Gene therapy for sickle cell disease

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Sickle cell disease (SCD) is potentially curable after allogeneic hematopoietic stem cell transplantation (HSCT) or autologous HSCT after ex vivo genetic modification. Autologous HSCT with gene therapy has the potential to overcome many of the limitations of allogeneic HSCT that include the lack of suitable donors, graft-versus-host disease, the need for immune suppression, and the potential for graft rejection. Significant progress in gene therapy for SCD has been made over the past several decades, now with a growing number of clinical trials investigating various gene addition and gene editing strategies. Available results from a small number of patients, some with relatively short follow-up, are promising as a potentially curative strategy, with current efforts focused on continuing to improve the efficacy, durability, and safety of gene therapies for the cure of SCD.

LEARNING OBJECTIVES

- Describe the advances in gene therapy for patients with sickle cell disease
- Identify the risks and strategies to overcome these limitations associated with gene therapy for patients with sickle cell disease

CLINICAL CASE

A 27-year-old man with homozygous sickle cell disease on hydroxyurea is interested in transplantation as a one-time treatment strategy. His disease course has been complicated by frequent vaso-occlusive pain crises, recurrent severe acute chest syndrome, frequent transfusions with subsequent iron overload, and proteinuria. His pain crises have increased in frequency and intensity in his late adolescence and early adulthood, requiring more frequent and longer hospitalizations and chronic opioid use. He has been on hydroxyurea for more than 10 years, and despite good compliance and a maximally tolerated dose, his fetal hemoglobin remains <20%. His pain worsened with crizanlizumab, and he does not have a matched sibling.

Introduction

Sickle cell disease (SCD) is a life-limiting inherited hemoglobinopathy with significant complications that worsen over the life span of a patient. The currently available disease-modifying therapies are necessary but insufficient to address the growing burden of disease,[1] and therefore, treatment options that seek to fully eliminate disease complications, particularly for those with the more severe forms of SCD, are needed.

The current treatment paradigm for individuals with SCD centers on supportive care for acute complications, drug therapies to reduce disease severity, or potential cure through hematopoietic stem cell transplantation (HSCT).[2] Whereas the health and survival of children with SCD have improved considerably with the use of newborn screening, penicillin prophylaxis, and immunizations, mortality rates for adults have worsened in the same time frame.[3] Treatment has therefore shifted from a life-threatening disease of children to a chronic disease of adults, although irreversible and debilitating complications such as stroke can occur at any age. Disease-modifying therapies are not universally used, have variable clinical responses, must be continued indefinitely, require chronic monitoring, and do not fully eliminate disease complications. Allogeneic HSCT from a human leukocyte antigen–matched sibling is currently the only established curative intervention for SCD; however, broad use of this option is significantly limited by donor availability. While important strides have been made with the use of alternative donor HSCT, specifically haploidentical HSCT, to decrease graft rejection and graft-versus-host disease, higher rates of transplant-related mortality and morbidity limit the broad use of this therapy. Furthermore, specialized treatment centers that can manage the transplant and complex posttransplant care restricts access for many patients. Given the lack of universally beneficial

