



Precision medicine approaches in sickle cell disease

by **Srila Gopal MD and
Catriona Jamieson MD, PhD**

Background

Sickle Cell Disease (SCD) is an inherited, life-threatening blood disorder, affecting nearly 100,000 Americans, with greatest incidence in the African American population.¹ The disease is defined by the presence of abnormal hemoglobin that polymerizes under conditions of stress leading to the formation of “sickle” shaped red blood cells (RBCs). Accumulation of these abnormal RBCs causes occlusion of blood vessels and triggers a massive inflammatory response. This leads to acute episodes of severe pain, also referred to as sickle cell pain crises, which may eventually cause end organ damage.² End organ dysfunction in adults with SCD can manifest as stroke, cognitive impairment, pulmonary hypertension, bone necrosis and chronic kidney disease.

With enhanced supportive care options and medical facilities in developed countries, median survival in SCD patients has improved from 20 years in the 1970s to 60 years today.^{3,4} While there are a handful of treatment options for SCD, it continues to be a life-threatening disease with significant disease burden globally. With advances in our understanding of the pathobiology of disease, developments in genetic engineering and molecular biology

techniques and improvements in precision medicine technologies, we are poised to intervene in the disease in a manner not possible previously, with the eventual goal of curing the disease. The accompanying Review article “Unsicling the Sickle: A Review of State of the Art and Emerging Applications of iPSCs and CRISPR/Cas9 for Gene Editing Solutions for Sickle Cell Disease” by Arushi Dogra and Deepak Asudani summarizes the various precision medicine strategies currently under evaluation in this complex and otherwise deadly disorder.

Molecular basis of disease and modifying factors

The defining mutation in SCD is a single base substitution in the first exon of the beta globin gene leading to a single amino acid change. All pathophysiological effects stem from this single genetic defect. However, despite being a monogenic disease, there is considerable heterogeneity in clinical phenotype. Several genetic and environmental factors have been proposed as contributors to this observed heterogeneity in phenotype. Genetic factors that determine clinical severity include fetal hemoglobin (HbF)⁵ levels and co-inheritance

of alpha thalassemia.⁶ Additional studies including genome wide association studies (GWAS) have described various loci of interest, but do not completely explain the clinical heterogeneity observed in the disease.⁷

Current treatment options

Treatment approaches in SCD focus largely on best supportive care and pain management. Hydroxyurea has been shown to decrease complications associated with SCD, with proven efficacy in decreasing vaso occlusive crises, chronic sickle cell pain, and acute chest syndrome. In addition, hydroxyurea also appears to have a survival benefit in SCD.⁸ The US FDA approved hydroxyurea for adult patients with sickle cell disease in 1998 and expanded the label to include children with SCD in 2017. L- Glutamine was shown to decrease acute complications in SCD,⁹ especially when used in combination with hydroxyurea and approved by the FDA in 2017. Despite the incorporation of these therapeutic strategies, however, SCD remains a very challenging disorder that is still difficult to treat and has significant morbidity and mortality risk. In addition to these approved therapies, several agents aimed at targeting cell adhesion, inflammatory pathways, ▶

platelet activation, inhibition of hemoglobin polymerization and sickling are in development - however, none of these strategies offer curative potential.

Offering the promise of “cure” – Gene therapy

Hematopoietic stem cell transplantation can be a potential curative strategy in SCD and showed great promise in initial studies. Applying this in clinical practice, however, has been extremely challenging. Limiting factors include the lack of a suitable donor in the majority of cases and transplant-related complications, thereby precluding its use widely. Gene therapy offers tremendous promise in the management of this complex disorder and has been proposed as a potential curative option for decades.

Being a monogenic disease, SCD is an ideal candidate for gene therapy. There are 3 therapeutic approaches currently under study for gene therapy in SCD, as discussed in the Review. These include gene addition techniques, gene editing techniques, and induction of HbF synthesis. The permanent delivery of an anti-sickling gene into hematopoietic stem cells (HSCs) offers the promise of production of non-sickle normal RBCs, thereby mitigating the numerous complications associated with this disease.

