Title

Diverse Approaches to Gene Therapy of Sickle Cell Disease.

Authors

White, Shanna
Hart, Kevyn
Kohn, Donald

Publication Date

2023-01-27

DOI

10.1146/annurev-med-042921-021707

Peer reviewed

HHS Public Access

Author manuscript

Annu Rev Med. Author manuscript; available in PMC 2024 April 08.

Published in final edited form as:

Annu Rev Med. 2023 January 27; 74: 473–487. doi:10.1146/annurev-med-042921-021707.

Diverse Approaches to Gene Therapy of Sickle Cell Disease

Shanna White[1], **Kevyn Hart**[2], **Donald B. Kohn**[1,3,4,*]

[1]Department of Pediatrics, Division of Hematology/Oncology, David Geffen School of Medicine, University of California, Los Angeles

[2]Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles

[3]Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles

[4]The Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, David Geffen School of Medicine, University of California, Los Angeles

Sickle Cell Disease

Genetics, biochemistry and clinical manifestations

Sickle cell disease (SCD) is a hemoglobinopathy, caused by a single common mutation in the β-globin chain of hemoglobin in all affected persons, leading to an abnormal hemoglobin protein. It is most prevalent in sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries; affecting 300,000 to 400,000 newborns per year and about 20 million people worldwide.[1] While the prevalence in the United States is significantly less, it is estimated that about 100,000 persons have SCD.[2] Sickle cell disease causes life-long complications leading to significant morbidity and a shortened life expectancy.

The classical mutation causing SCD, or hemoglobin SS disease (HbSS), is a homozygous point mutation (A-T) in the sixth codon of the β-globin gene on chromosome 11. This mutation leads to a single amino acid substitution of glutamic acid to valine at position six within the β-globin chain. This single substitution characterizes sickle hemoglobin (HbS) and leads to the many clinical manifestations. Persons with HbSS disease do not synthesize hemoglobin A (HbA) and instead have >75% HbS. Deoxygenation of HbS leads to polymerization of the hemoglobin and a subsequent gelatinous network of fibrous polymers and the transformation of red blood cells (RBC) into rigid, sickle-shaped cells. Coinheritance of hemoglobin C (HbSC), hemoglobin E (HbSE), or β-thalassemia alleles including $\beta\text{-thal}^0$ or $\beta\text{-thal}^+$, among others, all lead to forms of SCD with varying phenotypes.[3,4]

The sickled red blood cells, being less deformable, cause capillary blockage, sustain cell membrane damage and undergo hemolysis, all of which contribute to the clinical manifestations of SCD. The phenotypic presentation of patients with SCD range

*Corresponding Author: Donald B. Kohn, M.D., University of California, 3163 Terasaki Life Science Bldg., 610 Charles E. Young Drive East Los Angeles, CA 90095, dkohn@mednet.ucla.edu.

significantly in severity, including both acute and chronic complications. Most individuals in developed nations are diagnosed early in infancy via newborn screening. If undiagnosed at birth, symptoms may begin to manifest as early as six months of age, coinciding with the natural decline of fetal hemoglobin synthesis and the onset of production of adult hemoglobin containing β-globin.

Persons with SCD experience life-long intravascular hemolysis and chronic anemia. The most common acute complication is veno-occlusive events (VOE); episodic microvascular occlusions.[5] These events may manifest as dactylitis (mostly as an infant or toddler), bone pain and abdominal pain due to microvascular occlusion of mesenteric blood vessels and infarction of liver, spleen or lymph nodes. Other acute complications include acute chest syndrome, stroke, priapism, splenic sequestration, and transient pure red cell aplasia.

Great progress has been made in preventing childhood mortality, now with >95% of children in developed countries surviving to adulthood.[6] With increased survival, there has become an increasing number of persons with SCD living longer, revealing the chronic sequelae of the disease. These chronic manifestations of SCD are largely secondary to chronic tissue damage and vasculopathy with secondary end-organ damage. Any organ system can be affected. The chronic complications most commonly noted include effects from silent stroke, pulmonary hypertension, renal and liver dysfunction, avascular necrosis of bones, retinopathy and functional hyposplenism.

HbS can also be co-inherited with alpha-thalassemia.[7] Coinheritance is now well documented, as about one-third and one-half of patients of African and Middle Eastern or Indian descent, respectively, are noted to have alpha-thalassemia. This coinheritance is most often seen with deletion of one or two of the alpha globin genes and leads to an overall decreased concentration of hemoglobin in the RBCs. Because the rate of HbS polymerization is dependent on the concentration of hemoglobin within the erythrocytes, this decrease in hemoglobin level results in a subsequent decrease in cellular damage. The phenotype of patients with coinheritance is characterized by higher hemoglobin levels, lower mean corpuscular volume, less hemolysis and therefore fewer complications associated with hemolysis.

It is known that when HbF levels are higher, such as at birth, patients with SCD have fewer or no clinical manifestations. Most patients do not have the onset of complications until following the decline in HbF synthesis after birth. It has also been observed that persons who have coinciding hereditary persistence of HbF (HPFH) have a milder disease phenotype.[8] HbF interferes with polymer formation of the HbS within RBCs.[9] The known benefits of increasing HbF in patients with SCD has been the basis for development of various treatment options.

Standard Care for Sickle Cell Disease

Much of the routine care for patients with SCD involves close follow-up for early indications of known serious complications or active management of acute or chronic complications. Routine screenings include frequent laboratory monitoring for anemia and hemolysis markers, transcranial doppler studies for monitoring of intracranial blood vessel

flow velocities, urinalysis to evaluate for micro albuminurea and close monitoring for signs of infection, among others. Although there has been much progress in reducing early childhood mortality in developed countries, this has largely been due to early detection with newborn screening, penicillin prophylaxis, and vaccination against encapsulated micro-organisms, which can cause sepsis in SCD patients who develop functional asplenia early in life.

Until recently, Hydroxyurea was the only disease modifying treatment option for patients with SCD (Table 1). Hydroxyurea is an oral chemotherapeutic that raises levels of HbF. First tested in SCD in 1984, it has been shown to decrease frequency of pain episodes, acute chest, transfusions, acute stroke, and hospitalizations.[10] However, Hydroxyurea does not completely ameliorate symptoms and does not fully prevent progression to the chronic complications of SCD.