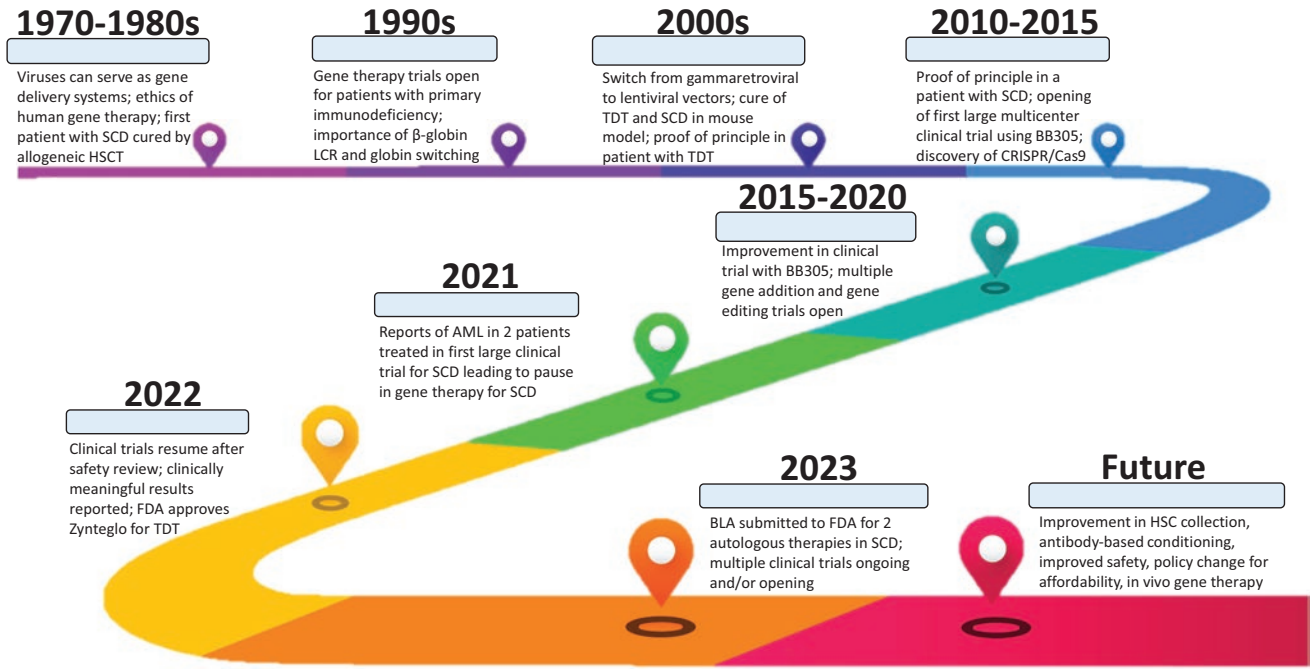

Figure 1. Historical timeline of gene therapy for sickle cell disease to present date. Highlights of decades of preclinical and clinical research leading to a possible FDA-approved autologous cell therapy product for sickle cell disease are presented. BLA, biologic license application; LCR, locus control region.

disease-modifying therapies, the lack of available human leukocyte antigen–identical sibling donors, the increased risks of complications with alternate donor HSCT, and the complex care following allogeneic HSCT, autologous HSCT after gene therapy is a theoretical universal cure for SCD that could eliminate major limitations of allogeneic transplantation. The historical perspective, current state, and future considerations of autologous gene therapy for SCD are reviewed herein.

Historical perspective

The concept that gene therapy may ameliorate human genetic diseases emerged in the 1970s after it was discovered that viruses could serve as a gene delivery system[4,5] (Figure 1). Proof of principle was first demonstrated in patients with advanced melanoma,[6] and the first clinical trials in gene therapy opened shortly thereafter. The first trials focused on ex vivo correction of hematopoietic stem cells (HSCs) from patients with primary immunodeficiencies (PIDs) using murine gammaretroviruses (specifically the murine leukemia virus), as this vector system permanently integrates into the target cell, which is a necessary feature for vector systems aimed at self-renewing stem cells and their progeny. The PIDs were an obvious early target given the relatively low-threshold gene transfer efficiency needed in the setting of a disorder where there existed a strong natural in vivo selection for gene-corrected cells. The long-awaited successful application of human gene therapy was indeed demonstrated first in X-linked severe combined immunodeficiency, but genotoxicity from insertional mutagenesis halted these initial studies after the retroviral vector inserted into protooncogenes driving a leukemia process in several patients.[7-9]

Initial challenges for gene transfer in hemoglobinopathies included an inability of gammaretroviral vectors to carry the large β-globin gene and the necessary regulatory elements, the potential for silencing, and the lack of high-level globin gene expression.[10] To overcome these challenges, the use of lentiviral vectors containing the β-globin locus control region enabled the first reversal of β-thalassemia in a mouse model by demonstrating viral transgene expression of nearly 20% of the total hemoglobin using a human β-globin lentiviral vector with crucial locus control region elements (TNS9 vector).[11,12] A similar lentiviral vector that substituted glutamine for threonine at amino acid 87 (β^{T87Q}) resolved anemia and reduced organ damage in 2 SCD transgenic mouse models.[13,14] The first clinical trials investigating gene therapy in patients with β-thalassemia and SCD therefore evaluated ex vivo delivery of β^{T87Q} using lentiviral transduction. Simultaneously, the discovery of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 for programmable editing was reported[15] ushering in a novel genome editing strategy for the cure of hemoglobin disorders.