More recent developments in gene editing technologies also offer non-viral forms of genome editing techniques aimed at correcting

the underlying genetic defect associated with the disease. The third approach involves induction of HbF production by targeting BCL11A regulation and forced chromatin looping.

The accompanying Review article provides the history of gene manipulation techniques adopted in sickle cell disease, the challenges and difficulties in incorporating gene therapy and the current approaches aimed at solving some of these critical challenges. In addition, the Review also provides a summary of clinical trials currently evaluating these gene therapy techniques.

Precision medicine techniques involving genome manipulation have the potential to cure and significantly alter the course of sickle cell disease and offers tremendous hope for patients afflicted by this condition. [JCPM](#)

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Unsickling the Sickle:

A Review of State of the Art and Emerging Applications of iPSCs and CRISPR/Cas9 for Gene Editing Solutions for Sickle Cell Disease

by **Arushi Dogra and Deepak Asudani MD, MPH**

I. Introduction

Sickle Cell Disease (SCD) is a group of autosomal, recessive, genetically-inherited disorders of the red blood cells that affect approximately 100,000 Americans and 4.4 million people worldwide.^{1,2} In patients

with SCD, red blood cells (RBCs), when deoxygenated, form insoluble, rigid aggregates that can be observed as the morphologic changes that highlight this condition (**Figure 1**). These sickled blood cells (so-called because of their characteristic “C,” or “sickle” shape)

have lifespans of 10 to 20 days, significantly shorter than the typical 90 to 120 days.^{1,3,4} Furthermore, these cells have a “sticky” nature that leads to occlusions in smaller blood vessels and results in insufficient oxygenation of nearby tissues.

This sickling presents with a constellation of signs and symptoms attributable both to lowered oxygen carrying capacity and mechanical effects. Some of these include acute chest syndrome, chronic hypoxemia, delayed body growth, development of vasocclusive conditions like strokes, pulmonary hypertension, and pain crises or episodes.^{3,5} The management of SCD and associated morbidity has historically relied on symptom management, blood transfusions, chelating agents, pain control and corrective supportive care. The pain crises and mechanical tissue damage due to deformed cells contribute to irreversible organ damage and a lower life expectancy.⁵

While SCD is most commonly diagnosed through routine blood testing after birth and in the first few years of life, its presence can also be discovered during prenatal stages. However, signs and symptoms of the disease are not typically expressed until 5 months of age.¹ The only currently implemented treatment for the disorder is an allogeneic hematopoietic stem cell transplantation of the blood or bone marrow.³ Only a small proportion of patients who receive stem cell transplant, as fewer than 10% of people have a matched donor in

“While SCD is most commonly diagnosed through routine blood testing after birth and in the first few years of life, its presence can also be discovered during prenatal stages”

their family.⁶ Besides complexities with finding suitable match, the transplant itself leads to significant risks and morbidity.

II. SCD as a Gene Editing Target

Fortunately, a better understanding of genetic basis of disease and the rise of newer technologies in stem cell engineering and gene editing is driving several possibilities for permanent SCD treatments. Researchers are currently investigating and developing at least three different gene therapy strategies involving the alteration of the patient’s hematopoietic stem cells. These include:

1. Inserting a sickle-resistant hemoglobin gene into hematopoietic stem cells (a form of gene therapy);^{7,8}
2. Editing the mutated HBB to be a healthy version of the gene;^{7,8} and
3. Gene editing to boost fetal hemoglobin production.^{7,8}

The following sections review the rationale, preclinical testing and advances made so far using these techniques.

