Molecular Therapy
Review

# Autologous gene therapy for hemoglobinopathies: From bench to patient's bedside

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**In recent years, a growing number of clinical trials have been initiated to evaluate gene therapy approaches for the treatment of patients with transfusion-dependent β-thalassemia and sickle cell disease (SCD). Therapeutic modalities being assessed in these trials utilize different molecular techniques, including lentiviral vectors to add functional copies of the gene encoding the hemoglobin β subunit in defective cells and CRISPR-Cas9, transcription activator-like effector protein nuclease, and zinc finger nuclease gene editing strategies to either directly address the underlying genetic cause of disease or induce fetal hemoglobin production by gene disruption. Here, we review the mechanisms of action of these various gene addition and gene editing approaches and describe the status of clinical trials designed to evaluate the potentially for these approaches to provide one-time functional cures to patients with transfusion-dependent β-thalassemia and SCD.**

## INTRODUCTION

Hemoglobinopathies are the most common monogenic diseases, with an estimated 7% of the worldwide population being carriers who have at least one DNA variant that results in defective hemoglobin synthesis.[1] Annually, approximately 60,000 people are diagnosed with transfusion dependent β-thalassemia (TDT) and 500,000 infants are born with sickle cell disease (SCD) around the world.[2–4]

Both TDT and SCD are the result of mutations in the hemoglobin β subunit gene (*HBB*), which is located on chromosome 11.[5,6] β-Thalassemia can result from any of approximately 350 mutations (i.e., substitutions, minor deletions or insertions, or rare gross deletions) in either *HBB* or its flanking, non-coding regions. The heterogeneity of potential *HBB* mutations, which can lead to either the complete absence of β-globin (denoted $\beta^0$) or a reduction in β-globin synthesis (denoted $\beta^+$), explains the range of clinical severity seen in patients with β-thalassemia and associated morbidities.[7] Abnormal production of β-globin causes an excess of unstable α-globin to accumulate in erythroid cells, leading to both ineffective erythropoiesis and hemolysis, which manifests clinically in severe anemia (Figure 1).[7,8] Those patients with TDT, who are generally either homozygous or compound heterozygous for $\beta^0$ or severe $\beta^+$, require life-long red blood cell transfusions for survival. In contrast with β-thalassemia, SCD is caused by a single amino acid change at position 6 in the β-chain wherein a glutamic acid is replaced with valine leading to the production of the aberrant sickle hemoglobin (also known as $\beta^s\beta^s$ disease). Polymerization of deoxygenated sickle hemoglobin in erythrocytes causes the deformation of the red blood cell into the characteristic sickle shape, leading to downstream complications of impaired blood flow in the microvasculature and hemolysis, with patients subsequently experiencing painful vaso-occlusive events, severe anemia, end-organ damage, and reduced life-expectancy (Figure 1).[3] For patients with SCD, current treatment options primarily involve symptomatic pain management, red blood cell transfusion, and the use of hydroxyurea, which has been shown to reduce pain episodes and other SCD-related complications for some patients by raising the levels of fetal hemoglobin among other possible actions.[9,10] Other treatment options for patients with TDT and SCD that have recently emerged include luspatercept, which has been shown to reduce ineffective erythropoiesis, resulting in lower transfusion need in patients with TDT,[11] and voxelotor, which was shown to increase hemoglobin levels and reduce markers of hemolysis in patients with SCD.[12] However, it is important to note that none of these therapies address the underlying biochemical cause of either disease nor do they fully ameliorate the clinical disease symptoms. Additionally, these therapies must be administered life-long to obtain a persistent therapeutic effect.

To date, allogeneic hematopoietic stem cell transplantation (HSCT) remains the only potentially curative option for patients with TDT or SCD. However, it has been estimated that less than 20% of eligible patients have a related human leukocyte antigen (HLA)-identical donor,[13–15] seriously limiting the widespread availability of this standard-of-care treatment option. While matched unrelated donor transplantation, umbilical cord blood transplantation, and haploidentical transplantation may allow for expanded access HSCT for

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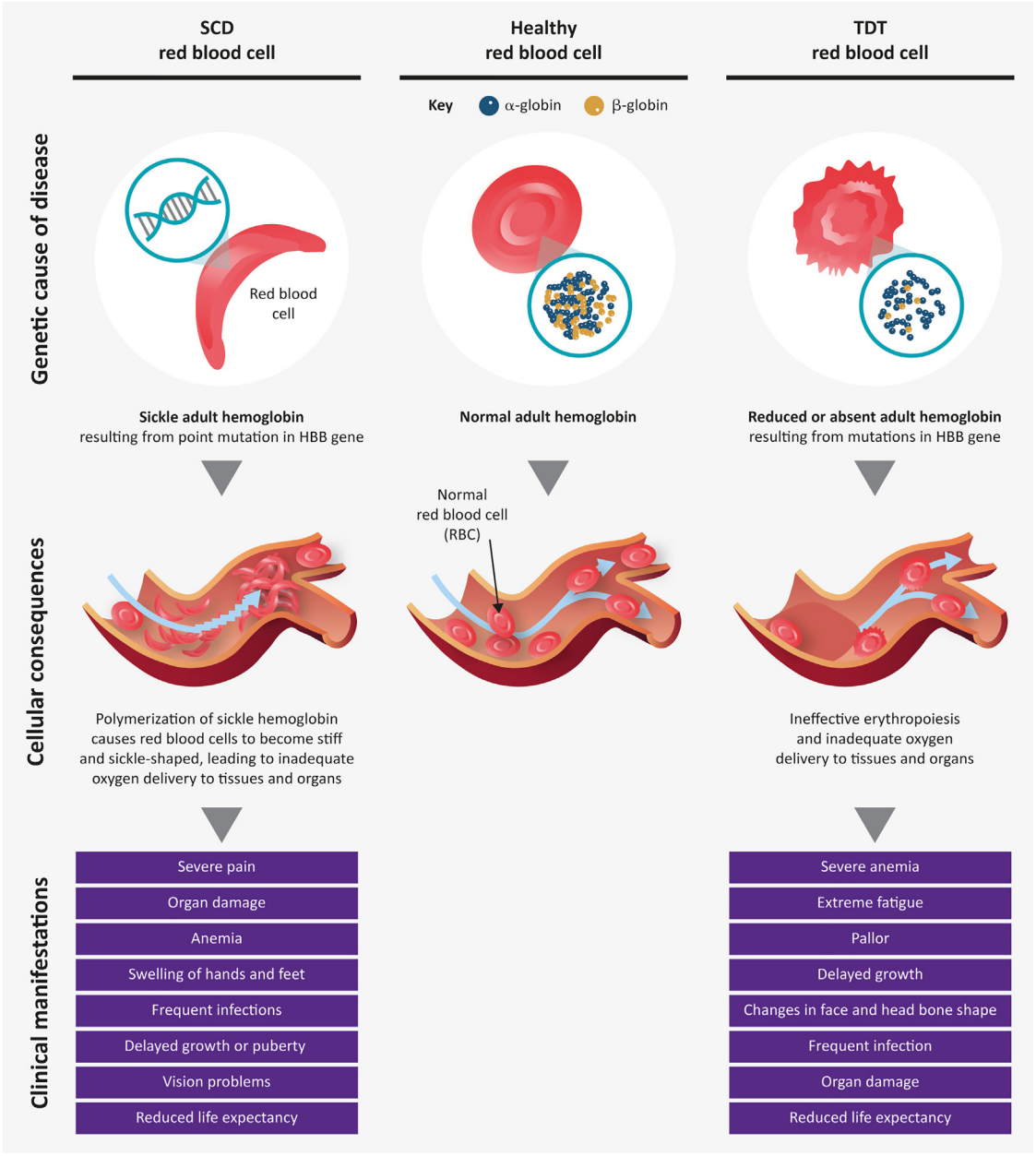


**Figure 1. Molecular biochemical, and clinical mechanisms of TDT and SCD disease and clinical manifestations. HBB, β-globin**

more patients, high complication rates limit a broader use of these approaches.[16] Finally, allogeneic HSCT, even with matched related HLA-identical donors, carries the risk of inducing severe complications, including graft-versus-host disease (GvHD), graft rejection, graft failure, and transplant-related mortality.[10]

Recently, a growing number of clinical trials evaluating novel therapeutics that use gene addition or gene editing approaches to address the underlying causes of TDT and SCD have been initiated. Here, we review the mechanisms of action of these gene addition and editing technologies and describe the status of therapies in clinical trials that use these approaches to assess novel treatments with the potential to provide one-time functional cures for TDT and SCD.