A decade of lentiviral-based gene therapy for SCD

Proof of concept in a patient with β-thalassemia, and later in a patient with SCD, was reported in 2010[16] and 2017,[17] respectively, using ex vivo lentiviral delivery of β^{T87Q} into autologous HSCs. These first patients were among the group of initial patients treated with a lentiviral vector encoding β^{T87Q}, initially tested in phase 1/2 studies known as HGB-204 (NCT01745120), HGB-205 (NCT02151526), and HGB-206 (NCT02140554). HGB-204 focused on patients with transfusion-dependent thalassemia (TDT); HGB-205 treated 4 patients with TDT and 3 patients with SCD. Early reports demonstrated stable and durable

engraftment of gene-modified cells after myeloablative conditioning, leading to stable production of the therapeutic hemoglobin (HbA^{T87Q}).[17,18] Success in HGB-204 led to several phase 3 clinical trials in patients with TDT (NCT02906202 and NCT03207009), resulting in US Food and Drug Administration (FDA) approval for the product Zynteglo (LentiGlobin BB305, betibeglogene autotemcel) in 2022 as the first cell-based gene therapy to treat adult and pediatric patients with TDT.[19]

Following proof of concept in a single patient with SCD in HGB-205, HGB-206 was initiated as a large, multicenter study in patients with severe SCD. Data from the first cohort of patients (group A, n = 7) using bone marrow–derived HSCs demonstrated a relatively low peripheral blood vector copy number (VCN) and modest HbA^{T87Q} production, and therefore patients continued to have stress erythropoiesis and lacked a robust clinical benefit.[20] Improvements were subsequently made to the manufacturing process, resulting in higher VCN values in group B (n = 2) and later to the collection of a peripherally mobilized stem cell source with plerixafor, which was demonstrated to be safe and effective in patients with SCD and demonstrated a superior $CD34^+$ quality[21-24] in group C (n = 35).[20,25] Such improvements now demonstrate high and sustained near-pancellular expression of HbA^{T87Q} that was nearly 50% of total hemoglobin by 6 months posttreatment. Clinically, there was a marked reduction in sickle hemoglobin, reduction in the propensity of red blood cells to sickle, near normalization of hemolysis markers, and resolution of severe vaso-occlusive events. LentiGlobin BB305 (bb1111, lovotibeglogene autotemcel) for SCD is being evaluated in a phase 3 study (NCT04293185) in both children and adults with SCD and was submitted for FDA approval for adults with severe SCD in the spring of 2023 (Table 1).

Additional phase 1/2 studies have investigated lentiviral vector delivery of either wild-type β^A-globin (NCT01639690, NCT02453477, NCT03276455), β^{AS3} antisickling globin (NCT03964792), γ-globin (NCT02186418), or delivery of a transgene that results in erythroid-specific expression of a short hairpin RNA targeting *BCL11A*, leading to knockdown of this critical repressor of fetal hemoglobin (HbF) (NCT03282656) (Table 1). Of these, lentiviral delivery of a short hairpin RNA targeting BCL11A demonstrated stable HbF induction (percentage HbF/[F + S] at most recent follow-up, 20.4% to 41.3%), with HbF broadly distributed in red cells (F cells 58.9% to 93.6% of untransfused red cells) and HbF per F cell of 9.0 to 18.6 pg per cell,[26] and is now being investigated in a phase 3 study (NCT05353647).