Transfusion therapy is another management option with the purpose being to decrease the circulating HbS. There are various indications for either simple or exchange transfusions. Simple transfusions are indicated in patients with acute, symptomatic anemia, aplastic crisis, symptomatic acute chest associated with anemia below baseline, splenic sequestration and severe anemia, as well as pre anesthesia.[11,12] Exchange transfusions are indicated in patients with acute or impending cerebrovascular accident and symptomatic severe acute chest syndrome. Some patients also require continued, chronic simple or exchange transfusions based on the severity and frequency of SCD complications. Multiple or chronic transfusions can have serious side effects, including alloimmunization and transfusional hemochromatosis and often require concurrent iron chelation therapy.[6]

There has been little progress in the development of disease-modifying therapeutics until recent years (Table 1). L-Glutamine was approved in 2017, almost 20 years following hydroxyurea.[13] The exact mechanism of action is unknown; L-glutamine acts by increasing nicotinamide adenine dinucleotide (NAD+) synthesis which should help in preventing oxidative damage to RBC and subsequently decreasing hemolysis.[13] It is an oral powder and has been shown to decrease VOEs and hospitalizations, although nearly three-quarters of patients in the phase 3 trial were also on hydroxyurea, suggesting it should be used in combination.[6,7]

Following the approval of L-Glutamine, Voxelotor, a HbS polymerization inhibitor, was approved in 2019.[15] It is an oral medication, taken daily. Voxelotor binds reversibly to hemoglobin, stabilizing the oxygenated form and therefore preventing the HbS polymerization by increasing hemoglobin's affinity to oxygen.[16] Voxelotor has demonstrated a dose-dependent increase in hemoglobin affinity and an increase in baseline hemoglobin by at least 1.0 g/dL and a decrease in hemolysis markers. While this may be beneficial in helping patients to discontinue chronic transfusions, it has not been shown to decrease VOE frequency.[15]

Crizanlizumab is another recent disease-modifying option for patients with SCD. Crizanlizumab is a humanized, anti-P-selectin monoclonal antibody that is given intravenously. P-selectin is a cell adherence molecule expressed on the surface of endothelial

cells and platelets when activated and is involved in the complex process which results in VOEs. It was shown to decrease the annual rate of VOEs, regardless of concomitant hydroxyurea use.[17]

Like hydroxyurea, all of the disease modifying agents have a benefit in the short term and act to prevent acute complications. The newer agents have not yet been evaluated longitudinally to evaluate long-term prevention of chronic complications of SCD.

Allogeneic Hematopoietic Stem Cell Transplant (HSCT) for Sickle Cell Disease

Allogeneic hematopoietic stem cell transplant (HSCT) is currently the only proven curative option for patients with SCD. Highest overall survival (OS) rates are seen in HSCT performed with a matched sibling donor (MSD); however less than 20% of patients have a MSD available.[18–20] Along with the best OS (93%−97%), MSD HSCTs also have the highest disease-free survival (DFS) (82%−100%) and lowest rates of graft rejection (8% −18%) and graft-versushost disease (GVHD) (6%−35%).[6] The lack of availability of MSDs for most patients with SCD has led to multiple studies evaluating the use of matched unrelated donor (MUD) and haploidentical donor sources. In general, results have been consistent, with haploidentical HSCT having lower rates of GVHD and higher rates of graft rejection, while MUD HSCTs have demonstrated the reverse - lower rates of rejection and higher rates of GVHD.[6] Use of umbilical cord blood as a stem cell source is typically limited by the fixed number of stem cells within a product, which make them less likely to be utilized in adult patients given that required stem cell dose is dictated by weight of the recipient. The SCURT trial (NCT00745420), published in 2016, aimed to evaluated unrelated donor sources in children with SCD and closed the umbilical cord blood arm of the trial early, due to high rates of rejection.[21]

Overall, age has been shown to have a significant impact in prognosis and outcome following allogeneic HSCT. Increasing age (16 years and older) has also been associated with worsened OS and event-free survival (EFS); increasing age has been correlated with graft failure and death. The development of GVHD has also been significantly higher in patients over the age of 16 years.

While HSCT is the only curative option, it carries significant potential risks. Given that the phenotypic variation in SCD patients is so broad and that there are not good predictive models of life-long disease severity, evaluation for HSCT eligibility is largely based on current disease severity and transplant is often reserved for those with severe disease.[22] Severe disease is not typically present in pediatric patients, thus leading to the frequent transplantation of adult patients that are known to be at higher risk for HSCT complications.

Gene Addition: γ-Retroviral and Lentiviral Vectors

Autologous HSCT with gene modified cells (gene therapy) has become a relevant approach as a curative option for sickle cell disease (SCD). One strategy involves genetically modifying hematopoietic stem cells (HSCs) with viral vectors to incorporate globin genes including γ-globin, γ/β-globin hybrids, and anti-sickling β-globin[1] (Figure 1).[23] Initially, γ-retroviruses were commonly used for clinical application for gene therapy because they

had many advantageous aspects including stable and efficient integration, flexibility of target cell types that can be transduced, and the simplicity of γ-retroviral genomes allowed for easy manipulation to produce replication-incompetent vectors and stable packaging cell lines.[24–25]

β-globin γ-retroviral vectors that were developed and studied first in murine HSCs suffered from minimal transgene expression following hematopoietic reconstitution.[26] These vectors were further optimized by the inclusion of the β-globin locus control region (LCR), an erythroid super-enhancer containing major regulatory elements that induced high levels of β-globin expression in murine erythroleukemia cells and in murine bone marrow cells in vivo.[27] Apart from expression challenges, γ-retroviral vectors had limitations in their capacity to carry the complex β-globin transgene intact; they require *ex vivo* culture of the target stem cells for several days to induce mitosis, due to the fact that integration can only occur during cell division; and carry risks for insertional mutagenesis and oncogene activation.[28] Gamma-retroviral vectors have a preference to integrate near transcriptional start sites of genes and, due to their strong enhancer elements, they have the potential to alter expression of endogenous genes adjacent to sites of vector integration.[29]