## GENE THERAPY APPROACHES FOR HEMOGLOBINOPATHIES

The expansion in the number of TDT and SCD clinical trials using gene therapy approaches is the direct result of the development of

improved viral vectors for gene addition therapy as well as the refinement of novel molecular techniques, such as CRISPR-Cas9 gene editing, that enable robust and precise editing of DNA. Crucially, the use of gene therapy-based treatments can offer advantages over traditional HLA-identical donor allogeneic HSCT as most current approaches in clinical trials are autologous approaches using a patient's own stem cells for *ex vivo* gene addition or editing. An autologous HSCT approach for gene therapy eliminates the need to identify a suitable allogeneic donor and is not associated with the risks of alloreactive bidirectional complications, including GvHD and graft rejection, seen in allogeneic transplantation.[10] The use of autologous approaches could, therefore, expand the availability of a functional cure for TDT and SCD to a greater number of patients.

## Gene addition techniques

Trials using gene therapy to attempt to treat hemoglobinopathies have a more than 42-year history. In 1980, a study was conducted to transfect a recombinant form of β-globin into bone marrow cells, derived from two patients with β-thalassemia, which were then infused back into the patients.[17] Although this study proved unsuccessful, in ensuing years researchers refined these methods and developed improved techniques using viral vectors to transduce cells with exogenous genetic material for cell and gene therapy applications.[18] During this same time, viral vectors themselves have gone through a series of iterations to improve efficiency and safety and address the particular challenge associated with hemoglobinopathies where there is a requirement to provide controlled expression of large structural proteins in defined cell types such as blood cells.

Four viral vector systems have emerged for gene therapy applications: (1) adenoviral vectors, (2) adeno-associated viral vectors, (3) retroviral vectors, which include murine leukemia virus (MLV)-based vectors, and (4) lentiviral vectors. These vector systems can be classified based on their mechanisms of action with transduction using adenoviral and adeno-associated viral vectors primarily resulting in the persistence of extrachromosomal episomes in the nucleus of the cell, although it has been reported that recombinant adeno-associated viral vector DNA can be integrated into genomic DNA at low frequencies,[19] while retroviral and lentiviral vectors integrate directly into host cell chromatin.[20] There are advantages and disadvantages associated with each viral vector system that should be considered for gene therapy applications. While adenoviral and adenoviral-associated vectors have a broad host range and can infect a wide variety of cell types, they have also been shown to elicit host inflammatory and immune responses.[21] Lentiviral vectors have the ability to transduce both dividing and non-dividing cells, including $CD34^+$ hematopoietic stem and progenitor cells (HSPCs), making this viral vector of keen interest for gene therapy applications designed to treat genetic diseases, including hemoglobinopathies.[22]

Lentiviral vector particles enter host cells through interactions with glycoproteins on the outer envelope and fuse with the host cell membrane, after which viral genetic cargo is released into the cell cytoplasm. In the cytoplasm, the RNA genome of the lentivirus is converted into DNA using reverse transcriptase, after which the DNA is integrated into the host genome. This integration event has been shown to be non-random, with a preference for transcriptionally active sites in the genome,[23] which raises the concern for insertional mutagenesis events with lentiviral vectors. Third-generation, self-inactivating versions of lentiviral vectors have been developed in an attempt to mitigate the potential risk for insertional mutagenesis events and a recent meta-analysis of gene therapy trials using HSPCs for monogenic disorders (406 treated patients) showed a safer profile for patients who received lentiviral vector transduced HSPCs.[24] However, lentiviral vectors that use strong promoter or enhancer elements can still activate neighboring genes.[18] In terms of efficacy, a recent study found that the amount of hemoglobin produced by cells transduced with a lentiviral vectors carrying a β-globin gene depends on the median value of vector copy number and on the percentage of transduced $CD34^+$ cells.[25]

## Gene editing techniques

### *Gene editing using zinc finger nucleases and transcription activator-like effector protein nucleases*

Structurally, both zinc finger nucleases (ZFNs) and the transcription activator-like effector protein nuclease (TALEN) gene editing approaches have similarities as both systems take advantage of a non-sequence-specific *Fok*I endonuclease that is fused to a site-specific DNA binding domain.

In the case of ZFNs, the DNA binding domain is composed of synthetic peptide units that specify zinc finger repeat binding domains, which can be altered in the amino acid sequence and number to enhance the DNA target site specificity of the ZFN.[26] These engineered zinc fingers are small in size (approximately 30 amino acids) and interact with DNA through contact with specific protein side chains and functional groups in the major groove of the double helix.[27] By increasing the number of zinc fingers, it is possible to increase the specificity and precision of editing by a ZFN. Mechanistically, two ZFNs that bind the DNA sequence in opposite orientations are required for DNA cleavage due to the need to dimerize the non-specific cleavage domains of the *Fok*I endonuclease[28] (Figure 2).

The transcription activator-like effector (TALE) that is used in the TALEN gene editing system was discovered in bacteria. TALEs are secreted by plant pathogenic bacteria into hosts, where they can bind to specific host DNA sequences to aid in the infection of plant species.[29] In the case of the TALEN gene editing system, DNA targeting specificity comes from the TALE protein, while DNA cleavage is completed by fusing the TALE protein to *Fok*I.[30–32] Unlike the CRISPR-Cas9 system where a single guide RNA (gRNA) can be used to target the Cas9 protein to a specific genomic location, TALEs have an array of 33–35 amino acids that comprise the DNA-binding domain, which can be engineered to achieve a desired target site specificity.[29] Modifications to the TALE protein, including fusing to the catalytic domain of the *Fok*I endonuclease, led to the TALEN architecture that is used most often for gene editing.[33]

In the TALEN system, editing is accomplished through the use of pairs of TALENs that can bind DNA in opposite orientations, such

