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Mini Review

CRISPR9 SCD Gene Therapy

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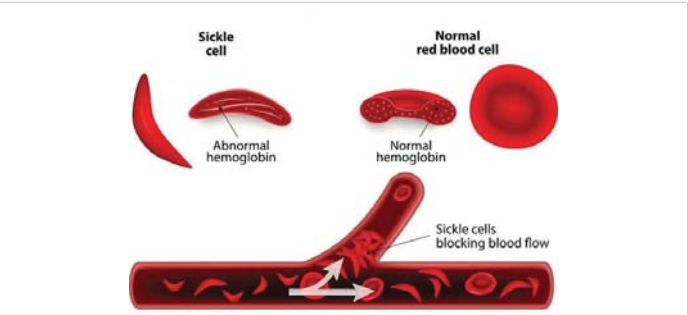
Introduction

The most prevalent monogenic blood illness is sickle cell disease (SCD), which affects millions of people globally and around 100,000 Americans. A single nucleotide mutation in the -globin gene results in the replacement of a hydrophobic valine for a hydrophilic glutamic acid at the sixth position, which results in SCD (HBB). Under hypoxic or acidic conditions, the resultant hemoglobin S (HbS) polymerizes, resulting in rigid, sickle-shaped RBCs with reduced deformability and shorter lifespans. Patients with SCD who have RBC damage experience extreme pain, end-organ destruction, chronic hemolysis, hemolytic anemia, and early death.

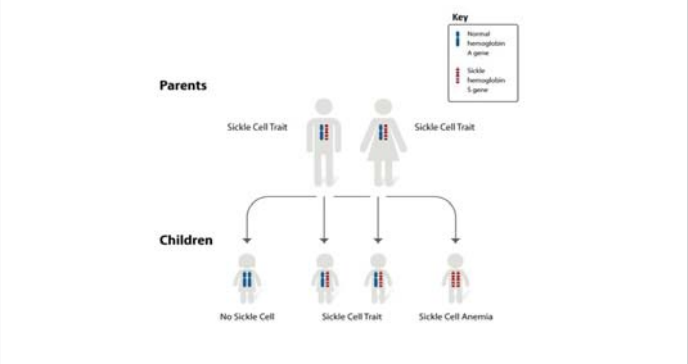
The average lifetime of individuals with SCD has not grown over the past few decades, although SCD was the first molecular disease with a discovered genetic basis more than 60 years ago. Only four drugs have been licensed by the FDA to treat acute complications: hydroxyurea, which was first used in 1998; l-glutamine; and crizanlizumab-tmca, which was first used in 2019 and voxelotor, which received its initial approval in 2020. The only therapeutic option for SCD patients is a hematopoietic stem cell transplant (HSCT), which is usually obtained from a related donor who is matched to the patient. However, only about 15% of SCD patients are suitable for HSCTs. When matched but unrelated donors or haploidentical donors are employed, HSCT-related morbidity and mortality are markedly enhanced. Additionally, there are significant risks and consequences associated with the treatment, making it inappropriate for widespread use without modifications to current regimens.

Autologous gene therapy, in which a patient's own cells are utilized to add a copy of the "healthy" gene, fix a faulty gene, or mute genes, does not require a donor who is compatible with the patient. Without the risk of graft-versus-host disease, ex vivo engineering of autologous hematopoietic stem and progenitor cells (HSPCs) and subsequent transplantation of genetically altered cells could offer patients a lasting treatment. Because sickle RBCs mature inefficiently and live shorter lives than healthy RBCs, gene-corrected HSPCs seem to have an advantage over SCD HSPCs in vivo. With as low as 2% - 5% donor chimerism, post-allogeneic transplantation can relieve SCD-related symptoms in SCD patients, supporting the use of gene therapy. This means that a tiny number of HSCs could successfully add or correct genes, resulting in a degree of RBC chimerism in the peripheral blood that is clinically significant. However, both preclinical and clinical investigations have shown that gene therapy for SCD using a lentiviral vector is successful. With the insertion of an antisickling HBB into autologous HSCs through lentiviral vector, the first patient with SCD was successfully treated, and 15 months later, there was a significant amount of therapeutic antisickling-globin. LentiGlobin, a self-inactivating (SIN) lentiviral vector containing the human anti-sickling HBB, is currently the subject of clinical research. Preclinical and clinical studies have demonstrated the efficacy of lentiviral vector-based gene therapy for SCD. The first patient with SCD was effectively treated after having an antisickling HBB inserted into autologous HSCs using a lentiviral vector, and 15 months later, there was a sizable amount of therapeutic antisickling-globin. Clinical studies are now being conducted on LentiGlobin, a self-inactivating (SIN) lentiviral vector expressing the human anti-sickling HB.