As gene therapy progressed, there was a shift in technology from γ-retroviral to lentiviral vectors (LV). Lentiviral vectors, typically derived from HIV-1, showed greater promise for clinical application due to their ability to deliver larger and more complex DNA cassettes, which is crucial for globin vectors to achieve high level expression. Lentiviruses can also transduce and integrate into non-dividing HSCs, promoting stable transgene expression, and have a better safety profile than retroviruses due to their preference of integration across gene units, and the ability to produce lentiviral vectors at high titer without strong enhancer elements.[30] The integration profile of lentiviral vectors tends to be across regions of actively expressed genes with increased chromatin accessibility.[8,9]

However, even with a safer integration profile, safety concerns still exist due to the potential for insertional mutagenesis. To combat this, LVs have been modified to be self-inactivating (SIN), where the viral enhancer and promoter sequences have been removed, limiting *cis*-acting effects of the LTR on cellular genes adjacent to vector integration sites.[31,32] A further modification to the 5'LTR has also been implemented in the vector plasmid, where the U3 region is deleted and replaced with the cytomegalovirus (CMV) promoter.[11] Both of these changes enhance safety and decrease the chance of recombination to produce replication-competent lentivirus. Apart from lentiviral safety modifications, β-globin vectors faced challenges due to low expression of the transgene, which limited therapeutic applicability. Recombinant LVs with the incorporation of the key transcriptional regulatory elements of the β-globin locus control region (DNase hypersensitive sites (HS): HS2, HS3, HS4) into the β-globin cassette addressed this limitation by allowing for stable and long-term expression in transduced bone marrow of β-thalassemic mice in primary and secondary transplant studies, which was the first time therapeutic levels of β-globin expression were demonstrated.[33]

Gamma-globin-Like Transgenes

Induction of fetal globin expression has been a popular strategy to combat SCD. The idea first arose through witnessing SCD and β-thalassemia patients who also had HPFH, where red blood cells have higher levels of fetal hemoglobin. Individuals with HPFH were shown to have less severe forms of SCD or β-thalassemia in comparison to individuals without HPFH.[34] A genome-wide association study (GWAS) was conducted evaluating levels of HbF in patients with β-thalassemia. Variants in the *BCL11A* erythroid enhancer region led to HPFH, and the higher fetal globin expression resulted in less severe forms of β-thalassemia[35]. Further studies showed that generating a *BCL11A* knockout in SCD mouse models corrected the pathogenic defects associated with SCD through increased HbF expression.[36]

One strategy to elevate fetal globin expression is to design LVs to deliver the γ-globin gene. Vectors containing the γ-globin gene along with β-globin regulatory elements were shown to induce therapeutic levels of HbF to ameliorate SCD in Berkeley sickle mouse models (BERK).[37] Other modifications to γ-globin vectors involved replacing the γ-globin 3'untranslated region (UTR) with the β-globin 3'UTR to enhance γ-globin expression[38] Gamma-globin vectors with the β-globin 3'UTR had overall better correction of SCD in Berkeley mouse models through increased mRNA stability, HbF expression, and RBC counts, compared to γ-globin vectors with the γ-globin 3'UTR.[38] γ-globin vectors have been evaluated in clinical trials (Table 2).

Gamma-Like Transgenes (β^{T87Q}-globin)

Utilizing LVs expressing β-globin in RBCs already producing HbS is another potential approach to ameliorating SCD. One drawback of over-expression of β-globin gene is the need for very high levels of expression of HbA to prevent HbS polymerization.

Generating a mutated codon 87 ($\beta^{A\text{-}T87Q}$) in β-globin which changes the amino acid from threonine to glutamine, promotes anti-sickling activity by disrupting lateral contacts in the sickle hemoglobin fibers. This codon change is derived from the amino-acid from γ-globin that is responsible for its ability to inhibit HbS polymerization.[39] $\beta^{A\text{-}T87Q}$-globin was assessed in vivo and kinetic studies showed that BERK mouse bone marrow transduced with $\beta^{A\text{-}T87Q}$-globin lentivirus had delayed HbS polymerization through turbidimetry of RBC lysates, and also corrected hematological parameters in mice.[40] Currently, clinical trials conducted by Bluebird Bio are utilizing $\beta^{A\text{-}T87Q}$-globin (HBG-206) for patients with severe SCD (Table 2). CD34+ cells were transduced with the LentiGlobin BB305 LV and, after autologous transplantation, patients had an average HbA^{T87Q} expression of ~40% of the total hemoglobin after infusion (median 17.3 months). A single infusion of LentiGlobin BB305-transduced autologous hematopoietic stem and progenitor cells (HSPC) led to reduced hemolysis and complete resolution of severe veno-occlusive crises (VOC) and acute chest syndrome in the 25 patients that could be evaluated.[41]

Gamma-Like Transgenes (β^{AS3}-globin)

Additional anti-sickling modifications were studied and incorporated into LVs. Mutating glutamic acid at amino acid position 22 to alanine further enhances anti-sickling properties

by disrupting axial contact in the sickle fiber.[42] E22A in combination with T87Q significantly inhibits HbS polymerization. An additional modification (G16D) increases the affinity for the α-globin polypeptide which gives the anti-sickling β-globin subunit a competitive advantage over the sickle subunits for forming hemoglobin tetramers. An anti-sickling globin containing all three anti-sickling codon modifications (β^{AS3}-globin) was incorporated into LVs (Lenti/β^{AS3}).[43] This vector corrected hematologic and clinical findings in a Sickle Cell mouse mode, and was also shown to efficiently transduce SCD patient BM CD34+ cells and induce therapeutic levels of Hbβ^{AS3}–globin to correct RBC physiology.[44] Clinical trials are currently utilizing β^{AS3} SIN LVs for patients with SCD (Table 2).

