Current Sickle Cell Disease Gene Therapy Treatments Literature Review

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ABSTRACT

Sickle cell disease (SCD) consists of haemoglobin-mutation related blood disorders caused by mutations of the *HBB* gene. Current treatments for SCD are symptom-based or preventive treatments. The only curative treatment for SCD- an allogeneic hematopoietic stem cell transplant- is inaccessible to the majority of SCD patients. The transplant required a donor graft is unavailable for most individuals. Currently, research in gene therapy treatment for SCD attempts to provide long-lasting treatments in two distinct techniques. The first technique is to change the mutation-containing genotype to produce a normal or functional haemoglobin protein. The second technique is to bypass the production of the mutated adult haemoglobin and product fetal haemoglobin instead. This literature review compares three gene-editing methods; Lentiviral Vectors, CRISPR/Cas9, and Base Editors. A review of previously published research papers was conducted and compared over a 2 month period during a summer student research program to determine the progression of each of the three gene-editing methods in the two techniques of SCD gene therapy treatment. While all three were successful in both genotype correction and fetal haemoglobin induction, only the Lentiviral Vector and CRISPR/Cas9 treatments for fetal haemoglobin induction have published data on human trials. However, the Base Editor shows promise in its ability to surpass many issues faced with both viral vectors and CRISPR/Cas9 such as off-target DNA breaks. Progression in SCD gene therapy can provide a treatment option for all affected individuals and can even provide a basis for gene therapy for other blood disorders.

Introduction

Sickle cell disease (SCD) is a term used to describe a multitude of inherited blood disorders that annually affects approximately 312,000 newborn children globally.[1] It is caused by the substitution of an amino acid in the *HBB* gene on chromosome 11 , which codes for the β-globin subunit of haemoglobin [2]. Depending on the type of genotype, the severity of SCD can differ. Other blood disorders caused by a mutation of the *HBB* gene such as β-thalassemia, present similar to SCD.

With access to symptom-based treatments and curative treatments for some, many adults with SCD in high-income countries survive past their sixties. Although there is limited data available on mortality rates of SCD in sub-Saharan Africa and India (which have a high prevalence of SCD births), one study reports that early-life mortality of

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[1] Kato, Gregory J., Frédéric B. Piel, Clarice D. Reid, Marilyn H. Gaston, Kwaku Ohene-Frempong, Lakshmanan Krishnamurti, and Wally R. Smith et al. 2018. "Sickle Cell Disease". Nature Reviews Disease Primers 4 (1). doi:10.1038/nrdp.2018.10.

[2] Kato et al. 2018

sickle cell anemia in Africa is 50%-90%.[3]

While short-term treatments for SCD provide temporary pain relief from specific symptoms, their long-term side effects, expensive sessions, and time-consuming treatment plans prove to be difficult for many patients. The only curative treatment for SCD; allogeneic hematopoietic stem cell (blood and bone marrow) transplant (HSCT), is inaccessible for the majority of patients due to the rare donor grafts it requires to be effective. Prospects in SCD treatments lie in gene therapy methods that edit the *HBB* substitution mutation in HSCs and allow for autologous HSCTs without donor grafts. The purpose of this review of gene therapy methods is to investigate the current developments in gene editing of *HBB* mutations and whether an optimal treatment currently exists to replace allogeneic HSCTs.

Pathophysiology

Haemoglobin Structure

Haemoglobin (Hb) is a heterotetrameric protein that enables red blood cells (erythrocytes) to transport oxygen. It has two pairs of identical subunit chains that vary depending on the type of haemoglobin. The *HBB* mutation affects the β-globin chain in Hb. Hb molecules are found in both developing blood cells (reticulocytes) and mature red blood cells (erythrocytes) in the bone marrow and bloodstream respectively. Hb plays a critical role in the body's major functions as it is the component of erythrocytes that enables them to transport oxygen from the lungs to the body's peripheral tissues. Each subunit chain has one iron molecule in its reduced iron II (Fe^{2+}) state as its heme group. The Fe^{2+} heme group is what allows one haemoglobin protein to transport four oxygen molecules simultaneously. A single O_2 molecule binds to the Fe^{2+} ion through a synthesis reaction.

Genetics of Sickle Cell Disease

SCD is caused by a substitution mutation of a single amino acid in either one or both of the β-globin subunits of the HbA molecule. Depending on the mutation, the SCD genotype and its severity vary. The most common SCD mutation is the $β^S$ allele. In the $β^S$ allele, the codon for the sixth amino acid position in the β-globin chain changes from GAA (Glu) to GTA (Val) in one or both β-globin subunit chains. The mutation is caused by a single adenine-thymine base pair inversion. In sickle cell anaemia (SCA), sickle Hb or HbS is formed when the individual is homozygous for the $β^S$ allele. Sickle cell trait (SCT) occurs in individuals who are heterozygous for the $β^S$ allele, forming HbAS. Since SCD is a recessive trait, heterozygous individuals are only carriers of the HbS mutation. The presence of the $β^S$ allele can also cause another type of blood disorder called β-thalassemia. In HbS$β^0$ thalassemia, the $β^S$ allele is paired with Hbβ^0 or non-functional HBB.

