaptable than ZFNs and TALENs (118, 119). The specificity of Cas9-mediated DSBs may be increased via at least five methods: using dimeric versions that require two distinct adjacent gRNA-programmed binding domains to install a DSB; developing Cas9 variants with reduced catalytic activity at regions of DNA with partial homol-



ogy to the on-target site; modifying the structure or length of the programming gRNA; employing short gRNAs as decoys against potential off-target loci; and using phage-derived proteins that antagonize CRISPR/Cas9 nuclease activity (118, 119, 127–129).

In contrast to standard genome-editing nucleases and LVs, base editors act through mechanisms that are independent of DSBs, facilitating more precise DNA modifications and reducing some genotoxicities (49, 124, 130). However, base editors also carry unique potential to create undesired modifications. At target sites, base editors can produce “bystander” edits of nearby A or C nucleotides or induce low-frequency DSBs with resultant indels. Base editors can also induce low-level off-target deamination of A or C nucleotides in DNA or RNA through Cas-dependent and -independent mechanisms (131, 132). Several laboratories have created variants of adenine and cytosine base editors by altering their Cas9 and/or deaminase domains (131–143).

Similar to base editors, prime editing does not act through the creation of DSBs. Early studies indicate that prime editing is less prone to off-target modifications than is conventional Cas9 nuclease with the same gRNAs (105, 144–146).

## Bone marrow conditioning and infusion regimens

Myelotoxic chemotherapy and/or irradiation before infusion of genetically modified autologous HSCs eliminates resident HSPCs that compete for the hematopoietic niche, facilitating engraftment. Most autologous HSCT protocols use high-dose myeloablative conditioning to promote full replacement of bone marrow with corrected HSCs (32, 33, 53, 147, 148). Major toxicities include multi-organ damage, infertility, and myeloid neoplasms (149). One individual who received LV gene therapy with high-dose busulfan for SCD developed myelodysplastic syndrome after approximately 3 years (13). As the malignant clone did not harbor LV DNA, transformation was attributed to busulfan conditioning. The myelodysplastic syndrome transformed to acute myeloid leukemia, and the patient subsequently underwent induction chemotherapy followed by haploidentical transplantation. Unfortunately, relapsed disease ultimately led to his death. Genotoxic agents like busulfan create mutations that can synergize with preexisting germline or somatic cancer susceptibility mutations, and/or an abnormal bone marrow microenvironment, to induce malignant evolution (150).

Because normal or genetically corrected RBCs survive longer in the circulation than SCD RBCs, therapeutic effects may be gained by establishing partial bone marrow chimerism with normal or HbAS HSCs (151–153). Sub-ablative conditioning has been incorporated into one LV gene therapy clinical trial for SCD (154) and may reduce HSCT toxicities for all SCD patients. However, a recent report raises caution (155). Three of 76 adult SCD patients who received allogeneic HSCT with reduced-intensity conditioning developed graft rejection followed by myeloid leukemia. Two individuals studied further were found to harbor somatic *TP53* mutations in bone marrow HSPCs prior to HSCT. Most likely, outgrowth of these preleukemic clones was favored by low-intensity conditioning, similar to what has been observed with HSCT for myelodysplastic syndrome or acute myeloid leukemia (156, 157). Thus, it may be prudent to screen adults with SCD for somatic mutations associated with clonal hematopoiesis before autologous HSCT.

Reduced-toxicity HSCT conditioning regimens using antibodies against HSPC cell surface receptors are under study for treating refractory leukemia (158) and may also be effective for autologous HSCT. Unconjugated anti-CD117 (c-KIT) antibody is being tested as a non-genotoxic conditioning agent for allogeneic HSCT in infants with severe combined immunodeficiency, with promising early data (159, 160). Killing of anti-CD117-bound HSPCs is inhibited by their expression of CD47 and its interaction with signal regulatory protein- $\alpha$  (SIRP $\alpha$ ) on the surface of immune effector cells (161). Thus, coadministration of anti-CD47 may enhance the efficacy of anti-CD117 for pre-HSCT conditioning (162). Studies in mice and nonhuman primates indicate that bone marrow conditioning with toxin-linked antibodies against CD45 or CD117 can facilitate donor HSC engraftment with minimal toxicity (163–167). Reduced-toxicity bone marrow conditioning with radioisotope-linked antibodies has been examined in allogeneic HSCT for hematological malignancies (168–172). Similar approaches using  $\alpha$ -emitters with high linear energy transfer and short path lengths (40–90  $\mu$ m) (173, 174), such as actinium-225 and astatine-211, may provide reduced-toxicity conditioning in autologous HSCT for nonmalignant blood disorders, including SCD.