II.1 Inserting Sickle-resistant Hemoglobin Gene: Rationale and Pre-clinical Testing

The first approach to treating SCD involves inserting sickling-resistant versions of the hemoglobin gene into induced pluripotent stem cells (iPSCs) using vector-mediated gene transfer.⁷ The initial research in 1988 explored the possibility of utilizing retroviruses, primarily of murine *Moloney leukemia virus*, to create retroviral vectors that could transfer the HBB gene into hematopoietic stem cells. However, these experiments demonstrated less than 1% of successful gene expression.⁹ To improve these results, locus control region ▶



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(LCR) elements were added to the viral vector to enhance gene expression in linked genes at target chromosomal locations.^{9,10} When LCR elements were added, however, site analysis showed that retroviruses tended to integrate around cell 'promoters' and 'oncogenes', essentially leading to vector-mediated insertional mutagenesis. The strong enhancement of the LCR upregulated expression of cancer genes around the targeted sites and contributed towards oncogenesis. Another challenge posed by the use of retroviruses was that only dividing cells could be transduced – that is, when cell membranes were already disrupted.^{7,9}

Research on viral vectors gained momentum with better understanding of the properties of the HIV-1 virus, which could transduce genetic material across membranes, even when cells were not dividing. Based on the structure of HIV-1, HIV-based lentiviruses were successfully tested to be able to deliver alternate versions of the HBB gene into iPSCs, enabling a sustainable solution to the hemoglobin defect.⁹ Removal of all HIV regulatory genes from the vector's plasmid mitigated the concerns about generating replicative lentiviruses.¹⁰ These vectors were then developed into retroviral vectors containing alternative forms of the HBB gene. This altered hemoglobin fell into two main categories: γ -globin-based hemoglobin vectors and modified β -globin-based vectors.⁷

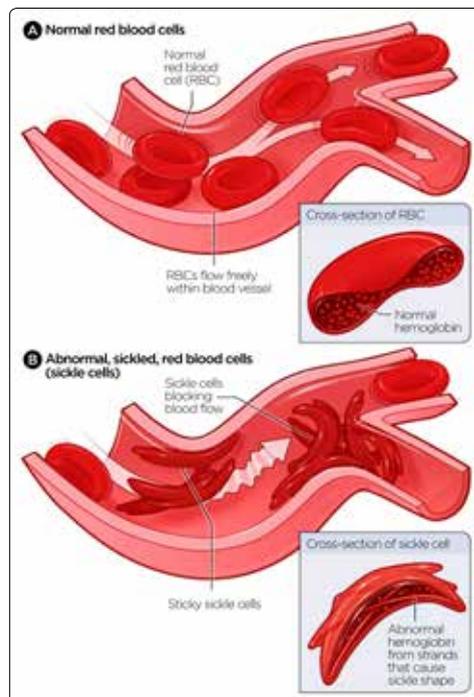
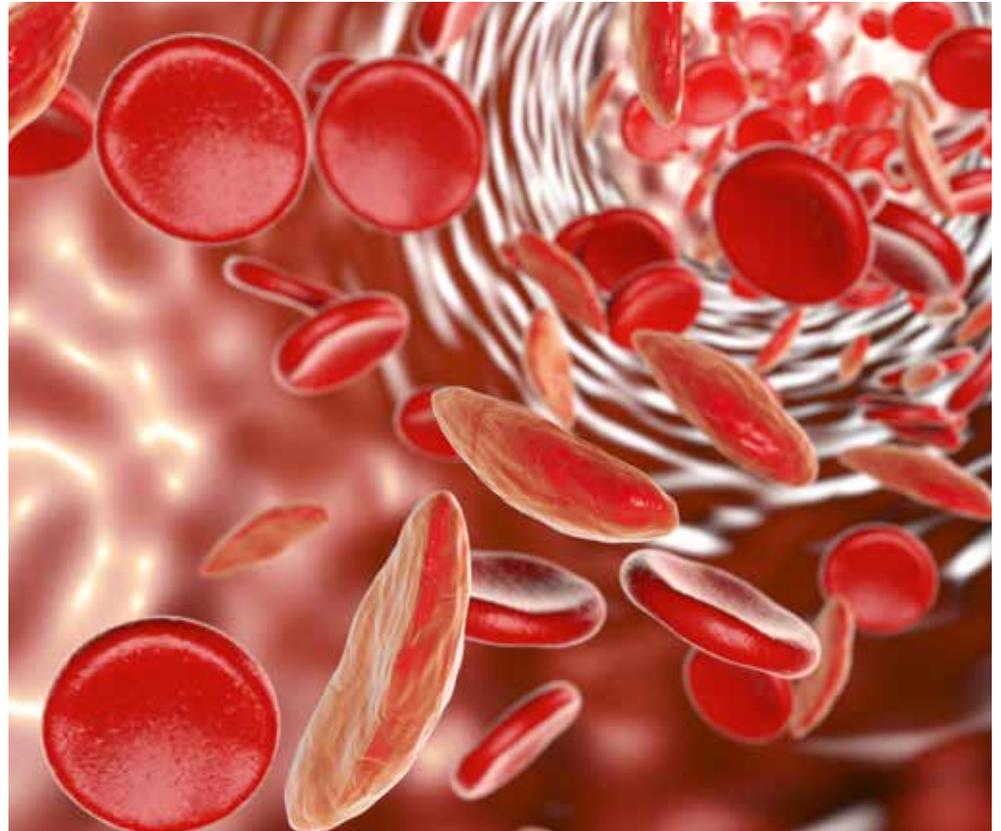