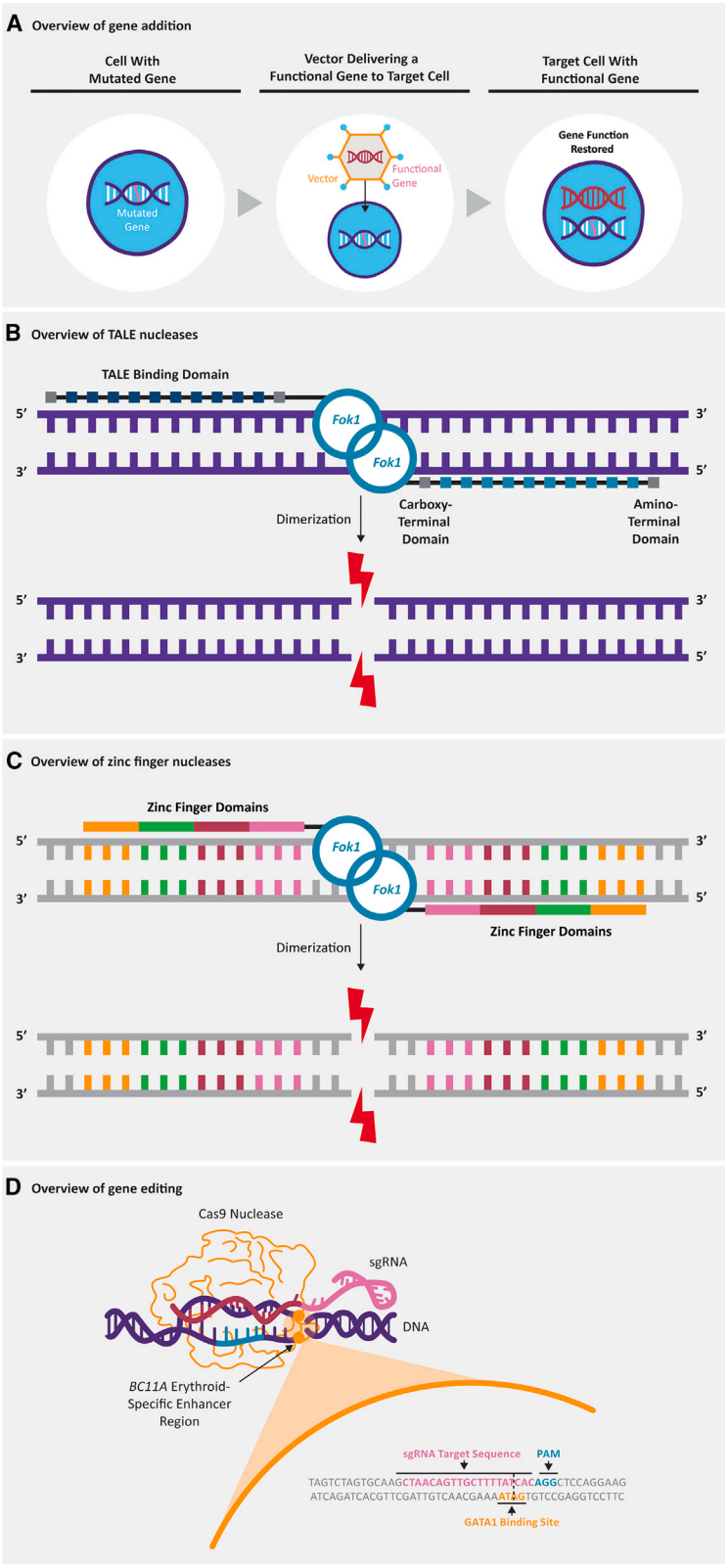


**Figure 2. Overview of key genetic therapy and gene editing approaches designed to treat TDT and SCD**

(A) Gene addition involves adding a functional gene into the genome to impact protein expression. (B) TALEN system relies on the dimerization of the *FokI* nuclease to generate a double-stranded DNA break that can be repaired by cellular repair pathways. (C) Similar to TALENs, ZFNs operate as dimers; dimerization enables the *Fok1* nuclease to generate a double-strand break in DNA that is then repaired by naturally occurring DNA repair systems. (D) CRISPR-Cas9 enables modification of DNA at a precise location; the Cas9 and sgRNA complex assembles to generate a DNA double-strand break at the target site and naturally occurring DNA repair systems are activated to resolve the break. sgRNA, single guide RNA.

that the *Fok*I domains of the two TALE proteins dimerize enabling cleavage of the target DNA within the defined spacer region, similar to ZFNs (Figure 2).[34] This cleavage event leads to the introduction of a double-stranded DNA break that is repaired by the cell using either the non-homologous end-joining (NHEJ) or homology-directed repair (HDR) pathways.[34]

### Gene editing with CRISPR-Cas9

CRISPR-Cas9 is a two-component nuclease system that allows for targeted repairs, insertions or deletions in specific genomic regions that was initially identified as an innate bacterial immune system capable of cleaving bacteriophage or plasmid DNA.[35,36] In CRISPR-mediated DNA cleavage, mature CRISPR RNAs (crRNAs) base pair with a *trans*-activating crRNA (tracrRNA) to form a two-RNA structure capable of guiding the Cas9 protein to a target DNA region. The part of the crRNA sequence that is complementary to the target DNA sequence is known as a spacer. For Cas9 to function, a specific protospacer adjacent motif (PAM) is also required, which varies depending on the bacterial species of the Cas9 gene. The most commonly used Cas9 nuclease, derived from *S. pyogenes*, recognizes a PAM sequence of NGG that is found directly downstream of the target sequence in the genomic DNA, on the non-target strand. Recognition of the PAM by Cas9 nuclease is thought to destabilize the adjacent sequence, allowing interrogation by the crRNA, and RNA-DNA pairing when a matching sequence is present.[37,38] Upon binding the target region, the two domains of Cas9 (HNH nuclease domain and RuvC-like domain) cleave the complementary and non-complementary strands to induce a double-stranded break (Figure 2).[39] While researchers found that the tracrRNA:crRNA duplex was required for proper site-specific cleavage by Cas9, it was also noted that the structure of this RNA duplex suggested the possibility that a single chimeric RNA might be able to capture all features required for DNA cleavage by Cas9 in a single chimeric gRNA.[39] The use of a single gRNA to target a specific DNA sequence is now standard in the CRISPR-Cas9 system to maximize editing precision. The ability of the CRISPR-Cas9 system to cleave DNA in human cells in a programmable fashion[39–41] led to considerations of the potential for CRISPR-Cas9 gene editing to be used in clinical therapies.

The specific double-stranded DNA breaks created by the Cas9 protein in the CRISPR-Cas9 editing system are primarily repaired in mammalian cells using one of two naturally occurring DNA repair pathways: NHEJ or HDR. NHEJ is a cellular process whereby the homologous or non-homologous broken ends of DNA created by Cas9 are joined together. While NHEJ is generally an accurate repair process, imprecise repair is possible, especially when DNA ends are not homologous, which can lead to the introduction of insertions or deletions at target sequences. Microhomology-mediated end joint (MMEJ) is another repair pathway of the cell that uses short microhomology sequences (5–25 base pairs) on each side of the double-strand break to define the location for break repair.[42–44] Unlike NHEJ and MMEJ, the HDR pathway takes advantage of a homologous donor DNA template to repair the double-stranded break induced by Cas9.[45,46] This combination of a programmable CRISPR-Cas9 editing system and cellular DNA repair pathways can be leveraged to enable the generation of precise and durable insertions, deletions, or gene repair events to change the function of a specific region of DNA.[47–49]

As with all gene editing approaches, the precision of editing is an essential consideration for the successful and safe implementation of any CRISPR-Cas9-based clinical therapy. In the CRISPR-Cas9 system, the precision of the gene editing is primarily a consequence of the complementarity between the first 20 nucleotides of the gRNA sequence and the target genomic sequence of interest,[50] although the duration of exposure to Cas9 and the version of Cas9 have also been shown to impact target specificity.' Identification of a gRNA that maximizes complementarity with the target sequences to eliminate the potential occurrence of off-target editing events is critical for the design of a highly effective and safe CRISPR-Cas9 therapeutic. To accomplish this, researchers can use *in silico* prediction tools to model potential off-target events based on the sequence of a given gRNA, as well as use next-generation sequencing assays such as GUIDE-seq, DISCOVER-seq, and CHIP-seq to survey the genome for instances of off-target editing to verify the precision of editing.[51] Since being first described in 2012, the precision of the CRISPR-Cas9 system has been extensively reviewed. A range of sgRNAs have been characterized in both *in vitro* and cellular assays to identify parameters that influence the binding of sgRNAs to target sequences, including the identification of the seed region of the sgRNA as being critical for target recognition.[52,53] These and other studies have suggested that the outcome of CRISPR-mediated editing can be predicted, and have led to a deeper understanding of how the structure and composition of the gRNA correlates with the precision of genome editing by CRISPR-Cas9,[54] enabling the advancement of highly precise CRISPR-Cas9-based therapies into early- and late-stage clinical trials.[55]