One major challenge for clinical applications of gene therapy that utilizes β-LVs is their low titer due to the large vector proviral length.[45] It has been shown that LVs that are smaller can be produced at 10–100-fold higher titers than β-LVs.[46] In addition to larger genomes, β-LVs tend to contain complex expression cassettes that also have a negative impact on vector titer. Low titers make producing clinical scale vector preparations under good manufacturing practices (GMP) more expensive, leading to increased costs per patient dose. β-LVs have also been shown to have reduced efficiencies for transduction of primary human HSCs, compared to simpler LVs, which can lead to sub-optimal gene transfer and transgene expression levels, resulting in sub-optimal therapeutic benefit.[46] A β^{AS3}-LV (GLOBE-AS3) designed with a short human β-globin promoter and a reduced LCR containing HS2 and HS3 elements, resulted in higher titer while driving high transgene expression.[47] β^{AS3}-globin LVs have been even further optimized to reduce the pro-viral length (~4.7kb) by modifying the LCR size, which produced higher titers and gene transfer to HSPCs, and these smaller β^{AS3}-globin LVs are able to ameliorate the sickle phenotype in SCD mouse models.[45]

siRNA to BCL11A

Another approach to increasing fetal globin expression is through knockdown of *BCL11A* through RNA interference. Erythroid lineage restricted knockdown of *BCL11A* is essential for allowing successful engraftment of HSPCs, while ubiquitous knockdown of *BCL11A* causes toxicity in HPSCs leading to poor reconstitution after transplantation.[48] Vectors containing a *BCL11A* microRNA adapted short hairpin RNA ($shRNA^{miR}$) (BCH-BB694) expressed under the control of the β-globin promoter and regulatory elements derived from HS2 and HS3 of the LCR ameliorated the sickle phenotype in mice and induced 40% HbF induction in erythroid differentiated SCD CD34+ cells.[49] A Phase 1 clinical trial (BCH-BB694) showed a sustained increase of HbF levels, with a median of 30.5% of all hemoglobin levels, in six patients; none of the patients have had a severe VOC or stroke post transplantation (Table 2).[50]

Gene Editing

In the past decade, new methods have been developed to perform direct editing in the genome of cells, which are being applied in a multitude of approaches for the treatment of SCD. Genetically engineered site-specific nucleases (SSN) have been developed that

can direct editing, ideally to a single base-pair in the entire genome. These SSN include Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector Nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) associated nuclease (Cas9).[51–55] Each of these SSN can be introduced into target HSPC by electroporation of expression plasmids, *in vitro* transcribed mRNA or as pre-formed ribonucleoprotein complexes (RNP) of recombinant Cas9 protein with the single-guide RNA (sgRNA) that will be present transiently to initiate editing, but do not persist in the cells long-term.

Upon introduction to the HSPC, these SSN produce DNA double-stranded breaks (DSB) at their target site in the genome, which induces cellular DNA repair pathways. One repair pathway that is active throughout the cell cycle is Non-Homologous End Joining (NHEJ) that reanneals the ends of the DNA, but often in an error-prone manner, leading to the insertion or deletion of some bases (Indels) at the repair site. The indels may cause disruption of the target gene, disrupting the translational reading frame, or adding or deleting some amino acids in the encoded protein when multiples of three bases have been added or deleted.

Another DNA repair pathway that may be induced is Homology Directed Repair (HDR), which uses a DNA template to repair the break gap, provided by a sister chromatid during cell division, or provided as an additional nucleotide sequence reagent to serve as a donor of the intended sequence change. HDR is restricted to the S and G2 phases of cell cycle, when DNA replication occurs, and thus gene editing using HDR is also restricted to cells that are actively cycling. Inducing a DSB near a target site for editing greatly increases the efficiency of HDR.[56]

More recently, Base Editing (BE) has emerged as a highly precise method to modify single base-pairs.[57] Base editors use a fusion protein between Cas9, to locate the genomic target using a sgRNA, and an enzyme capable of deaminating a nucleotide (cytosine deaminase or adenosine deaminase). The Cas9 is modified to eliminate one of its nuclease domains, so that a single-strand nick, not a double-stranded break, is made. Instead, the deaminase enzyme is “parked” near the target base to modify it by deamination. During repair of the single stranded nick made on the opposite strand by the modified Cas9, deaminated cytosine is interpreted as uracil and deaminated adenosine as guanosine. Thus, BE can produce transversion type base pair changes (C:G→T:A or A:T→ G:C). Serial improvements of the architecture of the Cas9-deaminase molecules have led to highly active enzymes that act largely at the intended target site.[58] Further advances in BE technology are continuing, with Prime Editing and other variations allowing complex sequence over-writes to be made, and these will likely be applied to gene editing for SCD.

BCL11A – A prime Target for Gene Therapy of SCD:

A highly studied target for gene editing for SCD is the *BCL11a* gene, encoding a transcriptional factor that serves to repress expression of fetal (γ)-globin, as described above.[35,59] Clinical efforts using ZFN and CRISPR/Cas9 are targeting the erythroid enhancer of *BCL11a*, to preserve its expression in non-erythroid blood cell lineages where it is needed for proper stem cell function and multi-lineage differentiation.[60–62] Using

optimized CRISPR editing reagents and HSPC manipulation techniques can lead to highly efficient disruption of the *BCL11A* erythroid enhancer in a large percentage of treated HSPC that retain engraftment capacity and stem cell function.[63]

Results from one clinical trial using CRISPR-mediated *BCL11A* disruption were reported by Frangoul et al (Table 2).[64] Two patients, one with sickle cell disease and one with severe beta-thalassemia, were transplanted with autologous HSPC that were edited *ex vivo* using CRISPR/Cas9 to disrupt the erythroid enhancer of *BCL11A*. Both patients developed high levels of fetal globin in circulating erythrocytes and the SCD patient did not have further VOE. Other trials with this approach are expected to open soon. Base editing has also been used to mutagenize the *BCL11A* erythroid enhancer to block its repression of fetal globin.[73]

A parallel approach to induce fetal globin expression uses CRISPR/Cas9 to introduce indels in the promoters of the fetal globin genes to disrupt the binding sites for BCL11A protein.[65,66] These deletions mimic a subset of naturally-occurring HPFH-associated γ-globin gene promoter deletions. This approach may have the advantage of maintaining all functions of *BCL11a* protein, except its repression of γ-globin.

Direct Correction of the E6V Amino Acid Substitution in HBB

Several groups have developed HDR-mediated approaches to correct the canonical E6V glutamic acid to valine amino acid change in β-globin that causes SCD.[67–70] Using ZFN or CRISPR to introduce a DSB near the sickle-causing mutation and providing a homologous repair template that contains the wild-type base at the mutation site can revert the mutation of HBB^S to HBB^A, if the donor is used for HDR. Different methods have been used to provide the homologous donor, such as single-stranded oligodeoxynucleotides, or via viral vectors that do not persist, such as adeno-associated virus 6 (AAV6), integrase-defective lentiviral vectors, adenovirus, and others. Because of the restriction of HDR to the S/G2 phases of cell cycle, with NHEJ occurring at all times, it is challenging to achieve high levels of HDR-mediated gene correction in long-term primitive HSC. Clinical trials of direct correction of the SCD-causing mutation are expected to begin in 2022.