Figure 1: Sickling leads abnormal mechanical changes to red cells and impedes the vascular flow



γ -globin lentiviral vectors were created based off of early observations that fetal hemoglobin had greater anti-sickling properties than adult forms of hemoglobin and the SCD patients with greater amounts of fetal hemoglobin faced less severe forms of the disease.^{7,9} Studies have found that patients with approximately 30% fetal hemoglobin faced little to no manifestations of SCD. Because of this, the addition of a gene encoding the fetal gamma-hemoglobin subunit to hematopoietic stem cells would help treat Sickle Cell Disease.⁹

Effects of γ -globin lentiviral vectors around the turn of the century were often limited by low expression, recombination, and silencing. New research in 2009 and 2010 found that lentiviral vectors carrying γ -globin were more effective when driven by β -globin regulatory elements.^{7,9} These lentiviruses successfully corrected SCD in mice in a 2009 study conducted at University of California, Berkeley.

The other alternate forms of hemoglobin carried by lentiviral vectors were synthetic β -globin variants with substitution mutations that increased their anti-sickling properties. The most effective of these mutations was the T87Q mutation, forming the first HBB alternate, β_{T87Q} .⁷ This form of hemoglobin delivered in lentiviral vectors was found to adequately correct SCD in mice populations.⁹ The second

“The strong enhancement of the LCR upregulated expression of cancer genes around the targeted sites and contributed towards oncogenesis”

modified version of HBB was created to be more effective as a triple-mutant of G16D, E22A, and T87Q and is known as β_{AS3} .⁷ More recent studies have found β_{AS3} -globin to correct SCD in mice as well.

A 2015 study by the Dana-Farber Cancer Institute and the Boston Children's Hospital compared the effects of the γ -globin vector and β_{AS3} -globin vector. The researchers found that both vectors resulted in equivalent anti-sickling and phenotypic correction of red blood cells in SCD cell cultures. This suggests that both methods would be equally viable for clinical trials, which have started to be conducted for this method.

In the next two sections (II.2 and II.3), methods using more recent gene editing technologies to repair and alter the expression levels of hemoglobin will be discussed.

II.2 Correcting the HBB Mutation: Rationale and Pre-clinical Testing

The most direct and most obvious of the three solutions involves correcting the HBB mutation that is understood to be a strong basis of the disease; however, this is also the riskiest of the three options.^{6,8} This approach initially made progress in 2015, when it was tested at Johns Hopkins University, where researchers were able to successfully combine the technologies of CRISPR/Cas9 and iPSCs to correct the single-base substitution in the mutant beta-globin gene of SCD.⁶ After utilizing specific guide RNA CRISPR/Cas9 to correct and deliver the point mutation in iPSCs of SCD patients, the

researchers differentiated the stem cells into human erythrocytes.¹

SCD makes a particularly good candidate for CRISPR-Cas9 gene editing as this is a condition with one of the most well-understood mutations: a single A to T nitrogenous base pair substitution at position 6 in the first exon of the β -globin (HBB) gene, leading to a substitution of glutamic acid (Glu) by valine (Val).⁶