The generation of DNA double-strand breaks has been shown to cause translocations and more extensive chromosomal rearrangement, such as chromothripsis.[56,57] However, it is important to note that (1) the risk of translocations occurring in CRISPR-Cas9 editing applications is mitigated by the precision of the editing and a lack of off-target editing events because the creation of translocations requires edits at two sites and in the absence of off-target edits there are no conditions promoting the occurrence of translocations, and (2) chromothripsis has only been shown to occur in cell line systems where tumor suppressor pathways have been altered and has never been show to occur in primary human HSPCs.[57]

Similar to the CRISPR-Cas9 system, CRISPR-Cas12a is a programmable single RNA-guide endonuclease system. While the Cas12a and Cas9 proteins have functional similarities, there are also substantial differences between these proteins.[58,59] The Cas12a protein contains a single nuclease domain, unlike the dual nuclease domains of Cas9, and Cas12a possesses intrinsic RNA processing activity, allowing for processing of the crRNA array and thus multigene editing of RNA transcripts.[59] In addition, whereas CRISPR-Cas9 editing results

in blunt DNA ends, Cas12a editing leads to the generation of staggered DNA ends, enabling multiplex gene editing applications and promoting HDR cell repair instead of NHEJ.[59] Potential therapies based on the use of CRISPR-Cas12a gene editing, such as those based on Cas12a-mediated targeting of HBG1 and HBG2 promoters, have now begun to enter early-stage clinical trials.

### Emerging techniques for gene editing

#### *Base gene editing*

The need to rely on the NHEJ pathway to repair the double-stranded breaks induced by gene editing systems such as CRISPR-Cas9, TALEN, and ZFN has led to the development of alternative gene editing techniques that can generate precise point mutations without double-stranded DNA breaks. Base editing is an emerging gene editing approach where nucleotide changes can be introduced at genomic locations without using cellular repair pathways. Base editors consist of a deactivated version of the Cas9 protein with a gRNA that enables the localization of a nucleobase deaminase enzyme to a specific genomic location where a DNA point substitution can be generated.[60] Upon a base editor binding to the target locus in DNA, base pairing between the gRNA and the target DNA leads to displacement of a small segment of single-stranded DNA. The deaminase enzyme modifies DNA bases within the single-stranded portion of DNA. The efficiency of the base editing is increased through the use of a catalytically disabled nuclease that generates a nick in the non-edited DNA to induce cellular repair of the non-edited strand using the edited strand as a template.[60–62] Two types of base editors have been developed: cystine base editors can facilitate the conversion of C:G base pairs to T:A base pairs, while adenine base editors can facilitate the conversion of A:T base pairs to G:C base pairs.[60] Although studies have shown that base editors have high gene editing efficiency and can edit non-dividing cells, an important limitation to current base editing technology is the generation of random mutations and off-target effect in both DNA and RNA due to the non-specific activity of nucleobase deaminase enzymes.[63] Therefore, further refinement of base editing technologies, including potential modifications to the deaminase enzymes to overcome non-specific activity, may be required before this gene editing technology finds wider use, especially in clinical applications.

Prime editing is another gene editing strategy that also relies on the specificity of the CRISPR-Cas9 system, but includes the addition of an edit containing extension sequence in the gRNA and an M-MLV reverse transcriptase fused to the C-terminus of Cas9 nickase to avoid double-strand breaks.[64] The advantage of prime editing over base editors is the potential to create a larger number of potential changes at the target site. Base editors can generate single base substitutions for four transitions, whereas prime editing can be used to generate any potential base substitution, including transversions. The possibility for prime editing to correct the A/T to T/A transversion in the *HBB* gene that causes SCD was recently assessed in a preclinical study using human HSPCs obtained from patients with SCD. Overall, HSPC-derived erythrocytes in which prime editing had been used to correct the SCD mutation had less sickle hemoglobin, contained $HBB^{A}$-derived adult hemoglobin at between 28% and 34% of normal, and resisted hypoxia-induced sickling.[65] These early results support the feasibility of prime editing to correct the single mutation in the *HBB* gene that leads to SCD.

## CLINICAL TRIALS USING GENE THERAPY FOR TDT AND SCD

### Gene addition approaches for TDT and SCD

In 2019, betibeglogene autotemcel (beti-cel), a gene addition therapy for TDT, received conditional marketing authorization from the EMA for use in Europe; however, market authorization was withdrawn in 2021 by the developer (not due to safety or efficacy reasons). Beti-cel has since been approved by the U.S. Food and Drug Administration (FDA) (2022). Beti-cel therapy is based on the infusion of autologous $CD34^{+}$ HSPCs that have been transduced with the replication-defective, self-inactivating BB305 lentiviral vector encoding a recombinant β-globin ($\beta^{A-T87Q}$) gene[25] (Table 1). *Ex vivo* transduction of patient stem and progenitor cells with this lentiviral vector leads to insertion of functional copies of the $\beta^{A-T87Q}$ gene, a variant of the normal HBB chain, where the amino acid substitution in position 87 has anti-sickling properties; the cell product is then infused into patients following myeloablative conditioning regimen with pharmacokinetically adjusted busulfan.[25]

In a phase 3 clinical trial, 20 of 22 patients (91%) with TDT and a non-$\beta^{0}/\beta^{0}$ genotype who were infused with beti-cel and were evaluable met the primary endpoint of transfusion independence for at least 12 months with a weighted hemoglobin of 9 g/dL or greater.[25] Patients who achieved transfusion independence also showed improvements in erythropoiesis and decreases in liver iron concentration. The safety profile was considered to be consistent with busulfan-based myeloablation.

The BB305 lentiviral vector that is used as the basis for beti-cel is also being assessed for the treatment of patients with SCD in a phase 1/2 trial of lovotibeglogene autotemcel (Table 2).[66] Similar to beti-cel, lovotibeglogene autotemcel is based on the infusion of autologous $CD34^{+}$ HSPCs that have been transduced with a replication-defective, self-inactivating lentiviral vector encoding the recombinant β-globin ($\beta^{A-T87Q}$) gene. In the first two parts of this trial (part A and part B), 7 patients (part A) and 2 patients (part B) were infused with lovotibeglogene autotemcel, while results from 35 patients in part C (pivotal trial population) were reported in February 2022.[66] For those patients in part C, following busulfan myeloablative conditioning and lovotibeglogene autotemcel (obtained transducing peripheral blood-mobilized autologous $CD34^{+}$ HSPCs) infusion, increases in hemoglobin and decreases in markers of hemolysis were observed; of the 25 patients with sufficient follow-up to be evaluable, all had resolution for severe vaso-occlusive events, which were defined in the trial as events that resulted in either hospital or emergency department visits that exceed 24 h, two or more visits to a day unit or emergency department during a 72-h period (both visits required intravenous treatment), or a priapism episode that lasted more than 2 h and led to a medical facility visit. When a broader definition of vaso-occlusive event was used (defined as acute pain with no medically determined cause other than vaso-occlusion, including acute episodes of pain, acute chest syndrome, acute