Two cases of acute myeloid leukemia (AML) were reported following gene addition therapy for SCD; importantly, in both cases, it was determined the AML was unrelated to the lentiviral vector, as was previously documented in patients with early PID treated with gammaretroviral vectors.[27,28] The 2 patients who developed AML were among the first 7 patients treated in the phase 1/2 trial of LentiGlobin BB305 for SCD (NCT02140554) produced from bone marrow–harvested HSCs using an earlier version of the drug product manufacturing process. Current theories suggest the stress of switching from homeostatic to regenerative hematopoiesis by transplanted cells drives clonal expansion and leukemogenic transformation of preexisting premalignant clones, eventually resulting in AML/myelodysplastic syndrome.[29] Bone marrow harvests as the cell source resulted in lower cell doses with fewer $CD34^{hi/+}$ long-term HSCs, likely increasing proliferative stress on the repopulating cells. Low drug product VCNs for these patients resulted in modest transgene expression and an inadequate therapeutic response. These patients continued to experience hemolysis and persistent anemia, resulting in transplanted and endogenous bone marrow cells continuing to experience hematopoietic stress posttreatment, providing a further opportunity for accumulation of mutations. To date, no cases of insertional oncogenesis have been reported in gene addition trials using lentiviral vectors for TDT or SCD. There are no reports of leukemia in the 63 patients with β-thalassemia who received drug products manufactured with the identical BB305 LVV used for manufacture of LentiGlobin for SCD in separate clinical trials, suggesting a uniqueness to the pathophysiology of SCD. Constant erythropoietic stress with dysregulated hematopoiesis, chronic inflammation, repeat bony infarction, and the possibility of preexisting clonal hematopoiesis of indeterminant potential–related mutations compound the already known risks associated with genotoxic conditioning, autologous HSCT, and an increased relative but low absolute risk of AML/myelodysplastic syndrome in this patient population.[30-33]

In totality, the reported data from the completed or ongoing trials encompassing gene addition therapies using lentiviral vectors support this mode of treatment to be an efficacious option with an acceptable safety profile for patients with TDT and SCD. To date, no cases of graft-versus-host disease or immune rejection have been reported, there have been no cases of insertional oncogenesis, and the overall safety profiles of the ongoing and completed studies are generally consistent with that of HSCT requiring myeloablative conditioning and of the underlying

Table 1. Investigational lentiviral vector–based autologous cell therapy products for sickle cell disease

Product	Sponsor	Technology	Effect	Activation	Status
Lovotibeglogene autotemcel	Bluebird bio	Lentiviral gene addition	BB305 LVV/$b^{A\text{-}T87Q}$ (antisickling)	2014	Submitted BLA spring 2023
ARU-1801	Aruvant Sciences GmbH	Lentiviral gene addition	LVV/γ-globin (G16D) (antisickling)	2014	Active, not recruiting
DREPAGLOBE	Assistance Publique–Hopitaux de Paris	Lentiviral gene addition	GLOBE LVV/b^A (nonsickling)	2019	Active, not recruiting
BCH-BB694	Boston Children's Hospital	Lentiviral gene addition	shRNA targeting *BCL11A* (antisickling)	2018	Phase 3

BLA, biologic license application; LVV, lentiviral vector; shRNA, short hairpin RNA.

disease. While the 2 reports of AML after gene addition have excluded the genetic technology itself, the long-term risks of secondary malignancy after gene therapy are unknown. Given limited follow-up to date and potentially significant risks associated with genetic modifications, patients are required to be followed long term in accordance with the FDA's guidelines for participants who receive investigational genetically modified cellular products.

Advances in gene editing for SCD

Multiple gene editing therapies for SCD are currently in clinical development and include gene knockdown of regulators of fetal hemoglobin, gene editing of globin regulatory elements, and direct globin gene editing, with targeted nucleases such as zinc-finger nucleases, CRISPR/Cas9, and, more recently, with base or prime editors. The major advantage of these methods over gene addition strategies is the ability to significantly reduce or entirely avoid nonspecific integration that may lead to insertional oncogenesis, although risks of off-target editing, deleterious on-target editing, or consequences of double-strand breaks remain significant.[34-40]

Most gene editing clinical trials for SCD have sought to increase endogenous HbF expression through knockdown of *BCL11A*, either targeting the *BCL11A* erythroid enhancer on chromosome 2 or targeting the HbF repressor binding sites on chromosome 11. Two studies (NCT04819841 and NCT04774536) aimed to correct the underlying b^S glutamate to valine amino acid substitution at position 6 (E6V) in SCD by editing the β-globin gene and achieving repair via homology-directed repair by providing template DNA, but 1 study closed after the first patient developed pancytopenia,[41] and the other is not yet recruiting patients.