These *in vitro* results were duplicated in experiments at University of California, Los Angeles, where researchers delivered Cas9 systems into iPSCs using lentiviral vectors and were able to produce over 18% successful gene correction in human CD34+ hematopoietic

stem cells, as well as some success with using CRISPR/Cas9 to correct the mutation in bone marrow. The latter experiment led to production of wild-type hemoglobin from the corrected marrow.¹²

This method made another major step forward in 2016, when several researchers from California published their findings about engrafting cells with the HBB mutation corrected into mice and have them produce normal hemoglobin in response.^{7,3}

Still, the gene editing involved in the direct alteration of the HBB gene does hold a great chance of error and degree of complication, as Cas9 must both target and repair the DNA and

Table 1: Brief overview of selected SCD Gene Editing Clinical Trials

Number	Title	Brief Description from ClinicalTrials.gov (abridged)		
NCT02186418*	Gene Transfer for Patients with Sickle Cell Disease	The purpose of this Phase 1/2 study is to determine the efficacy and safety of gamma-globin gene transfer in subjects with sickle cell disease. (Open label)		
NCT02151526*	A Study Evaluating the Efficacy and Safety of LentiGlobin BB305 Drug Product in Beta-Thalassemia Major and Sickle Cell Disease	This is a Phase 1/2, open label, safety, and efficacy study of the administration of LentiGlobin BB305 Drug Product to subjects with either beta-thalassemia major or severe sickle cell disease (SCD).		
NCT03282656*	Gene Transfer for Sickle Cell Disease, a single infusion of autologous bone marrow derived CD34+ HSC cells transduced with lentiviral vector containing a short-hairpin RNA targeting BC11A	The investigators have recently discovered a gene that is very important in the control of fetal hemoglobin expression. Increasing the expression of this gene in sickle cell patients could increase the amount of fetal hemoglobin while simultaneously reducing the amount of sickle hemoglobin in their blood, and therefore potentially cure the condition.		
NCT03745287*	A Safety and Efficacy Study Evaluating CTX001 in Subjects with Severe Sickle Cell Disease	This is a single-arm, open-label, multi-site, single-dose Phase 1/2 study in up to 12 subjects 18 to 35 years of age with severe sickle cell disease (SCD). The study will evaluate the safety and efficacy of autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) using CTX001.		
DR1-01452**	Stem Cell Gene Therapy for Sickle Cell Disease	Objective of this grant is to develop a stem cell gene therapy for the Sickle Cell disease. Team has obtained FDA approval of the IND to start a Phase 1 clinical trial See also: β -globin gene transfer to human bone marrow for sickle cell disease PubMed: 23863630)		
Trial Number	Study - Institute	Title	Arm	Status
NCT02186418*	Cincinnati Children's Hospital Cancer and Blood Diseases Institute/Caribbean Institute for Health Research, University of the West Indies	Gene Transfer for Patients with Sickle Cell Disease	Gene transfer of ARU-1801 drug product, autologous CD34+ hematopoietic stem cells transduced ex-vivo with a gamma-globin lentiviral vector	Phase 1-2 ongoing
NCT02151526*	Imagine Institute at Necker-Enfants Malades Hospital, Paris	A Study Evaluating the Efficacy and Safety of LentiGlobin BB305 Drug Product in Beta-Thalassemia Major and Sickle Cell Disease	LentiGlobin BB305 Drug Product, autologous CD34+ hematopoietic stem cells transduced with lentiviral vector encoding the human beta ^{A1870} -globin gene	Phase 1-2 completed 22 teen or adult patients Phase 3 ongoing
NCT03282656*	Dana-Farber Cancer Institute/Boston Children's Hospital Cancer and Blood Disorders Center	Gene Transfer for Sickle Cell Disease, a single infusion of autologous bone marrow derived CD34+ HSC cells transduced with lentiviral vector containing a short-hairpin RNA targeting BC11A	Single infusion of autologous bone marrow derived CD34+ HSC cells transduced with lentiviral vector containing a short-hairpin RNA targeting BC11A	Phase 1 ongoing 7 patients, ages 3-40
NCT03745287*	Columbia University Medical Center/The Children's Hospital at TriStar Centennial Medical Center/ Sarah Cannon Center for Blood Cancers/Methodist Children's Hospital/Texas Transplant Institute	A Safety and Efficacy Study Evaluating CTX001 in Subjects with Severe Sickle Cell Disease	Single infusion of CTX001, autologous CD34+ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the BCL11A gene	Phase 1-2 ongoing
DR1-01452**	UCLA Center of Regenerative Medicine and Stem Cell Research/California Institute for Regenerative Medicine	β -globin gene transfer to human bone marrow for sickle cell disease	IV administration of autologous HSC, genetically corrected ex vivo by lentiviral vector mediated addition of a hemoglobin gene that blocks sickling	Phase 1-2 ongoing