## Preclinical endpoints that predict therapeutic responses

Ideally, genetic correction of autologous SCD patient HSCs will generate 100% nonsickling RBCs with normal circulatory life span, eliminate disease symptoms, and arrest end-organ damage. Modification of all HSCs is not required to achieve this goal because corrected RBCs have a survival advantage in the circulation. The clinical benefits of any autologous HSCT for SCD depend on the fraction of modified HSCs in the bone marrow and the relative survival advantage conferred to RBC progeny, which can be modeled mathematically (151, 175). In general agreement with these models, allogeneic HSCT studies with normal or SCD-heterozygous donors indicate that chimerism as low as 20% can result in 100% circulating donor RBCs (151–153, 176). By this analogy, heterozygous or homozygous correction of the mutant SCD codon via HDR must occur in at least 20% of repopulating HSCs. The same may hold true for base editor conversion of HbS to Hb G-Makassar, assuming that the HbGS heterotetramer is nonsickling under normal physiological conditions.

*Lentiviral vector gene therapy.* Variables that predict successful LV gene therapy for SCD include the fraction of modified human HSCs, expression levels of the  $\beta$ -like globin transgene, its antisickling properties, and its ability to outcompete endogenously expressed  $\beta^S$ -globin for binding to  $\alpha$ -globin during Hb assembly. Clinical trial data using the BB305  $\beta^{A-T87Q}$ -globin LV (HGB-206, NCT02140554) demonstrate a relatively high VCN in the preinfusion CD34<sup>+</sup> cell product (mean 3.8 copies per diploid genome), which may be required for full therapeutic efficacy (177). This VCN generates approximately 16–20 pg  $\beta^{A-T87Q}$  per cell in an immortalized erythroid line harboring an engineered  $\beta^S$  mutation (178). Interestingly, this study showed that expression of the  $\beta^{A-T87Q}$  transgene caused VCN-dependent reductions in endogenous  $\beta^S$ -globin mRNA and protein.

*Genetic induction of HbF.* Predicting the therapeutic requirements for  $\gamma$ -globin gene induction is complex because the protec-

tive effects of HbF on SCD can be partial and the levels required to inhibit SCD pathologies are likely organ specific. For example, high HbF is associated with longer life span, reduced pain episodes, and fewer leg ulcers (179, 180). A protective role for HbF against cerebrovascular disease is less clear. Several studies show that silent cerebral infarcts or ischemic strokes associate with low HbF levels (181–183). However, these associations were not replicated in two large, multicenter studies (184, 185).

Genetic modifications that induce HbF pancellularity are likely to attenuate SCD-related morbidities more effectively than those that induce HbF heterocellularity (179, 180). Many patients with SCD in India and parts of the Middle East carry the Arab-Indian haplotype at the  $\beta$ -like globin locus, which is associated with approximately 20% HbF expressed heterocellularity. These individuals experience SCD-related morbidities, although later in life compared with patients in the United States and other regions where the Arab-Indian haplotype is less common (186, 187). In contrast, individuals with SCD and HPFH possessing more than 30% HbF distributed pancellularity appear to be symptom free (179, 188, 189). Thus, while the protective thresholds for HbF induction may vary across different organ systems, genetic manipulations that induce more than 30% pancellularity should produce substantial clinical benefits and may induce cures. For comparison, six individuals who received autologous HSCs that were transduced with LV encoding erythroid-expressed *BCL11A* shRNA exhibited a median of 30.5% (range, 20.4%–41.3%) HbF in RBC lysates, with 70.8% (range, 58.9%–93.6%) of individual RBCs expressing HbF, as detected by immunoflow cytometry (F cells) (33). One individual who received autologous HSCs harboring Cas9-disrupted *BCL11A* erythroid enhancer has maintained 42%–49% HbF expressed pancellularity in RBCs at 15 months after therapy (32). All of these patients have exhibited markedly reduced pain episodes and improved laboratory parameters of hemolysis. It will be important to correlate laboratory studies with detailed multiorgan assessments at 5 and 10 years on these and future gene therapy research participants.

Induction of HbF inhibits RBC sickling by reducing the effective concentration of HbS, which is the primary determinant of its polymerization at low oxygen tension (71). It is estimated that 9–12 pg HbF per RBC can block HbS polymerization in venous capillary beds (179). The fraction of RBCs that achieve this HbF threshold is poorly predicted by standard clinical tests such as percentage HbF in RBC lysates and detection of F cells with anti-HbF antibodies. Moreover, these tests imprecisely predict SCD severity. Thus, improved analytical methods to quantify HbF and HbS concentrations in individual RBCs and to measure their propensity for sickling under physiological oxygen concentrations should enhance preclinical testing of genetic strategies to treat SCD (190–195).

## Perspectives and future

While studies of SCD over the past 50 years have benefited medical science, it may be argued that affected patients have fared less well. The 2020 NASEM report notes that “the health care needs of individuals living with SCD have been neglected by the U.S. and global health care systems, causing them and their families to suffer” (27). Now we are poised to develop new potentially curative therapies based on autologous HSCT. Enabling technologies are

advancing rapidly and the future is promising. However, the field is in its infancy, the best strategies remain unknown, and each step (Figure 1) requires further optimization. Moreover, advances are also occurring in alternative donor allogeneic HSCT, including the use of haploidentical donors with non-myeloablative bone marrow conditioning (196–198). Ensuring that patients and families are fully informed about the potential risks and benefits of different curative approaches represents a major ethical challenge (19, 199).