**Table 1. Summary of clinical trials using gene addition or gene editing for TDT**

| Drug product (clinical trial identification number) | Mechanism of action | Phase of development | Study population | Key primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| Gene addition | | | | | | | | |
| beti-cel[25] *NCT02906202* | BB305 is a self-inactivating and replication-defective lentiviral vector adds functional copies of theβ-globin gene ($\beta^{A-T87Q}$-globin) to hematopoietic stem cells using BB305 lentiviral vector produces $\beta^{A-T87Q}$-globin, which is considered gene-therapy derived adult hemoglobin. | phase 3 | patients aged ≤50 years with TDT and non-$\beta^0/\beta^0$ genotype | proportion of participants who meet the definition of transfusion independence (i.e., weighted average hemoglobin level of ≥9 g/dL without red cell transfusions for ≥12 months) | myeloablative conditioning regimen with busulfan | primary endpoint met: 91% (n = 20) of evaluable participants (n = 22) achieved transfusion independence for ≥12 months after treatment[25] | safety was consistent with busulfan myeloablation; four participants had ≥1 AE considered related to beti-cel, one of which (thrombocytopenia) was considered to be serious; there was no evidence of insertional oncogenesis[25]; there were no deaths and no malignancies in the study | integration of the modified gene is semi-random[87]; multiple insertion sites are possible using lentiviral vectors[87]; clinical effectiveness requires multiple insertions per cell; potential risk for insertional oncogenesis (Zynteglo USPI) |
| GLOBE lentiviral vector[88] *NCT02453477* | adds a functional copy of the β-globin gene using an inactivating lentiviral vector | phase 1/2 | adults and pediatric patients aged ≥ 3 to <65 years with TDT (any genotype) | overall survival; achievement of hematological engraftment; short-term safety and tolerability of the different conditioning regimens; overall safety and tolerability measured by AE recording; polyclonal engraftment; reduction in transfusion frequency up to transfusion independence | reduced toxicity myeloablative conditioning regimen with treosulfan and thiotepa | 4/9 patients achieved transfusion independence at 60 months[88] 4/6 pediatric participants achieved transfusion independence; 0/3 adult participants achieved transfusion independence; however, the frequency and volume of transfusions reduced by ≥50% in adult patients | no AEs related to GLOBE; no severe infectious-related AEs, except neutropenia as expected after conditioning[88] | integration of the modified gene is semi-random[87] multiple insertion sites are possible using lentiviral vectors[87] potential risk for insertional oncogenesis |

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**Table 1. *Continued***

| Drug product (clinical trial identification number) | Mechanism of action | Phase of development | Study population | Key primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| TNS9.3.55 [90] *NCT01639690* | adds functional copy of β-globin gene using an inactivating lentiviral vector | phase 1 | adult patients aged ≥18 years with TDT | safety: occurrence of insertional oncogenesis; generation of replication-competent lentivirus; safety of non-myeloablative conditioning regimen tolerability: occurrence of insertional oncogenesis; generation of replication-competent lentivirus; safety of low-dose non-myeloablative conditioning regimen | non-myeloablative conditioning regimen (reduced dose busulfan) | none of the patients (n = 4) achieved transfusion independence with a median follow-up of 90 months[89] results suggest need for myeloablative conditioning for engraftment of genetically modified cells[72] this is likely due to the endogenous cells that were not ablated competing against the incoming graft | no SAEs or unexpected safety issues related to TNS9.3.55 | integration of the modified gene is semi-random[87]; multiple insertion sites are possible using lentiviral vectors[87]; potential risk for insertional oncogenesis[90] |
| Gene editing | | | | | | | | |
| ST-400[82] *NCT03432364* | ZFN disrupts the BCL11A erythroid enhancer region to enable production of HbF | phase 1/2 | adults aged ≥18 and ≤40 years with TDT | safety and tolerability assessed by incidence of AEs and SAEs | myeloablative conditioning with busulfan | none of the patients (n = 3) achieved transfusion independence with a follow-up of 2, 6, and 9 months; minor increases in HbF observed in all patients[82] | one patient had an SAE of hypersensitivity during ST-400 infusion considered to be related to the product cryoprotectant, DMSO no other SAEs related to ST-400 were observed[82] other AEs were consistent with myeloablation. | high precision using ZFN approach but no published assessments of off-target events |
| Exa-cel[84,91] *NCT03655678* | a one-time, non-viral cell therapy designed to reactivate HbF via *ex vivo* CRISPR-Cas9 gene editing at the erythroid enhancer region of BCL11A in autologous | phase 1/2/3 | patients aged 12–35 years with TDT | proportion of participants achieving transfusion independence for ≥12 consecutive months with a weighted hemoglobin | myeloablative conditioning with busulfan | almost all patients (42 of 44 patients) stopped RBC transfusions with a median time since last transfusion of 9 months; 16 patients were transfusion free for ≥12 months[91] increases in HbF and mean total Hb levels | safety was consistent with busulfan myeloablative conditioning that was used prior to exa-cel all patients achieved neutrophil and platelet engraftment there were no deaths, discontinuations, or malignancies | CRISPR-Cas9 has been shown to only edit the target site in the erythroid-specific enhancer region of BCL11A[84]; computational and experimental assessments of exa-cel based |

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**Table 1. *Continued***

| Drug product (clinical trial identification number) | Mechanism of action | Phase of development | Study population | Key primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| | $CD34^+$ HSPCs increases levels of Hb and HbF in patients with TDT | | | average of 9 g//dL | | (>9 g/dL) were achieved by month 3, with mean total Hb levels increasing to and maintained at >11 g/dL thereafter. allelic editing in bone marrow and peripheral blood was stable and durable at month 6, the mean proportion of edited BCL11A alleles in bone marrow $CD34^+$ HSPCs and peripheral blood mononuclear cells was 74.3% and 63.4%, respectively these proportions remained stable in all patients with $\geq$1 year of follow-up | two patients had SAEs considered to be related to exa-cel One patient had concurrent events of hemophagocytic lymphohistiocytosis, headache and acute respiratory distress syndrome considered related to exa-cel, as well as idiopathic pneumonia syndrome considered related to both exa-cel and busulfan; another patient had delayed neutrophil engraftment and thrombocytopenia considered related to both exa-cel and busulfan all SAEs resolved | editing at the erythroid-specific enhancer region of *BCL11A* showed no evidence of off-target editing[84] |

AE, adverse event; Hb, hemoglobin; HbF, fetal hemoglobin ; RBC, red blood cell; SAE, serious adverse event.