Sickled Cell Structure and Mechanism (HbS)

The change in the sixth amino acid from Glu and Val (Glu6Val) is significant as the two amino acids have very different properties. Glu is a negatively charged acidic residue while Val is hydrophobic. The Glu6Val mutation creates a hydrophobic site on the outside of the HbS β-globin chain is formed.This mutated haemoglobin forms long HbS polymers within the red blood cells and has a deformed 'sickle' shape when deoxygenated. Many factors normally involved in respiration exacerbate polymer growth such as HbS and 2,3-diphosphoglycerate concentration within

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[3] Grosse, Scott D., Isaac Odame, Hani K. Atrash, Djesika D. Amendah, Frédéric B. Piel, and Thomas N. Williams. 2011. "Sickle Cell Disease In Africa". American Journal Of Preventive Medicine 41 (6): S398-S405. doi:10.1016/j.amepre.2011.09.013.

cells, the partial pressure of oxygen, temperature, and pH. The low oxygen affinity of sickled erythrocytes also promotes the formation of polymers which in turn further decreases oxygen affinity.

Symptoms

The abnormal nature of sickled cells results in a variety of complications including chronic pain, problems with their internal organs, increased susceptibility to pathogens, and anaemia.

Sickled cells often block the microcirculation in capillaries with their stiff and C-shaped sticky structure, which leads to a lack of blood flow to vital organs. A vaso-occlusive crisis is when sickled cells build up in the venous pole of capillaries, blocking circulation to vital organ systems, and consequently causing necrosis of tissue and bones. Vaso-occlusive crisis and osteonecrosis is the most common cause of hospitalization in SCA patients.

Many SCD individuals experience painful swollen joints, chronic back pain and spinal issues. Another major issue caused by the buildup of sickled cells is acute chest syndrome (ACS). ACS can refer to a multitude of conditions affecting the pulmonary system such as intrapulmonary infarctions resulting in necrosis, hypoxia, pulmonary infiltrate, and severe asthma. The blockage of blood vessels by sickled cells can cause a multitude of other conditions including girdle syndrome and priapism. Strokes and silent cerebral infarctions (SCI) in pediatric patients are primarily caused by SCD. The chance of developing a stroke in SCD patients is the highest from birth to 10 years. Many complications caused by SCD involve the spleen such as a splenic sequestration crisis (enlarged spleen) and a hepatic sequestration crisis. In SCD, sickled cells can get caught vessels leading to and from the spleen due to excessive hemolysis.[4]

Current Treatments

There are a range of treatment methods for SCD that tackle various aspects of the disease and its consequent patient outcomes. Advanced screening at a young age coupled with increased parental education and early preventative medication has proven to decrease mortality in young SCD patients in certain countries. A universal screening program implemented in the United Kingdom detected 300 newborns with SCD and 17 000 carriers per year since 2001.[5] A study on families who had a child with SCD in Jamaica revealed that teaching parents to palpate the abdomen for abnormalities reduced mortality due to splenic crisis by 90%.[6] Consent monitoring, as well as proactive vaccination of SCD patients at a young age is a vital component of the treatment plan.

Hydroxycarbamide is an agent that has been shown to reduce pain and ACS in randomised control trials of various age groups with low long-term toxicity. Although effective, barriers remain in the confidence of both prescribers and SCD patients due to a lack of certainty in case-by-case toxicity and efficacy. Chronic blood transfusion therapy is another repeated treatment that can be used to reduce pain and ACS. Blood transfusion can be used alongside hydroxycarbamide or as a primary treatment. However, constant transfusions can result in an overload of iron, organ damage, infections from transfusions, thrombosis, sepsis and social isolation. Due to their higher cost, chronic transfusions are saved primarily for cases where an oncoming stroke, renal failure or continuous painful episodes are present. The only cure currently available for SCD is an allogeneic HSCT. Allogeneic HSCT has proven to successfully provide a cure for SCD in 85-90% of severe pediatric cases treated. The treatment is limited by the requirement for human leukocyte antigen (HLA)-matched related donor (MRD) grafts following a rigorous myeloablative regimen.

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[4] Chakravorty, Subarna, and Thomas N Williams. 2014. "Sickle Cell Disease: A Neglected Chronic Disease Of Increasing Global Health Importance". Archives Of Disease In Childhood 100 (1): 48-53. doi:10.1136/archdischild-2013-303773.

[5] Chakravorty et al. 2014

[6] Emond, A.M., R. Collis, D. Darvill, D.R. Higgs, G.H. Maude, and G.R. Serjeant. 1985. "Acute Splenic Sequestration In Homozygous Sickle Cell Disease: Natural History And Management". The Journal Of Pediatrics 107 (2): 201-206. doi:10.1016/s0022-3476(85)80125-6.