CTX001 (exagamglogene autotemcel, exa-cel) is an investigational autologous cell therapy that uses CRISPR/Cas9 to disrupt the *BCL11A* erythroid enhancer to increase endogenous HbF (NCT03745287). As of February 2022, 31 patients with SCD (age 22.5 [12-34] years) had been infused with exa-cel (follow-up 9.6 [2.0-32.3] months).[42] The mean proportion of HbF was >20%, and total hemoglobin levels were >11 g/dL 3 months postinfusion, with editing rates of 86.6% in the bone marrow $CD34^+$ HSCs 6 months postinfusion. All patients remained vaso-occlusive crisis (VOC)-free, and there were no serious adverse events, including deaths, discontinuations, or malignancies. Exagamglogene autotemcel (exa-cel) was submitted for FDA approval for SCD and β-thalassemia in the spring of 2023[43] (Table 2).

Two other therapies in development, SAR445136 (formerly BIVV003) and OTQ923, have presented early data but have been stalled in further development (Table 2). SAR445136 (formerly BIVV003) is an autologous cell therapy in development that uses zinc-finger nucleases to disrupt the *BCL11A* erythroid enhancer (NCT03653247, PRECIZN-1 study). Thus far, 5 patients have been treated with SAR445136 with up to 125 weeks of follow-up.[44,45] HbF fractions increased to 12.2% to 41.2%, and 3 patients sustained a protective level of ≥10 pg HbF/F cell at follow-up without any further VOCs. One patient who did not sustain a HbF/F level ≥10 pg per cell experienced 2 severe VOCs at 9 and 16 months postinfusion. OTQ923 is an autologous CRISPR/Cas9-edited $CD34^+$ cellular product with a targeted disruption of the *HBG1/HBG2* promoters on chromosome 11. As of July 8, 2022, 2 participants had received OTQ923, with follow-ups of 9 months and 3 months, respectively, and HbF levels of 22% and 15.9%, respectively.[46] Further development of OTQ923 has been suspended, and the focus has shifted on developing in vivo editing.[47] EDIT-301 and BEAM-101 are strategies using CRISPR/Cas12a to target the gamma-globin promoter and a base editor to target BCL11A expression, respectively (Table 2). Data from these studies are not currently available.

To date, cumulative data from the ongoing gene editing clinical trials for SCD suggest gene editing of the *BCL11A* erythroid enhancer could be an effective mechanism to induce HbF expression, whereas other studies have demonstrated that the need for sufficient HbF/F-cell induction, regardless of methodology, is important to achieve therapeutic benefit.

The future of gene therapy for SCD

Future iterations of gene therapies for SCD are currently in preclinical development and focus on overcoming the existing barriers of current ex vivo strategies. Critical areas of understanding, improvement, and exploration of gene therapy for

Table 2. Investigational gene-edited autologous cell therapy products for sickle cell disease

Product	Sponsor	Technology	Effect	Activation	Status
CTX001	Vertex Pharmaceuticals Incorporated	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* erythroid enhancer (antisickling)	2018	Submitted BLA spring 2023
BIVV003 (now called SAR445136)	Sangamo Therapeutics	CRISPR/Cas9 editing	ZFN/*BCL11A* erythroid enhancer (antisickling)	2019	Further development discontinued
OTQ923/HIX763	Novartis Pharmaceuticals	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* (antisickling)	2020	Further development discontinued
GPH101	Graphite Bio, Inc.	CRISPR/Cas9 HDR	CRISPR-Cas9/b^S > b^A (nonsickling)	2021	Further development discontinued
EDIT-301	Editas Medicine, Inc.	CRISPR/Cas912a editing	CRISPR-Cas9/HGB1/2 (antisickling)	2021	Active, data not reported
CRISPR_SCD001	UCSF Benioff Children's Hospital Oakland	CRISPR/Cas9 HDR	CRISPR-Cas9/b^S > b^A (nonsickling)	2021	Not yet recruiting
BEAM-101	Beam Therapeutics	Base Editing	Base editing *BCL11A* erythroid enhancer (antisickling)	2022	Not yet recruiting

BLA, biologic license application; HDR, homology-directed repair; ZFN, zinc finger nuclease.

hemoglobinopathies are 3-fold: safe collection of an adequate quantity of long-term HSCs, long-term expression with adequate engraftment of gene-modified cells with minimal toxicity to patients, and safe, efficient, and cost-effective manufacturing techniques enabling equitable access to therapies, including in vivo gene delivery.