**Table 2. Summary of clinical trials using gene addition or gene editing for SCD**

| | Mechanism of action | Phase of development | Study population | Primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| Gene Addition | | | | | | | | |
| LentiGlobin for SCD (bb1111; lovotibeglogene autotemcel)[66] *NCT02140554* | BB305 is a self-inactivating and replication-defective lentiviral vector adds functional copies of the β-globin gene ($\beta^{A-T87Q}$-globin) to hematopoietic stem cells using BB305 lentiviral vector Similar to TDT approach produces HbAT87Q which is considered gene-therapy derived adult hemoglobin T87Q change is intended to inhibit polymerization of sickle Hb | phase 1/2/3 | patients aged 12–50 years with severe SCD and $\beta^S/\beta^S$ or $\beta^S/\beta^0$ or $\beta^S/\beta$+ genotype | proportion of participants achieving complete resolution of VOCs between 6 months and 18 months after drug product infusion | myeloablative conditioning with busulfan | among 25 evaluable patients, all achieved resolution of severe VOCs of these, 2 patients had VOCs[66] 35 patients infused with LentiGlobin; all engrafted; total hemoglobin increased over time with $HbA^{T87Q}$ responsible for ≥40% total Hb | safety profile is consistent with busulfan myeloablation and underlying SCD no drug-related SAEs observed 3 patients had nonserious AEs related to LentiGlobin; no cases of hematologic cancer with 17.3 months of median follow-up[66] one death due to cardiac arrest; the death was associated with cardiac fibrosis and other chronic cardiopulmonary injury two patients with low-level trisomy 8 and persistent anemia potentially associated with co-inheritance of two α-globin deletions two patients with SCD who received lovotibeglogene autotemcel in group A of the study were diagnosed with acute myeloid leukemia at 3 years and 5.5 years after infusion. Demonstration of lentiviral insertion in blast cells from 1 patient[66,69,70] | multiple insertion sites are possible using lentiviral vectors[87]; insertion of the modified gene is semi-random[87]; insertion into oncogenes possible and must be considered[90]; clinical effectiveness requires multiple insertions per cell |
| DREPAGLOBE[74,75] *NCT03964792* | adds a copy of βAS3, which is an anti-sickling β-globin protein, using lentiviral vector has 3 amino acid substitutions relative to wild type | phase 1/2 | patients aged 12–20 years with severe SCD and $\beta^S/\beta^S$ genotype and failed hydroxyurea therapy, was unable to tolerate hydroxyurea therapy, or chose not take hydroxyurea | incidence of transplant related mortality; incidence of the need for rescue autologous bone marrow transplant; frequency and severity of AEs post-transplant; incidence of vector-derived replication competent lentivirus; incidence of clinically detectable malignancy and/or abnormal clonal dominance assessed as related to study treatment | myeloablative conditioning with busulfan | among 3 patients treated, DREPAGLOBE was not effective in 1 patient and 2 patients had clinical benefit with non-severe VOCs not requiring hospitalization[75] variable efficacy of the treatment, likely dependent on gene marking achieved in HSCs and engraftment of modified HSCs in bone marrow | | semi-random integration[87] |
| ARU-1801[92] (MOMENTUM) *NCT02186418* | uses modified γ-globin lentiviral vector to produce $HbF^{G16D}$ in autologous $CD34^+$ HSC | phase 1/2 | patients aged 18–45 years with severe SCD and failed hydroxyurea therapy, unable to tolerate hydroxyurea therapy, or chose not take hydroxyurea | incidence of grade 3 allergic reaction; incidence of grade 4 infection; incidence of grade 4 neutropenia; incidence of grade 3 or 4 organ toxicity; incidence of AEs; incidence | reduced-intensity conditioning with single-dose melphalan | among 4 patients treated, 1 patient has been VOC free since administration and 2 patients have shown 93% and 85% fewer annualized reductions in VOCs[92] levels of HbF ranged from 14% to 36% | predominant AEs were transient neutropenia and thrombocytopenia | semi-random integration[87]; preclinical studies in SCD mice: G16D mutation enables γ-globin G16D to bind α-globin with higher affinity; lentiviral transfer of γ-globin G16D resulted in 1.5× to 2× more HbF |

(*Continued on next page*)

**Table 2. *Continued***

| | Mechanism of action | Phase of development | Study population | Primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| | | | | of SAEs; incidence of death due to study procedures; incidence of hematological malignancy; incidence of hematological cancer; time to neutrophil recovery; time to platelet recovery; number of participants with a total number of $CD34^+$ cells recovered from all collections combined (mobilized peripheral blood and bone marrow) of $\geq 8 \times 10^6$ kg viable $CD34^+$ cells; proportion of participants for which a minimum of $4 \times 10^5$ $CD34^+$ cells/kg body weight from all collections combined havebeen successfully transduced; number of participants with bone marrow aspirates at 1-year post-infusion with $\geq 1\%$ gene-marked cells | | | | per vector count number compared to analogous wild-type γ-globin vector; HbF G16D may be more potent for anti-sickling than HbF |
| Gene editing | | | | | | | | |
| BIVV003[83] (PRECIZN-1) *NCT03653247* | gene editing by ZFN at the *BCL11A* erthyroid enhancer region to enhance production of HbF in HSPC modified *ex vivo* | phase 1/2 | patients aged 18–40 years with severe SCD and βS/βS or βS/β0 genotype | percentage of participants who are alive at post-transplantation at 100 days, 52 weeks, and 104 weeks; percentage of participants with successful engraftment; number of participants with AEs and SAEs | myeloablative conditioning with busulfan | all 4 patients had no VOCs since infusion through 13–65 weeks of follow-up[83] 4 patients treated with PRECIZN-1 as of May 3, 2022, with up to 125 weeks of follow-up all subjects achieved levels of HbF/cell of >10 pg, which has been suggested to inhibit HbS polymerization F-cell levels >90%. 3 subjects had no VOCs following infusion of PRECIZN-1; 1 subject who did not sustain HbF levels had 2 VOCs after infusion | no AEs or SAEs related to BIVV003[83] most AEs consistent with conditioning regimen | high precision using ZFN approach, but no published assessments of off-target events |
| Nula-cel[93] (CEDAR) *NCT04819841* | gene editing of autologous HSPCs to correct *Hbb* point mutation using CRISPR-Cas9 RNP, with delivery through | phase 1/2 (paused) | patients aged 12–40 years old with severe SCD with recurrent severe VOC ($\geq 4$ episodes in the preceding 2 years) | proportion of patients who reach neutrophil engraftment; incidence rate of treatment-related mortality at 100 days and 12 months post- | myeloablative conditioning | not publicly available development of nula-cel discontinued by Graphite in February 2023[94] | first patient dosed had SAE of prolonged pancytopenia likely related to treatment[93] | high precision using CRISPR-Cas9 approach based on gRNA sequence, but no published assessments of off-target events |

(*Continued on next page*)

**Table 2. *Continued***

| | Mechanism of action | Phase of development | Study population | Primary endpoint(s) | Conditioning regimen | Efficacy outcomes | Safety outcomes | Precision |
|---|---|---|---|---|---|---|---|---|
| | a nonintegrating AAV6 with DNA repair template | | ACS ($\geq$2 episodes in the prior years with $\geq$1 episode in the past year), and Lansky/Karnofsky performance status of $\geq$80 | infusion; overall survival; frequency and severity of AEs/SAEs | | | | |
| Exa-cel[84,95] *NCT03745287* | a one-time, non-viral cell therapy designed to reactivate HbF via *ex vivo* CRISPR-Cas9 gene-editing at the erythroid enhancer region of BCL11A in autologous $CD34^+$ HSPCs increases levels of Hb and HbF in patients with SCD | phase 1/2/3 | patients aged 12–35 years with severe SCD and $\beta^S/\beta^S$ or $\beta^S/\beta^0$ genotype with a history of $\geq$2 severe vaso-occlusive crisis events per year for the previous 2 years prior to enrollment | proportion of participants who are VOC-free for $\geq$12 consecutive months | myeloablative conditioning with busulfan | all patients (n = 31) were VOC free (duration of follow-up: 2–32.3 months)[95]; increases in Hb,HbF, F-cells that happened early and were maintained; patients had mean increase in fetal hemoglobin of >40% that was maintained; allelic editing in peripheral blood and bone marrow was stable and durable; the mean proportion of edited *BCL11A* alleles in bone marrow $CD34^+$ HSPCs and peripheral blood mononuclear cells was 86.6% and 76.0%, respectively, at month 6 | safety was consistent with busulfan myeloablation that was used prior to exa-cel; all patients achieved neutrophil and platelet engraftment; no patients have had serious AEs considered to be related to exa-cel; one patient died due to COVID-19 infection resulting in respiratory failure; this SAE was not related to exa-cel; there were no discontinuation and no malignancies; safety findings were consistent with busulfan myeloablative conditioning that was used prior to exa-cel | CRISPR-Cas9 has been shown to only edit target site in the erythroid-specific enhancer region of *BCL11A*[84]; computational and experimental assessments of exa-cel based editing at the erythroid-specific enhancer region of *BCL11A* showed no evidence of off-target editing[84] |