*citations apply to entire row, see details <https://clinicaltrials.gov/ct2/show/NCTXXXXXXX>

** <https://www.cirm.ca.gov/our-progress/awards/stem-cell-gene-therapy-sickle-cell-disease>

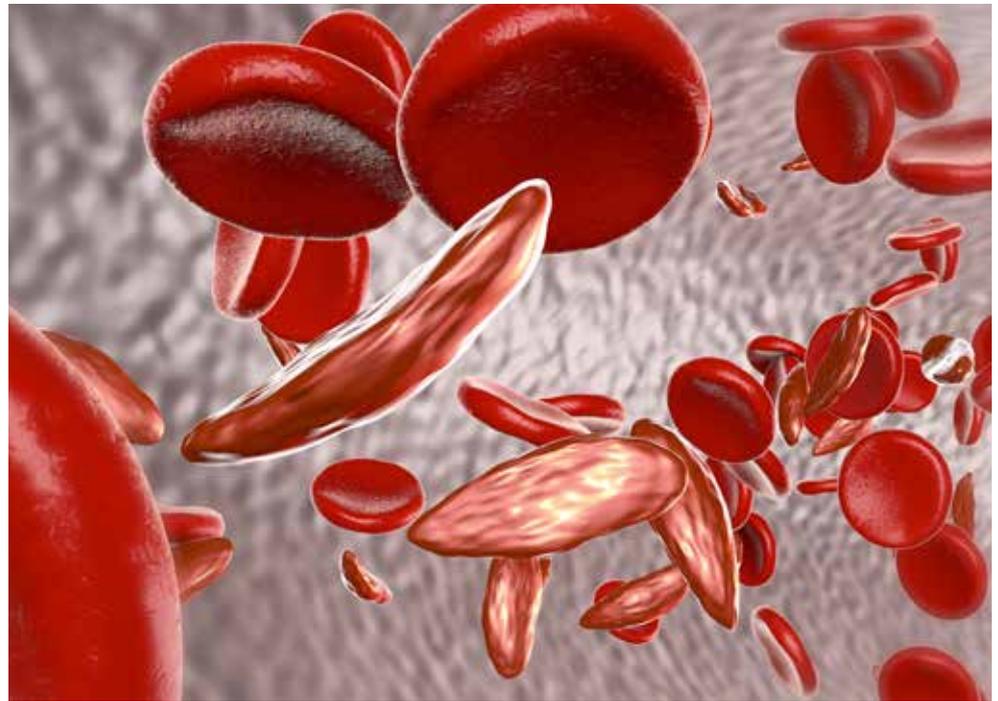
a high amount of precision is needed to edit the single-base mutation. This method also has yet to move into clinical trials and still needs to alleviate concerns, especially in terms of reliability and accuracy.¹²

II.3 Targeting BC11A to Increase Fetal Hemoglobin Production: Rationale and Pre-clinical Testing

Another method that appeals to many research groups involves minimizing SCD disease burden indirectly by altering genes that regulate levels of fetal hemoglobin.⁸ Fetal hemoglobin, or HbF is the form of hemoglobin produced prenatally from around 2 months to 6 months of the gestation period. Prenatal hemoglobin tends to have greater affinity with oxygen compared to the adult hemoglobin. During first few months to a year post-birth, most infants cease to have substantial amount of HbF and generate more of beta-globin hemoglobin. Some people with less severe forms of SCD have mutations that allow them to produce fetal hemoglobin late into life.⁶ In fact, this variation is a key regulator of the severity of SCD in patients, as the presence of fetal hemoglobin represses blood cell sickling.