Recent reports of SCD patients developing myeloid neoplasms after undergoing LV gene therapy remind us that experimental treatments come with unknown risks. Of 47 patients treated in the largest LV gene therapy trial to date, three have been diagnosed with myeloid malignancies. One of these has been attributed to busulfan conditioning, and studies are under way to investigate the etiologies of the two cases reported more recently (13, 14). Leukemia has not been reported in several hundred individuals who have been treated with LV gene therapy for indications other than SCD, including 63  $\beta$ -thalassemia patients who received the same vector used for SCD in separate clinical trials. Thus, SCD itself may predispose to gene therapy-related myeloid malignancies, possibly by enhancing the rate of preleukemic somatic mutations acquired before treatment (155, 200) (discussed in “Bone marrow conditioning and infusion regimens” above) and/or by creating an abnormal bone marrow microenvironment (150, 201). Similar risks could also apply to newer LV gene therapy or genome editing protocols. A promising trial of LV-mediated posttranscriptional silencing of *BCL11A* was also paused out of an abundance of caution, though no such events have been described in the nine individuals treated to date (Table 2 and ref. 202). These recent observations highlight the need for close long-term clinical and molecular monitoring, beginning before the initiation of gene therapy.

The notion that individuals with SCD may be uniquely susceptible to gene therapy-related myeloid malignancies raises concerns about hydroxyurea as a potential contributor. Hydroxyurea is antimetabolite that has been used to treat SCD since the 1980s. Some studies indicate that hydroxyurea can be mutagenic in vitro and enhance the rate of myelodysplastic syndrome/acute myeloid leukemia transformation in patients with myeloproliferative disorders (203). However, these studies have remained inconclusive, and a World Health Organization expert panel concluded that hydroxyurea is not classifiable as to its carcinogenicity to humans (203). Moreover, long-term studies have shown that hydroxyurea therapy reduces morbidity and mortality in SCD without increasing the rates of accumulated mutations or cancer (204). It has recently been reported that individuals with SCD are at increased risk for myeloid malignancies, yet no further increase was noted in the data after hydroxyurea’s FDA approval (205). We conclude that while the potential for chronic hydroxyurea administration to influence long-term outcomes of gene therapy should be investigated, current evidence indicates that the benefits of hydroxyurea therapy outweigh the risks for most patients.

Current autologous HSCT therapies for SCD are technologicaly complex, expensive, and high-risk. We believe that the development of reduced-toxicity bone marrow conditioning regimens to be used in conjunction with autologous HSCT represents a high-priority challenge. As safe and effective approaches become established, it will be important to streamline manufacturing and reduce costs

in order to disseminate cures more broadly. Initially, such therapies will occur in high-income countries where advanced tertiary care centers provide cell manufacturing and medical support. Delivery of autologous HSCT therapies to the vast majority of individuals with SCD, who reside mainly in low- and middle-income countries, will be facilitated by reduced-toxicity *in vivo* approaches that selectively modify HSCs via intravenous or bone marrow injection of targeted delivery vehicles such as engineered nanoparticles or nonintegrating viral vectors (206–208). The NIH and the Gates Foundation are collaborating to develop gene-based cures for SCD on a global scale (209). We are confident that this endeavor will succeed, although it will require time and advancing technologies. In the meantime, autologous HSCT for SCD must be considered as an essential component of a larger, multifaceted effort that also includes widespread newborn screening, institution of preventative therapies such as immunization, prophylactic penicillin and hydroxyurea, better drugs, allogeneic HSCT, and optimized infrastructures for delivering both basic and advanced medical care.

### Author contributions

PAD, AS, JSP, YZ, JFT, and MJW performed background research, wrote sections of the manuscript and edited the document. MJW

organized the manuscript and coordinated the writing. PAD and AS contributed equally to the authorship of this work; their order was assigned alphabetically.

### Acknowledgments

We apologize to all the colleagues whose contributions we could not properly acknowledge in this Review because of length restrictions. We thank SCD patients for participating in the research described here. The authors' research on genetic therapies for SCD is supported by NIH grants F32 DK118822 (to PAD), K01 HL125495 (to JSP), and P01 HL053749 (to MJW); the American Society of Hematology Scholar Award (to AS); Doris Duke Sickle Cell Disease/Advancing Cures grant 2017093 (to MJW and AS); the Assisi Foundation of Memphis (to MJW); and St. Jude/American Lebanese Syrian Associated Charities (ALSAC). We thank Michael Debaun, Greg Newby, and David Liu for helpful comments on the manuscript.

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