AE, adverse event; COVID-19, coronavirus disease 2019; Hb, hemoglobin; HbF, fetal hemoglobin; RNP, ribonucleoprotein; SAE, serious adverse event; VOC, vaso-occlusive crisis.

hepatic sequestration and acute priapism), there were two patients who had vaso-occlusive events between engraftment and last visit.[66]

Third-generation lentiviral-based vectors, such as the one used in the beti-cel and lovotibeglogene autotemcel therapies, have been engineered to be replication incompetent and self-inactivating, an effort to limit the potential risk of insertional oncogenesis.[67,68] Although no insertional oncogenesis events have been reported for patients with TDT given beti-cel, there have been two patients with SCD who received lovotibeglogene autotemcel (earlier version of the same lentiviral vector used in beti-cel) who were diagnosed with acute myeloid leukemia at 3 years and 5.5 years after infusion. Case studies of both patients did not show insertional oncogenesis, although analysis of peripheral blood samples did reveal leukemic blast cells that contained BB305 lentivector insertion in one of the patients; the vector was integrated in close proximity of *VAMP4*, a gene never described to be involved in leukemogenesis.[66,69,70] Still, these results would highlight the need for long-term monitoring of patients treated with BB305 lentiviral vector to better understand the insertion patterns associated with BB305 lentiviral vector transduction.

Several studies demonstrate that myeloablative conditioning is needed to maximize $CD34^+$ engraftment of lentiviral-transduced cells.[71,72] In the beti-cel and lovotibeglogene autotemcel trials, prior to infusion of $CD34^+$ HSPCs transduced cells, patient underwent myeloablative conditioning with single agent busulfan. In a different lentiviral gene therapy program, the phase 1/2 TIGET-Bthal trial, autologous hematopoietic stem cells are modified with the GLOBE lentiviral vector to express a transcriptionally regulated human β-globin gene, and patients were given a conditioning regimen based on the myeloablative combination of treosulfan and thiotepa[73] (Table 1). Of the three adults and six children with TDT who were treated, transfusion requirements were reduced, but not eliminated, in adults, while three of four children who were evaluable were transfusion independent. Given the reduced toxicity conditioning regimen used in this trial, it cannot be excluded that endogenous cells were not successfully ablated, which then competed against the transduced cells in the cell graft, resulting in insufficient chimerism. Similar findings were observed in a smaller phase 1 trial conducted in the United States of a lentiglobin-based therapy in adults with TDT that also used a reduced intensity busulfan conditioning regimen.[71] In this trial, four patients received a conditioning regimen of busulfan that was in the non-myeloablative range prior to infusion of cells transduced with the TNS9.3.55 lentiviral globin vector (Table 1). The patients, who were followed for 6–8 years and had no unexpected safety issues during either the conditioning regimen or following infusion of the cell product, had low but stable hematopoietic gene marking. There were reductions in transfusion requirements for two patients, but no patients were able to achieve transfusion independence during the follow-up period. While these findings demonstrate it is possible to achieve durable stem cell engraftment with a non-myeloablative conditioning regimen, myeloablative conditioning appears required to maximize the therapeutic benefit derived from $CD34^+$ engraftment of lentiviral-transduced cells.[71,72]

Several other clinical trials are in progress that are evaluating the use of gene addition via lentiviral vectors in patients with SCD (Table 2). In 2018, Weber et al.[74] reported the design of a lentiviral vector that contained an anti-sickling *HBB* gene (βAS3 globin) that, when transduced into hematopoietic stem progenitor cells from patients with SCD, led to an up to 50% reduction in the sickling of red blood cells under hypoxic conditions. Based on these findings, a phase 1/2 trial was initiated in 2020 with three patients with SCD recruited.[75] All patients received busulfan-based myeloablative conditioning prior to the infusion of transduced cells. While all drug products had similar vector count numbers, patients had variable gene marking in peripheral blood mononuclear cells, suggesting there was a challenge in self-renewal and engraftment potential of hemopoietic stem cells in the graft. Two out of the three patients showed some benefit in terms of reduced pain crises and transfusion need; however, the clinical data indicated variable efficacy associated with treatment which was most likely due to the extent to which gene addition was achieved and maintained in engrafted hemopoietic stem cells.

While the majority of lentiviral vector-based gene therapy approaches for individuals with either TDT or SCD have used vectors containing a modified version of the *HBB* gene to produce functional copies of the β-globin gene, ARU-1801 gene therapy uses cells that are transduced with a lentiviral vector containing a modified $\gamma\text{-globin}^{G16D}$ gene (Table 2). Adult hemoglobin is a tetramer composed of two alpha (α-globin) and two β (β-globin) subunits while fetal hemoglobin, which is developmentally regulated, is composed of two alpha and two gamma (γ-globin) subunits. Fetal hemoglobin is the predominant form of hemoglobin expressed throughout gestation until the transcriptional switch from γ-globin to β-globin synthesis shortly after birth which results in expression of adult hemoglobin.[76] It has been shown that infants with TDT or SCD are generally asymptomatic until their fetal hemoglobin levels decline during the first year of life and adults with hereditary persistence of fetal hemoglobin, a condition where production of fetal hemoglobin continues past the first year of life, similarly have little to no TDT or SCD disease manifestations.[76–78] These findings suggest that reactivating production of γ-globin and fetal hemoglobin can be a safe and effective way to protect against TDT and SCD disease symptoms. As of February 2022, five patients with SCD had been infused with ARU-1801 gene therapy. Prior to infusion, patients received a reduced-intensity conditioning regimen of single dose melphalan. Results show high engraftment and the capacity to reach effective levels of anti-sickling hemoglobin which the authors suggest indicate infusion of ARU-1801 along with a reduced intensity conditioning regimen may provide durable and clinically meaningful levels of γ-globin. The reactivation of fetal hemoglobin using lentiviral vector-based gene therapy is being evaluated in other clinical trials, as well. In 2021, researchers described the use of a lentiviral vector (BCH-BB694) to mediate the erythroid-specific knockdown of the *BCL11A* gene, a transcription factor that represses expression of γ-globin and fetal hemoglobin, using a microRNA (miRNA)-adapted short hairpin RNA to induce expression of fetal hemoglobin.[79] Six patients had been followed for 6 months after receiving BCH-BB694, with all patients showing

reductions or disappearance of clinical manifestations of SCD and all patients who could be fully evaluated had evidence of robust and stable fetal hemoglobin induction. While ARU-1801 clinical development program was discontinued in June 2022, additional approaches based on enhancing the production of fetal hemoglobin as a functional cure for TDT and SCD continue to be advanced in clinical trials.

### Gene editing approaches for TDT and SCD

Beyond gene addition approaches using viral vectors, progressive refinement of gene editing techniques, including those for CRISPR-Cas9, TALENs, and ZFNs, has enabled the expansion of gene edited cell-based therapies into early and late-stage clinical trials.