Genetic cause of sickle cell anemia

Sickle cell anemia is caused by a single base change in the DNA structure of the ß-globin gene (HBB). Position 6 of the resultant amino acid sequence is occupied by glutamic acid (GAG) in healthy people, while valine (GTG) replaces glutamic acid in individuals with sickle cell anemia. This mutation results in the production of hemoglobin S, the form associated with the disease.



Sickle cell anemia inheritance

Sickle cell anemia is a monogenic, autosomal recessive disease. Monogenic indicates that it is caused by a mutation in just one gene, autosomal means that the gene is not on a sex chromosome, and recessive means that a person only develops the disease if the mutation is passed down from both parents.

Inheritance of only one copy results in sickle cell trait, which typically has no symptoms but increases the risk of passing the mutation on to future generations. Sickle cell anemia is sometimes referred to as a Mendelian disease because of how it is passed along. It's widely used to explain Mendelian heredity and genetics as a result. Particularly those of African American ancestry are disproportionately impacted by the illness.

CRISPR/Cas9 CRISPR/Cas9 edits genes by accurately slicing DNA and letting the body›s own DNA repair mechanisms take control. The guide RNA and the Cas9 enzyme are the two components of the mechanism.

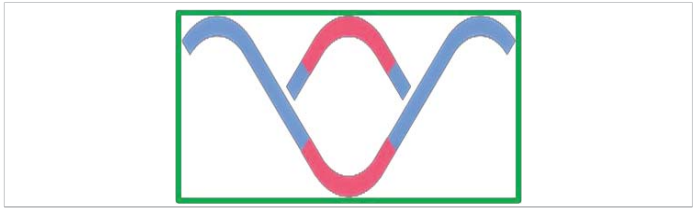
Dictionary of CRISPR: CRISPR is a bacterial antiviral defense mechanism used by some species, consisting of clustered, regularly interspersed, short palindromic repeats. Dr. Emmanuelle Charpentier, our co-founder, was one of the researchers who figured out how to use this system as a tool for altering genes Jinek, et al. Cas9: (Science, 2012) a CRISPR-associated Cas endonuclease or enzyme that acts as «molecular scissors» to cut DNA at a place designated by a guide RNA. The majority of organisms use ribonucleic acid (RNA), a molecule that provides the « instructions essential for life» and is used to create and store genetic material:

A DNA-related molecule employed for multiple functions, including transporting and reading DNA instructions.» A type of RNA molecule known as a guide RNA (gRNA) attaches to Cas9 and instructs Cas9 where to cut DNA based on the gRNA›s sequence [1-4].

With CRISPR/Cas9, three different types of genomic alterations can be made.

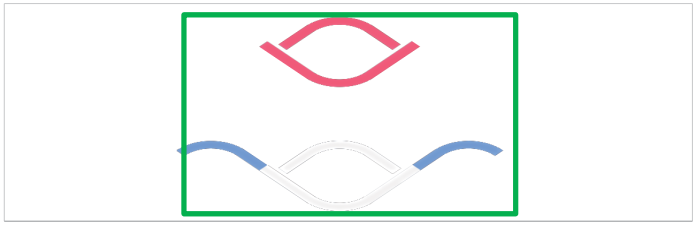
Disrupt

Non-homologous end joining, which can add or remove base pairs with a single cut, can alter the original DNA sequence and result in gene inactivation.



Delete

A bigger DNA fragment can be deleted by using two guide RNAs that each target a different location. After cleavage at each site, non-homologous end joining binds the two ends while erasing the previous sequence.