Base Editing to Convert E6V to Alternative Amino Acid That Does Not Cause Sickling

While current base editing methods are not capable of performing the transition base-pair change from T:A to A:T needed to revert the sickle-causing mutation in HBB^S, Newby et al[71] reported using an adenine base editor to convert the GTG sickle codon (encoding valine) to the novel GCG codon, which encodes alanine, which is present in a known non-pathogenic hemoglobin Makassar (HBB^G) variant.[72] They reported highly efficient base editing in a murine model of SCD and CD34+ cells from patients with SCD, largely preventing sickling manifestations. A clinical trial is expected to be performed in the near future.

In Vivo Gene Therapy for Sickle Cell Disease

The ultimate mode for using gene editing of HSC to treat SCD will be to perform the editing *in vivo*, rather than through *ex vivo* HSC isolation with chemotherapy conditioning, as is currently done for all of the approaches discussed above. If it were possible to administer

gene editing reagents systemically and achieve sufficiently high efficiency editing in HSC *in vivo*, the treatment may be much more widely applied and not reliant upon the high acuity medical setting of the bone marrow transplant unit.[74] The dream of a gene therapy that may be distributed world-wide, especially to the majority of patients with SCD in lower resourced areas, is highly motivating and a great deal of focus is being brought to bear for developing solutions.[75]

Acknowledgements.

K.H. is supported by U2CDK129496 KUH-ART: Advanced Research Training in Kidney Disease, Urology and Hematology and S.W. is supported by NIH T32 HL086345 Training Grant in Developmental Hematology.

References

1. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, Temperley WH, Williams TN, Weatherall DJ, Hay SI. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet. 2013 381(9861):142–51. [PubMed: 23103089]
2. Centers for Disease Control and Prevention - Sickle Cell Disease. https://www.cdc.gov/ncbddd/sicklecell/data.html
3. Sundd P, Gladwin MT, Novelli EM. Pathophysiology of Sickle Cell Disease. Annu Rev Pathol. 2019 Jan 24;14:263–292. [PubMed: 30332562]
4. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF, Vichinsky EP. Sickle cell disease. Nat Rev Dis Primers. 2018;4:18010. [PubMed: 29542687]
5. Lanzkron S, Carroll CP, Haywood C Jr. The burden of emergency department use for sickle-cell disease: an analysis of the national emergency department sample database. Am J Hematol. 2010 Oct;85(10):797–9. [PubMed: 20730795]
6. Quinn CT, Rogers ZR, McCavit TL, Buchanan GR. Improved survival of children and adolescents with sickle cell disease. Blood. 2010 Apr 29;115(17):3447–52. [PubMed: 20194891]
7. Piel FB, Steinberg MH, & Rees DC (2017). Sickle Cell Disease. New Engl J Med, 376, 1561–1573. [PubMed: 28423290]
8. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med. 1994 330(23):1639–44. [PubMed: 7993409]
9. Akinsheye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastiani P, Chui DH, Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood. 2011 Jul 7;118(1):19–27. [PubMed: 21490337]
10. Nevitt SJ, Jones AP, Howard J. Hydroxyurea (hydroxycarbamide) for sickle cell disease. Cochrane Database Syst Rev. 2017 Apr 20;4(4):CD002202. [PubMed: 28426137]
11. Howard J Sickle cell disease: when and how to transfuse. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):625–631. doi: 10.1182/asheducation-2016.1.625. [PubMed: 27913538]
12. Meier ER. Treatment options for sickle cell disease. Pediatric Clinics of North America, 2018 65(3), 427–443. 10.1016/j.pcl.2018.01.005 [PubMed: 29803275]
13. Niihara Y, Miller ST, Kanter J, et al. Investigators of the Phase 3 Trial of l-Glutamine in Sickle Cell Disease. A Phase 3 Trial of l-Glutamine in Sickle Cell Disease. N Engl J Med. 2018 379(3):226–235. [PubMed: 30021096]
14. Kapoor S, Little JA, Pecker LH. Advances in the Treatment of Sickle Cell Disease. Mayo Clin Proc. 2018 93(12):1810–1824. [PubMed: 30414734]
15. Vichinsky E, Hoppe CC, Ataga KI, et al. A Phase 3 Randomized Trial of Voxelotor in Sickle Cell Disease. N Engl J Med. 2019 381(6):509–519. [PubMed: 31199090]