This characteristic also lends itself to therapeutic approaches which shift the erythropoiesis towards making more of HbF, as commonly practiced with the use of hydroxyurea. From an applied genomics perspective, however, this observation makes it attractive to attempt to increase fetal hemoglobin levels in SCD patients by genetic methods.⁸

This fetal hemoglobin approach may result in potentially lower risk of making gene-modified cells due to simpler requirements to edit genes. Rather than transporting an improved



gene into the cell as a plasmid via viruses or guide DNA, this method needs the delivered gene-editing enzyme to make an incision in

“Another method that appeals to many research groups involves minimizing SCD disease burden indirectly by altering genes that regulate levels of fetal hemoglobin”

the DNA, thereby allowing the endogenous DNA repair mechanisms of the cell to rejoin the strands.¹⁴ With this approach, mutations in DNA are introduced in the cell and, as a consequence, an error prone reparative mechanism is set up. With decreased BCL11A expression in this milieu, the HbF repressive function is suppressed leading to an increased proportion of fetal hemoglobin.^{14,15}

This approach was utilized by a group of collaborative researchers from the Dana-Farber Cancer Institute and Boston Children’s Cancer and Blood Disorders Center. These researchers reported success with reducing sickled blood cells in a mouse model by suppressing the BCL11A gene with CRISPR/Cas9 systems delivered by lentiviral vectors. However, in this process, they discovered that BCL11A also plays an important role in allowing hematopoietic stem cells to engraft in bone marrow. Without BCL11A, the blood cells of the mice are eventually depleted, which not only rendered the therapy ineffective but led to issues with hematopoiesis as well. In order to circumvent this issue, the researchers created a gene therapy virus that repressed BCL11A expression solely in precursors of red blood cells by attaching it to a promoter of beta hemoglobin and regulatory elements that are only active in these cells. This selective engineering allowed the cells to successfully both engraft and reduce symptoms of SCD.¹⁵

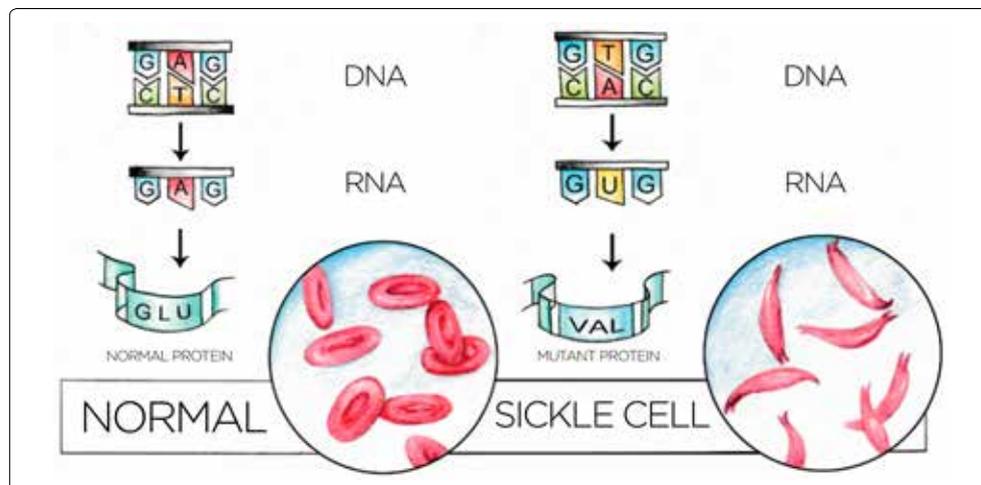


Figure 2: HBB showing normal and genetic mutation at position 6, with mutant protein and associated sickling