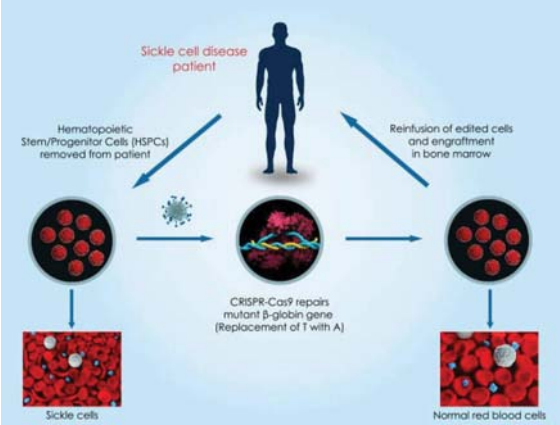
Correct or insert

By pairing the CRISPR/Cas9 system with a DNA template, the cell can correct a gene or even insert a new gene using a process known as homology directed repair.

Sickle cell gene therapy using CRISPR

Because sickle cell disease is brought on by a genetic mutation, it is a prime target for CRISPR-mediated gene therapy. Gene-edited cell therapy, an ex vivo treatment that uses CRISPR to treat sickle cell anemia, involves removing

the patient's hematopoietic stem cells, modifying them, and then replacing them.



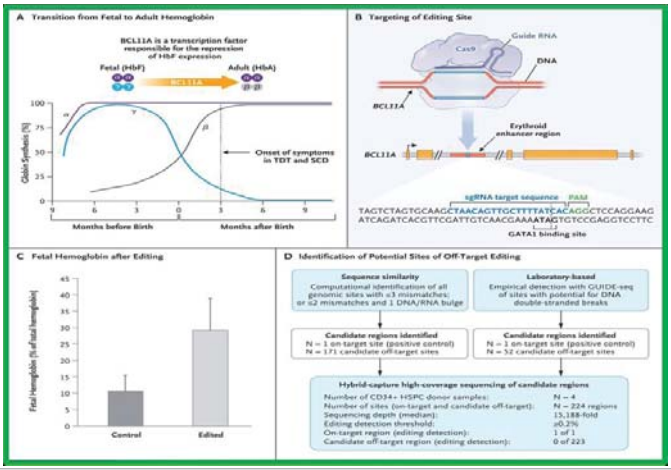
Repairing the adult hemoglobin gene mutation that causes sickle cell disease and causing the creation of the healthy, normal adult hemoglobin is one of the key elements of CRISPR sickle cell gene therapy (hemoglobin S).

Then, using homology-directed repair, the gene can be modified at the breakpoint (HDR). When the cell employs HDR to repair the DNA break, a donor template with the normal gene sequence is supplied to rectify the mutation. This process is known as a gene knock-in. The modified cells are then reintroduced into the patient›s bloodstream to create normal hemoglobin

The most prevalent monogenic disorders in the world, transfusion-dependent thalassemia (TDT) and sickle cell disease (SCD), respectively, afflict 60,000 and 300,000 people annually.

Respectively: Mutations in the gene that produces the hemoglobin component cause both illnesses (HBB). The TDT-related mutations in HBB lead to an imbalance between the and globin (e.g., and) chains of hemoglobin, which impairs erythropoiesis. These mutations either decrease (+) or eliminate (0) the synthesis of -globin. A point mutation in HBB that changes glutamic acid to valine at position 6 of the amino acid chain results in sickle cell anemia or sickle hemoglobin. Deformation of erythrocytes, hemolysis, anemia, uncomfortable Vaso-occlusive events, irreversible end-organ damage, and a reduced life span are all consequences of polymerization of deoxygenated sickle hemoglobin.

For individuals with TDT, the main therapy options are transfusion and iron chelation, while for those with SCD, the main therapeutic options are pain management, transfusion, and hydroxyurea. Although luspatercept and crizanlizumab, two recently approved medications, have decreased the incidence of Vaso-occlusive events in SCD patients and the necessity for blood transfusions in TDT patients, neither one of these treatments effectively tackles the fundamental etiology of the disease. TDT and SCD can potentially be cured by allogeneic bone marrow transplantation, although less than 20% of eligible patients have a donor who matches their human leukocyte antigen. For the treatment of TDT patients with non-0 mutations who lack a compatible sibling donor, the European Union has granted approval for the gene-addition product betibeglogene autotemcel. Additionally, patients with SCD and 0/0 TDT are being examined. Additionally, it has been shown that reactivating the -globin gene can be accomplished via a short hairpin RNA molecule tailored to microRNAs and encoded by lentiviral vectors. The clinical development of this drug is still in its early phases.