16. Quinn CT, Ware RE. Increased oxygen affinity: to have and to hold. Blood. 2021 138(13):1094–1095. [PubMed: 34591098]
17. Kutlar A, Kanter J, Liles DK, et al. Effect of crizanlizumab on pain crises in subgroups of patients with sickle cell disease: A SUSTAIN study analysis. Am J Hematol. 2019 94(1):55–61. [PubMed: 30295335]
18. Hsieh MM, Kang EM, Fitzhugh CD, Link MB, Bolan CD, Kurlander R, Childs RW, Rodgers GP, Powell JD, Tisdale JF. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med. 2009 361(24):2309–17. [PubMed: 20007560]
19. Shenoy S Hematopoietic stem-cell transplantation for sickle cell disease: current evidence and opinions. Ther Adv Hematol. 2013 Oct;4(5):335–44. [PubMed: 24082994]
20. Eapen M, Brazauskas R, Walters MC, et al. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: a retrospective multicentre, cohort study. Lancet Haematol. 2019 6(11):e585–e596. [PubMed: 31495699]
21. Kamani NR, Walters MC, Carter S, et al. Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant. 2012 18(8):1265–72. [PubMed: 22343376]
22. Kanter J, Liem RI, Bernaudin F, et al. American Society of Hematology 2021 guidelines for sickle cell disease: stem cell transplantation. Blood Adv. 2021 5(18):3668–3689. [PubMed: 34581773]
23. Hoban MD, Orkin SH & Bauer DE Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood 127, 839–848 (2016). [PubMed: 26758916]
24. Bank A, Markowitz D & Lerner N Gene transfer. A potential approach to gene therapy for sickle cell disease. Ann N Y Acad Sci 565, 37–43 (1989). [PubMed: 2672970]
25. Maetzig T, Galla M, Baum C & Schambach A Gammaretroviral Vectors: Biology, Technology and Application. Viruses 3, 677–713 (2011). [PubMed: 21994751]
26. Karlsson S, Bodine DM, Perry L, Papayannopoulou T & Nienhuis AW Expression of the human beta-globin gene following retroviral-mediated transfer into multipotential hematopoietic progenitors of mice. Proc Natl Acad Sci U S A 85, 6062–6066 (1988). [PubMed: 3413076]
27. Plavec I, Papayannopoulou T, Maury C & Meyer F A human beta-globin gene fused to the human beta-globin locus control region is expressed at high levels in erythroid cells of mice engrafted with retrovirus-transduced hematopoietic stem cells. Blood 81, 1384–1392 (1993). [PubMed: 8443396]
28. Pawliuk R, Bachelot T, Raftopoulos H, Kalberer C, Humphries RK, Bank A, Leboulch P. Retroviral vectors aimed at the gene therapy of human beta-globin gene disorders. Ann N Y Acad Sci. 1998 Jun 30;850:151–62. [PubMed: 9668537]
29. Cattoglio C, Facchini G, Sartori D, et al. Hot spots of retroviral integration in human CD34+ hematopoietic cells. Blood. 2007 Sep 15;110(6):1770–8. [PubMed: 17507662]
30. Naldini L, Trono D, Verma IM. Lentiviral vectors, two decades later. Science. 2016 9;353(6304):1101–2. [PubMed: 27609877]
31. Miyoshi H, Blömer U, Takahashi M, Gage FH & Verma IM Development of a self-inactivating lentivirus vector. J Virol 72, 8150–8157 (1998). [PubMed: 9733856]
32. Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L, Trono D. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. J Virol. 1998 72(12):9873–80. [PubMed: 9811723]
33. May C et al. Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 406, 82–86 (2000). [PubMed: 10894546]
34. Murray N, Serjeant BE & Serjeant GR Sickle cell-hereditary persistence of fetal haemoglobin and its differentiation from other sickle cell syndromes. Br J Haematol 69, 89–92 (1988). [PubMed: 2454649]
35. Uda M et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci U S A 105, 1620–1625 (2008). [PubMed: 18245381]
36. Xu J et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. Science 334, 993–996 (2011). [PubMed: 21998251]

37. Perumbeti A et al. A novel human gamma-globin gene vector for genetic correction of sickle cell anemia in a humanized sickle mouse model: critical determinants for successful correction. Blood 114, 1174–1185 (2009). [PubMed: 19474450]
38. Pestina TI et al. Correction of Murine Sickle Cell Disease Using γ-Globin Lentiviral Vectors to Mediate High-level Expression of Fetal Hemoglobin. Molecular Therapy 17, 245–252 (2009). [PubMed: 19050697]
39. Nagel RL et al. Structural bases of the inhibitory effects of hemoglobin F and hemoglobin A2 on the polymerization of hemoglobin S. Proc Natl Acad Sci U S A 76, 670–672 (1979). [PubMed: 284392]
40. Pawliuk R et al. Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy. Science 294, 2368–2371 (2001). [PubMed: 11743206]
41. Kanter J et al. Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease. N Engl J Med 386, 617–628 (2022). [PubMed: 34898139]
42. McCune SL, Reilly MP, Chomo MJ, Asakura T & Townes TM Recombinant human hemoglobins designed for gene therapy of sickle cell disease. Proc Natl Acad Sci U S A 91, 9852–9856 (1994). [PubMed: 7937904]
43. Levasseur DN, Ryan TM, Pawlik KM & Townes TM Correction of a mouse model of sickle cell disease: lentiviral/antisickling beta-globin gene transduction of unmobilized, purified hematopoietic stem cells. Blood 102, 4312–4319 (2003). [PubMed: 12933581]
44. Romero Z et al. β-globin gene transfer to human bone marrow for sickle cell disease. J Clin Invest 67930 (2013) doi:10.1172/JCI67930.
45. Morgan RA et al. Improved Titer and Gene Transfer by Lentiviral Vectors Using Novel, Small β-Globin Locus Control Region Elements. Molecular Therapy 28, 328–340 (2020). [PubMed: 31628051]
46. Han J et al. β-Globin Lentiviral Vectors Have Reduced Titers due to Incomplete Vector RNA Genomes and Lowered Virion Production. Stem Cell Reports 16, 198–211 (2021). [PubMed: 33186538]
47. Urbinati F, Campo Fernandez B, Masiuk KE, et al. Gene Therapy for Sickle Cell Disease: A Lentiviral Vector Comparison Study. Hum Gene Ther. 2018 29(10):1153–1166. [PubMed: 30198339]
48. Guda S et al. miRNA-embedded shRNAs for Lineage-specific BCL11A Knockdown and Hemoglobin F Induction. Mol Ther 23, 1465–1474 (2015). [PubMed: 26080908]
49. Brendel C et al. Preclinical Evaluation of a Novel Lentiviral Vector Driving Lineage-Specific BCL11A Knockdown for Sickle Cell Gene Therapy. Mol Ther Methods Clin Dev 17, 589–600 (2020). [PubMed: 32300607]
50. Esrick EB et al. Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease. New England Journal of Medicine 384, 205–215 (2021). [PubMed: 33283990]
51. Bibikova M, Carroll D, Segal DJ, Trautman JK, Smith J, Kim YG, Chandrasegaran S. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol. 2001 Jan;21(1):289–97. [PubMed: 11113203]
52. Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics. 2010 Oct;186(2):757–61. [PubMed: 20660643]
53. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. Elife. 2013 Jan 29;2:e00471. [PubMed: 23386978]
54. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013 Feb 15;339(6121):819–23. [PubMed: 23287718]
55. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014 Nov 28;346(6213):1258096. [PubMed: 25430774]
56. Porteus MH, Baltimore D. Chimeric nucleases stimulate gene targeting in human cells. Science. 2003 May 2;300(5620):763. [PubMed: 12730593]

57. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016 533(7603):420–4. [PubMed: 27096365]
58. Richter MF, Zhao KT, Eton E, et al. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat Biotechnol. 2020 Jul;38(7):883–891. [PubMed: 32433547]
59. Bauer DE, Kamran SC, Lessard S, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science. 2013 Oct 11;342(6155):253–7. [PubMed: 24115442]
60. Bjurström CF, Mojadidi M, Phillips J, et al. Reactivating Fetal Hemoglobin Expression in Human Adult Erythroblasts Through BCL11A Knockdown Using Targeted Endonucleases. Mol Ther Nucleic Acids. 2016;5(8):e351. [PubMed: 28131278]
61. Psatha N, Reik A, Phelps S, Zhou Y, et al. Disruption of the BCL11A Erythroid Enhancer Reactivates Fetal Hemoglobin in Erythroid Cells of Patients with β-Thalassemia Major. Mol Ther Methods Clin Dev. 2018 Aug 14;10:313–326. [PubMed: 30182035]
62. Demirci S, Leonard A, Essawi K, Tisdale JF. CRISPR-Cas9 to induce fetal hemoglobin for the treatment of sickle cell disease. Mol Ther Methods Clin Dev. 2021 Oct 1;23:276–285. [PubMed: 34729375]
63. Wu Y, Zeng J, Roscoe BP, et al. Highly efficient therapeutic gene editing of human hematopoietic stem cells. Nat Med. 2019 May;25(5):776–783. [PubMed: 30911135]
64. Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N Engl J Med. 2021 Jan 21;384(3):252–260. [PubMed: 33283989]
65. 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 Sep;22(9):987–90. [PubMed: 27525524]
66. Métais JY, Doerfler PA, Mayuranathan T, et al. Genome editing of HBG1 and HBG2 to induce fetal hemoglobin. Blood Adv. 2019 Nov 12;3(21):3379–3392. [PubMed: 31698466]
67. Hoban MD, Lumaquin D, Kuo CY, et al. CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells. Mol Ther. 2016 Sep;24(9):1561–9. [PubMed: 27406980]
68. 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 Oct 12;8(360):360ra134.
69. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature. 2016 Nov 17;539(7629):384–389. [PubMed: 27820943]
70. Romero Z, Lomova A, Said S, et al. Editing the Sickle Cell Disease Mutation in Human Hematopoietic Stem Cells: Comparison of Endonucleases and Homologous Donor Templates. Mol Ther. 2019 Aug 7;27(8):1389–1406. [PubMed: 31178391]
71. Newby GA, Yen JS, Woodard KJ, et al. Base editing of haematopoietic stem cells rescues sickle cell disease in mice. Nature. 2021 Jul;595(7866):295–302. [PubMed: 34079130]
72. Blackwell RQ, Oemijati S, Pribadi W, Weng MI, Liu CS. Hemoglobin G Makassar: beta-6 Glu leads to Ala. Biochim Biophys Acta. 1970 Sep 29;214(3):396–401. [PubMed: 5509617]
73. Zeng J, Wu Y, Ren C, Bonanno J, Shen AH, Shea D, Gehrke JM, Clement K, Luk K, Yao Q, Kim R, Wolfe SA, Manis JP, Pinello L, Joung JK, Bauer DE. Therapeutic base editing of human hematopoietic stem cells. Nat Med. 2020 Apr;26(4):535–541. doi: 10.1038/s41591-020-0790-y. Epub 2020 Mar 16. [PubMed: 32284612]
74. Li C, Georgakopoulou A, Mishra A, Gil S, Hawkins RD, Yannaki E, Lieber A. In vivo HSPC gene therapy with base editors allows for efficient reactivation of fetal γ-globin in β-YAC mice. Blood Adv. 2021 Feb 23;5(4):1122–1135. doi: 10.1182/bloodadvances.2020003702. [PubMed: 33620431]
75. Cannon P, Asokan A, Czechowicz A, et al. Safe and Effective In Vivo Targeting and Gene Editing in Hematopoietic Stem Cells: Strategies for Accelerating Development. Hum Gene Ther. 2021 Jan;32(1–2):31–42. [PubMed: 33427035]

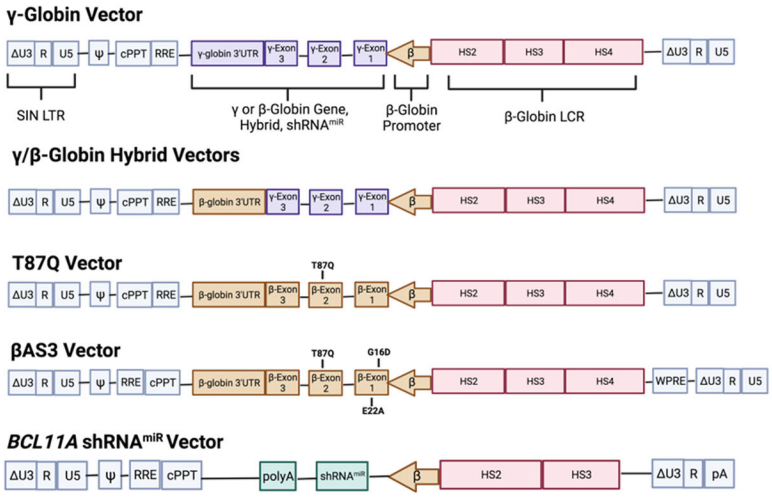

Figure 1: Lentiviral Vector Approaches for Gene Therapy of Sickle Cell Disease.

γ-Globin lentiviral vector containing γ-globin gene coding and non-coding regions, β-globin promoter and modified Locus Control Region (LCR) hypersensitive site (HS) elements HS2, HS3, HS4. γ/β-Globin hybrid lentiviral vector containing γ-globin gene coding regions, β-globin 3' untranslated region (UTR), β-globin promoter, and modified LCR elements HS2, HS3, HS4. T87Q lentiviral vector containing a modified β-globin gene containing a T87Q amino acid change to promote anti-sickling properties, β-globin promoter, and modified LCR elements HS2, HS3, and HS4. βAS3 lentiviral vector containing three anti-sickling amino acid changes (G16D, E22A, T87Q) in the β-globin gene, β-globin promoter, and modified LCR elements HS2, HS3, and HS4. *BCL11A* shRNA^{miR} lentiviral vector containing *BCL11A* shRNA^{miR}, synthetic polyadenylation signal (polyA), β-globin promoter, and modified LCR elements HS2, and HS3. All vectors are SIN lentiviral vectors (ΔU3). Ψ, packaging signal; LTR, long terminal repeat; cPPT, central polypurine tract; RRE, rev-response element; HS2, HS3, HS4, DNase hypersensitive sites 2, 3, and 4 from the β-globin locus control region (LCR); WPRE, woodchuck hepatitis virus posttranscriptional regulator element. *Not drawn to scale.