has received attention in commercial world. For instance, two different CRISPR-focused start-up companies, CRISPR Therapeutics and Editas Medicine, have presented early data. CRISPR Therapeutics has been exploring the role of five different gene families in relation to fetal hemoglobin production, one of which is BCL11A. At the 2017 European Hematology Association Annual Congress, in Madrid, Spain, they reported an 80% success rate in editing a target gene in mice (see, Re-creating hereditary persistence of fetal hemoglobin (HPFH) with CRISPR/Cas9 to treat sickle cell disease and beta-thalassemia). CRISPR Therapeutics has also recently begun to conduct clinical trials with this method in collaboration with Vertex Pharmaceuticals Incorporated. Meanwhile, Editas Medicine presented on altered cells that produced 25% greater fetal hemoglobin and were successfully implanted in mice⁶, as well (see, CRISPR-mediated Editing of Hematopoietic Stem Cells for the Treatment of β -Hemoglobinopathies, ASGCT, May 11, 2017).

III. Clinical Trials

Newer technologies and a better appreciation of applied genomics has led to an extensive research ecosystem, one that is primarily directed towards diseases caused by single-nucleotide mutations. A quick search on ClinicalTrials.gov (<https://clinicaltrials.gov/ct2/home>) for related terms (e.g., “CRISPR” or “gene transfer” OR “gene therapy” OR “gene editing”) discloses many trials are underway across a spectrum of conditions or diseases.

For sickle cell disease, interest is in the single-base mutation occurs in the sixth amino acid resulting in a valine, rather than a glutamate residue. Several SCD trials are underway with a noteworthy engagement of both public and

corporate entities. We include a selected list of clinical trials of genome editing candidate therapies for SCD in **Table 1**; brief summaries are provided for those interested in further reading and research. Although by no means exhaustive, these trials represent a cross section of approaches. Safety and efficacy of these treatments will be of great interest to develop future therapies, however, we also foresee that results from these trials will be reviewed for opportunities to optimize gene editing technologies for the next generation of therapies in relevant therapeutic areas.

IV. Conclusions

As we learn more from current and future trials, therapies will emerge for the safe and effective treatments of patients with SCD. All three methods (inserting a sickling-resistant hemoglobin gene, correcting the hemoglobin mutation, and stimulating the production of fetal hemoglobin) have demonstrated progress as seen by encouraging peripheral smears, favorable clinical responses, and abated transfusion dependency among several patients in the clinical trials. In addition, since these new methods of treating SCD are autologous (utilizing a patient’s own hematopoietic stem cells from iPSCs), patients would avoid the risks due to blood or bone marrow transplants, thereby improving the quality of life for patients. Furthermore, newer technologies, such as CRISPR/Cas9, allow researchers to manipulate the genetic scripts of these cells more effectively, altering hemoglobin production in early stages of stem cell development. Overall, these encouraging results and scientific advancements push us to envision a future with well-established, potentially permanent, and widely available curative options for Sickle Cell Disease. [JOPM](#)



Arushi Dogra is an intern at University of California, San Diego Health’s Moores Cancer Center and engaged in testing chemical oncological treatments on human tissue. As a UCSD Research Scholar, she has investigated the effects of ER stress and aging on yeast populations, and, at the Simons Research Fellowship at Stony Brook University, she has studied the role of the

complement system in Systemic Lupus Erythematosus. She has presented her research on Mantle Cell Lymphoma at the California Science and Engineering Fair. She also attends Del Norte High School and is interested in a career in medicinal research in oncology, immunology, and genomics. Passionate about sharing her enthusiasm for scientific research with others, she started her district’s first iGEM synthetic biology research team and presented that research at the Hynes Convention Center, Boston. Arushi is a Gold Medalist in the British Biology Olympiad. She is also the President of DNHS’ HOSA-Future Health Professionals chapter.



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