ST-400 is a cell product consisting of autologous hematopoietic stem cells that are edited *ex vivo* using ZFN technology to disrupt the erythroid enhancer region of the *BCL11A* gene.[80,81] Preclinical studies have shown that a gene editing approach that removes the repressive function of the BCL11A protein will lead to increases in fetal hemoglobin and subsequently amelioration of TDT and SCD symptoms. In the phase 1/2 trial of ST-400 (THALES), the first three patients with TDT infused had neutrophil and platelet engraftment, increases in fetal hemoglobin, and reductions in transfusion requirements, although all three patients continued to require transfusions following ST-400 infusion[82] (Table 2). The use of ZFN gene editing at the erythroid enhancer region of *BCL11A* to reactivate production of fetal hemoglobin is also being assessed in a phase 1/2 trial of the gene-edited cell product SAR445136 (PRECIZN-1 trial) for patients with SCD[83] (Table 2). In June 2021, preliminary proof-of-concept results from four patients with SCD infused with SAR445136 who had sufficient follow-up time showed patients successfully engrafted neutrophils and platelets, had increases in fetal hemoglobin that persisted for up to 65 weeks of follow-up, and had no recurrence of vaso-occlusive crisis (VOCs) following infusion. While these results demonstrate the potential for ZFN gene-edited cell products to treat patients with TDT and SCD, data from a greater number of patients who are followed over a longer period of time are needed to understand the full range of therapeutic benefits.

Exagamaglogene autotemcel (exa-cel) is a cell therapy that is also designed to reactivate fetal hemoglobin but uses *ex vivo* CRISPR-Cas9 editing at the erythroid enhancer region of the *BCL11A* gene in autologous $CD34^+$ HSPCs.[84] A ribonucleoprotein complex composed of Cas9 and a highly specific gRNA, SPY101, is used to target a critical binding site of the transcription factor GATA1 in the non-coding erythroid lineage-specific enhancer region of *BCL11A* on chromosome 2. Repair of these double-stranded DNA breaks by the cell using NHEJ produces insertions and deletions that disrupt GATA1 binding, thereby selectively lowering *BCL11A* transcription only in erythroid cells and preserving normal *BCL11A* function in other cell types. The permanent and precise *ex vivo* gene editing of the erythroid enhancer region of the *BCL11A* gene, which is a negative regulator of fetal hemoglobin production,[80,85] results in an increase in levels of fetal hemoglobin. As previously noted, during the first year of life, neonates and infants with TDT or SCD are typically asymptomatic, while fetal hemoglobin levels remain high and become symptomatic when fetal hemoglobin declines after the first year of life.[76,86] Furthermore, patients with TDT or SCD who co-inherit hereditary persistence of fetal hemoglobin (HPFH), in which fetal hemoglobin expression continues throughout adulthood, have little or no disease and are generally healthy.[78]

A 2021 article from Frangoul et al.[84] reported on preclinical studies assessing the precision of editing with CRISPR-Cas9 and initial clinical results from the first two patients, one with TDT and one with severe SCD, infused with exa-cel. The frequency of CRISPR-Cas9 gene editing at the erythroid enhancer of *BCL11A* was assessed in CD34+ hematopoietic stem cells from ten healthy donors showing high frequencies of edited alleles (mean 80%) across all subpopulations of $CD34^+$ cells that persisted over time. In immunocompromised mice, engraftment of $CD34^+$ HSPCs was shown to be equivalent for control and single-gRNA CRISPR-Cas9-edited cells. Computational methods using sequence similarity analyses along with experimental high coverage, next-generation sequencing using the GUIDE-seq found that CRISPR-Cas9-edited stem cells from four healthy donors had no evidence of off-target editing events.[84] Functionally, engraftment capabilities of edited and non-edited HSPCs were equivalent suggesting CRISPR-Cas9 editing did not impact hematopoietic stem cell function. Finally, isolated edited stem cells that were differentiated toward the erythroid lineage had increases in mean fetal hemoglobin levels compared to unedited cells.[84]

Clinically, following busulfan myeloablative conditioning and then exa-cel infusion, both the TDT and severe SCD patients engrafted neutrophils and platelets and had increases in total hemoglobin and fetal hemoglobin with pancellular distribution along with high levels of allelic editing in bone marrow and blood.[84] Critically, after more than a year of follow-up, the patient with TDT remained transfusion-independent and the patient with SCD had not had a vaso-occlusive event. These results established the potential for exa-cel to be curative treatment for patients with TDT and SCD.

Exa-cel has moved into pivotal phase 3 clinical trials (CLIMB THAL-111 and CLIMB SCD-121) (Tables 1 and 2). While both clinical trials are ongoing, in 2023, efficacy and safety data from 96 patients dosed with exa-cel were reported. Overall, 32 of 35 patients with at least 16 months of follow-up met the primary endpoint of the CLIMB THAL-111 trial being transfusion independent for at least 12 months (range, 13.3–45.1 months) following exa-cel infusion and 29 of 31 patients with at least 16 months of follow-up met the primary endpoint of the CLIMB SCD-121 trial being free of VOCs for at least 12 months (range, 14.8–45.5 months), with some of these patients having nearly 4 years of follow-up. The definition of VOC in this study included any event of acute pain that required a visit to a medical facility and administration of pain medication or red blood cell transfusion, acute chest syndrome, priapism lasting more than 2 h and requiring a visit to a medical facility, or splenic sequestration. Consistent with this observed elimination of transfusions and VOCs, patients with TDT

had increases in mean total hemoglobin and mean fetal hemoglobin by month 3 (>9 g/dL) after exa-cel infusion with mean total hemoglobin then increasing to more than 11 g/dL, which was maintained through the follow-up period. Patients with SCD had mean fetal hemoglobin levels of 36.8% by month 3, with pancellular distribution (>95% of red blood cells with fetal hemoglobin), which were sustained at approximately 40% through the follow-up period. The proportions of edited *BCL11A* alleles in bone marrow $CD34^+$ hematopoietic stem cells and peripheral blood mononuclear cells in patients with TDT and SCD were reported to be above 75% at month 6 and were maintained in those who had greater than 1 year of follow-up, suggesting durable CRISPR-Cas9 editing of long-term hematopoietic stem cells. The safety profile was considered consistent with the busulfan myeloablative conditioning regimen used prior to exa-cel infusion. One patient with SCD died because of severe acute respiratory syndrome coronavirus 2 pneumonia, occurring in an unvaccinated patient with a history of heavy smoking and recurrent episodes of asthma. These results, which were obtained from the largest clinical trial of a gene editing therapy for TDT or SCD to date, suggest that the use of CRISPR-Cas9 editing of stem cells represent a viable clinical modality for the treatment of genetic diseases, including TDT and SCD. In addition, the clinical results confirm the preclinical findings as well as the natural history of TDT and SCD patients in the neonatal period and in those who co-inherit HPFH that reactivation of fetal hemoglobin by exa-cel has the potential to functionally cure both TDT and SCD. Based on these data, the FDA approved exa-cel for treatment of patients aged 12 years and older with SCD in December 2023 and with TDT in January 2024. Additional data from the CLIMB THAL-111 and CLIMB SCD-121 trials, along with long-term safety and extension studies, will allow for further understanding of the durability of allelic editing and treatment effect, as well as the long-term safety profile, of exa-cel.

## CONCLUSIONS

Clinical trials of therapeutic modalities using gene therapy approaches in patients with TDT and SCD are now showing the potential to not only treat patients with these hemoglobinopathies, but the opportunity to provide one-time functional cure that was previously only possible through use of matched-donor allogeneic HSCT. While many of these approaches have demonstrated proof-of-concept as detailed in this review, there are some, including the CRISPR-Cas9-based gene editing therapy exa-cel and the gene addition approaches of beti-cel and lovotibeglogene autotemcel, that have been recently approved for use, which will provide new options for the treatment of patients with TDT and SCD in the coming years.

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## AUTHOR CONTRIBUTIONS

All authors contributed to reviewing the literature and the writing of the first draft and all subsequent revisions of this manuscript.

## DECLARATION OF INTERESTS

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