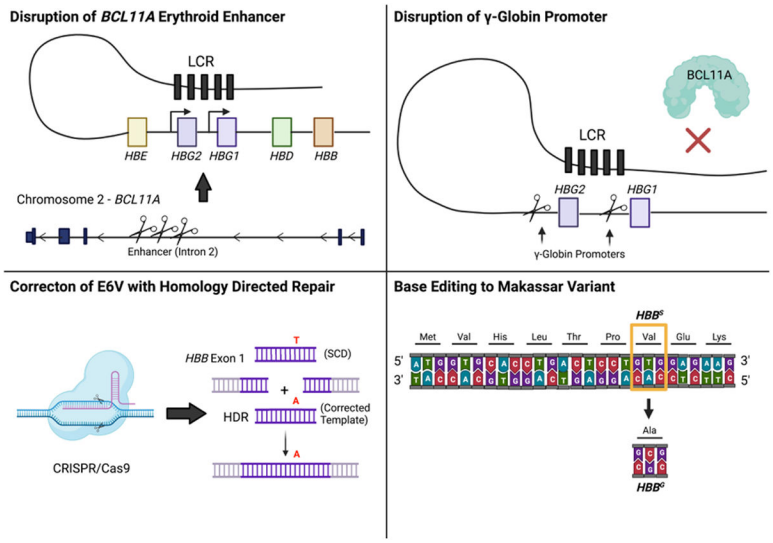

Figure 2: Gene Editing Approaches for Gene Therapy of Sickle Cell Disease.

Disruption of *BCL11A* erythroid enhancer. Applying CRISPR to disrupt the erythroid enhancer region in intron 2 of *BCL11A* on chromosome 2 leads to BCL11A downregulation. Without BCL11A repression of γ-globin, the LCR will interact with *HBG2* and *HBG1* promoting γ-globin expression. Disruption of γ-globin promoter. Altering the *BCL11A* binding sequence in the γ-globin promoters of *HBG2* and *HBG1* will inhibit BCL11A binding and γ-globin repression. The LCR will interact with *HBG2* and *HBG1* promoting γ-globin expression. Correction of E6V with Homology Directed Repair. Using CRISPR/Cas9 and sgRNA to target the point mutation (T) and suppling a corrected template to incorporate the wildtype nucleotide (A) with homology directed repair. Base Editing to Makassar Variant. Adenine base editing to convert the GTG (Val) to the codon GCG (Ala), to produce a non-pathogenic variant (HBB^G).

Table 1:

Current Approved Pharmacologic Therapies for Sickle Cell Disease

Medication (Year of FDA approval)	Approved Age Group	Mechanism of Action	Physiologic Effects	Clinical Effects
Hydroxyurea (1998 in adults and 2017 in children)	> 9 months	Ribonucleoside diphosphate reductase inhibitor	• Increases HbF production • Decreases intracellular HbS polymerization	• Decreases pain crises • Decreases Acute chest episodes • Decreases blood transfusions • Decreases overall mortality
L-Glutamine (2017)	> 5 years	Increases NADPH	• Increases RBC reducing potential	• Decreases pain crises • Decreases hospitalizations • Decreased acute chest episodes
Crizanlizumab (2019)	> 16 years	Monoclonal antibody against P-selectin	• Decreases erythrocyte and leukocyte adhesion	• Decreases rate of pain crises
Voxelotor (2019)	> 12 years	Increases Hb affinity for oxygen	• Delay production of deoxyhemoglobin • Decrease in HbS polymerization	• Increases baseline Hgb > 1g/dL • Decreases hemolysis markers; indirect bilirubin levels and % reticulocyte count

Table 2:

Ongoing or Completed Gene Therapy Trials for Sickle Cell Disease

Sponsor	Approach	ClincialTrials.gov Identifier	Status (Phase)
Lentiviral Vectors			
Bluebird Bio	LV encoding the human beta-A-T87Q globin gene	NCT02151526	Completed (1)
Bluebird Bio	LV encoding the human beta-A-T87Q globin gene	NCT02140554	Active, Not recruiting (2)
Bluebird Bio	LV encoding the human beta-A-T87Q globin gene	NCT04293185	Recruiting (3)
Aruvant Sciences GmbH	LV to express gamma-globin	NCT02186418	Recruiting (1/2)
Donald B Kohn (UCLA)	LV to express an anti-sickling (β^{AS3}-globin) gene	NCT02247843	Recruiting (1/2)
David Williams (BCH)	LV containing a short-hairpin RNA targeting *BCL11a*	NCT03282656	Active/Not Recruiting (1)
Assistance Publique – Hopitaux de Paris	LV expressing the β^{AS3}-globin gene	NCT03964792	Recruiting (1/2)
Gene Editing			
Vertex Pharmaceuticals Incorporated	Autologous HSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the BCL11A gene	NCT03745287	Active/Not Recruiting (2/3)
Novartis Pharmaceuticals	Two genome-edited HSPC products to reduce the biologic activity of BCL11A and increase HbF	NCT04443907	Recruiting (1/2)
Mark Walters (UCSF)	Autologous HSPCs with sickle allele modified by the CRISPR-Cas9 ribonucleoprotein	NCT04774536	Not Yet Recruiting (1/2)
Graphite Bio, Inc.	CRISPR-Cas9 edited and sickle mutation-corrected HSPCs to convert HbS to HbA	NCT04819841	Recruiting (1/2)
Editas Medicine, Inc.	Autologous Clustered Regularly Interspaced Short Palindromic Repeats Gene-edited HSPC	NCT04853576	Recruiting (1/2)

LV – Lentiviral vector

HSPC – Hematopoietic stem and progenitor cells

HbF – Fetal hemoglobin

HbS – Sickle hemoglobin

HbA – Hemoglobin A