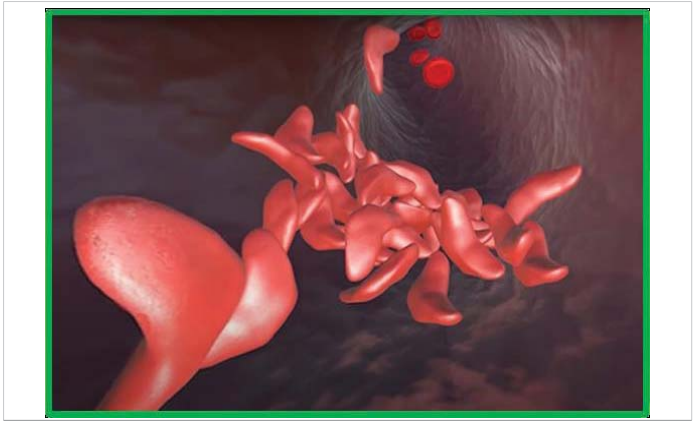
In individuals with TDT and SCD, fetal hemoglobin with two alpha and two gamma chains has been associated with lower mortality and morbidity. Due to the developmentally controlled generation of fetal hemoglobin, postnatally produced levels of -globin decline while those of adult hemoglobin (which is made up of two alpha and two beta chains) increase. The symptoms of TDT or SCD often appear in newborns and young children during the first year of life, when fetal hemoglobin synthesis declines but fetal hemoglobin levels remain high. Patients with TDT or SCD who also carry fetal hemoglobin that persists into adulthood due to inherited persistence have little or no symptoms.

Single-nucleotide polymorphisms (SNPs) have been connected to adult production of fetal hemoglobin in genome-wide association studies. Some of these BCL11A locus SNPs on chromosome 2 are connected to a decreased severity of TDT and SCD. Beta-globin and fetal hemoglobin cannot be produced by erythroid cells due to the zinc-finger transcription factor BCL11A; erythroid-specific enhancers include the SNPs linked to fetal hemoglobin. These SNPs upregulate fetal hemoglobin expression while decreasing BCL11A expression.

It is feasible to programmatically target insertions or deletions (indels) at a specific genomic DNA spot using the CRISPR-Cas9 nuclease system, a bacterial immune system that can cut plasmid or bacteriophage DNA. We used CRISPR-Cas9 gene editing methods in hematopoietic stem

and progenitor cells (HSPCs) targeting the erythroid-specific enhancer region of BCL11A to inhibit BCL11A expression in erythroid-lineage cells, restart globin synthesis, and mimic the phenotype of hereditary persistence of fetal hemoglobin.

Future CRISPR therapeutics IGI researchers are aiming to make the existing CRISPR therapy better so that, someday, the sickle cell mutation can be fixed within the body without extracting stem cells or destroying the bone marrow, as are being done by UC physicians. The infused, corrected stem cells can multiply and replenish themselves. However, destroying the bone marrow weakens the immune system and increases the danger of infection or even cancer for patients. This is due to the fact that white blood cells, which fight disease, are also produced by the bone marrow.



Wilson is optimistic that he and the IGI team will be able to use antibodies to target the CRISPR enzyme to the proper stem cells in order to deliver the CRISPR therapy directly to the bone marrow of the patient. Other researchers have sought to introduce the CRISPR enzyme into the body using modified viruses or fatty droplets, sometimes known as lipid nanoparticles, without success.

The chemical we are attempting to transport is physically smaller — an eighth of the diameter of the nanoparticles that other researchers are attempting to get to the bone marrow — and "This could give considerable benefits," he claimed. Our self-delivering enzyme ought to be able to go to the bone marrow.

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