Data suggest plerixafor mobilization is safe, efficient, and capable of yielding sufficient HSC quantities in most patients for clinical gene therapy applications,[21-24] although expanded options are urgently needed for those who do not mobilize sufficiently with plerixafor alone, particularly given the need for multiple mobilization cycles to collect sufficient quantities of HSCs for manufacturing.[48] Concerns regarding toxic conditioning, infertility, and secondary malignancy remain significant, leading to the development of multiple reduced toxicity conditioning strategies, largely antibody based.[49-51] Recently, a multiplex base-edited engineered HSC including a therapeutic edit at the gamma-globin promoter and a missense mutation in the extracellular domain of CD117 (cKIT), a receptor tyrosine kinase expressed by hematopoietic stem and progenitor cells (HSPCs) that regulates HSPC survival, proliferation, and differentiation, was reported.[52] Eighty percent of biallelic CD117 editing and near-complete editing of the HbF locus were achieved, and treatment of HSPCs with an anti-CD117 monoclonal antibody in vitro resulted in >85% reduction in viability of unedited HSPCs, while CD117-edited cells remained unaffected. A similar model is being trialed using base editing to produce HbG-Makassar along with a mutation in CD117.[53] Such strategies may enable less toxic pretransplant conditioning for autologous HSC-based SCD therapies and represent a promising potential alternative to busulfan-based myeloablative regimens that may preserve fertility.

Last, the overall projected costs of and equitable access to ex vivo gene therapies have fueled the ongoing preclinical development of in vivo gene therapies,[54] which could prove to be more portable and require less infrastructure, thus expanding access to these critical therapies. Whereas costs for gene therapy are high, gene therapy may be more cost-effective than a lifetime of emerging disease-modifying therapies[55] or more cost-effective than allogeneic transplantation as a result of significantly less costs in the posttransplant period.[56] Additional costs for gene therapy occur largely in the pretransplant and manufacturing stage; therefore, in vivo delivery methods that do not require stem cell mobilization or manufacturing to facilitate delivery are needed. Safe, in vivo delivery of CRISPR/Cas9 is possible for the treatment of human disease,[57] and targeting of CD117 lipid nanoparticles that can deliver RNA to HSCs in vivo is being investigated.[45,58] In vivo HSC prime editing was recently shown to rescue SCD in a mouse model[59] and may represent a simplified and portable strategy for autologous HSC-based SCD gene therapy.

Conclusion

Gene therapy for SCD has the potential to be curative, with preliminary data showing small successes that have improved over time, now with at least 2 cell therapy methods submitted for FDA approval. Early data in both gene addition and gene editing trials for SCD suggest the possibility of a future free from vaso-occlusive events, improvement in quality of life, and clinically meaningful improvements in patient outcomes. Data are limited by the small number of patients treated, a relatively short follow-up period, and concerns regarding long-term safety; however, hope remains for a large population of patients with SCD in need of curative therapeutic options. For the patient presented in this case, transplantation with autologous HSCs modified either by lentiviral vector gene addition or by gene editing to raise HbF is a viable strategy to offer this patient who does not have a matched sibling, whose disease is worsening with age, and who is interested in a curative option. The risks of these therapies should be well explained and well understood for this patient, who must weigh significant risks against the possible benefit of a clinically meaningful cure after autologous gene therapy.

Conflict-of-interest disclosure

Alexis Leonard: no competing financial interests to declare.
John F. Tisdale: no competing financial interests to declare.

Off-label drug use

Alexis Leonard: none to disclose.
John F. Tisdale: none to disclose.

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DOI 10.1182/hematology.2023000487