Over 85% of HSCT SCD patients are unable to access MRD grafts. Long-term studies on organ function, toxicity and efficacy are still required to definitively consider allogeneic HSCT as a curative treatment for patients with severe SCD.[7]

Gene Therapy Techniques

Gene therapy techniques work to alter the DNA of select cells in order to achieve a desired phenotype. There are three prominent gene therapy methods; lentiviral vectors, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9, and base editors, that are prominent in curative treatments for blood disorders caused by *HBB* mutations. All three have shown promise in editing HSCs to allow for autologous HSCTs (haematopoietic stem cell transplants) for both SCD and β-thalassemia patients. Current gene therapy in SCD works to either correct the mutation and achieve a normal phenotype or bypass the production of sickled Hb by inhibiting the *BCL11A* transcription factor. Gene therapy forgoes the need for an HLA-MRD graft and allows curative treatments to become accessible to the majority of individuals with SCD.

Viral Vectors

Viral vector-mediated gene therapy utilizes the natural behaviour of certain viruses to insert a desired genome into cells. This process is called transduction. Once in the cytosol of the cell, viral RNA containing the desired gene is transcribed into DNA by a reverse transcriptase. The desired gene is then integrated into the host cell's genome, and is replicated as a part of the genome. The choice in the species of virus used as a vector depends on different factors such as their capacity, size, safety, toxicity and production. There are multiple viruses currently being tested as viral vectors to deliver genetic material for gene therapy including adenoviruses, retroviruses, poxvirus, adeno-associated viruses, baculoviruses, and herpes simplex viruses.

Lentiviral Vector

Lentiviruses are a type of retrovirus whose genomes are organized in the gag, pol, and env regions. Gag encodes structural proteins, pol encodes enzymes for the ssRNA, and env encodes the viral envelope. One example of a lentivirus commonly used in gene therapy is the human immunodeficiency virus (HIV). Lentiviral vectors have become increasingly important in gene therapy because they can be transduced into quiescent (non-dividing) cells such as adult stem cells. Lentiviruses such as HIV are modified to remove their viral genes and insert the desired genes. Specific components of the virus are changed to maintain infectious efficacy while maintaining safety. Lentiviral vectors are created through the co-transfection of the three plasmids from the human embryonic kidney (HEK) 293T cells. When modifying HIV, new HIV viral vectors have been engineered without their four accessory genes, making them safer to use.[8]

CRISPR/Cas9

Structure and Method

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[7] Chakravorty et al. 2014

[8] Escors, David, and Karine Breckpot. 2010. "Lentiviral Vectors In Gene Therapy: Their Current Status And Future Potential". Archivum Immunologiae Et Therapiae Experimentalis 58 (2): 107-119. doi:10.1007/s00005-010-0063-4.

CRISPR/Cas9 systems are composed of two components, the CRISPR locus and the Cas9 enzyme. There are three types of CRISPR systems (I, II, III). Type II has proven to be the most efficient in gene editing. Type II CRISPR contains the Cas9 nuclease, which enables the CRISPR/Cas9 system to be used in a variety of different gene-editing scenarios. CRISPR locus are repeated sequences on the DNA of bacteria. The repeated DNA sequences are spaced out by protospacers. Within the targeted DNA sequence, each protospacer has an associated PAM. Protospacer adjacent motif (PAM) is a short DNA sequence that aids in sequence recognition. It is made up of 2-6 base pairs that follow the sequence after the targeted DNA sequence. The PAM helps the Cas9 determine where to bind on the DNA and aids the CRISPR in recognizing and distinguishing target DNA.Cas9 is an enzyme that catalyzes the cleavage at specific sites on DNA; once bound to the DNA, a double-stranded cut can be made and a new DNA sequence can be inserted. Cas9 consists of the crRNA (CRISPR RNA) and trans-activating crRNA (tracrRNA). Together crRNA and tracrRNA act as guide RNA (gRNA) that help the CRISPR/Cas9 system bind to the target DNA. crRNA is a shorter RNA sequence that directs the CRISPR/Cas9 complex to the specific DNA site and binds to the complementary bases. It has 20 nucleotide guide sequences that bind to 20 bases on the DNA. tracrRNA is made up of a longer, repeating sequence of bases that forms a stem loop and acts as a handle for the systems to be guided to the correct position.[9]

Cas9 Mutations

The Cas9 enzyme can be mutated by non-homologous end joining (NHEJ) or homology-directed repair (HDR). The Cas9 nickase mutant (Cas9n) creates single stranded DNA breaks that are repaired via HDR. Cas9n has the potential to increase the accuracy of DNA breaks and prevent off-site breaks. The Cas9 system is easier to customize than other gene-editing nucleases such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Cas9 can be modified to edit a variety of different genes by generating a unique 20 nucleotide guide sequence.[10]

Base Editor

Base editors (BEs) create point mutations in the genomic DNA or the cell's RNA to alter a base pair in the sequence. The precision of base editors allows them to edit a DNA or RNA strand without creating DSB or a reference DNA sequence. DNA BEs can be classified as cytosine base editors (CBEs) and adenine base editors (ABEs).

CBEs

CBEs were developed before ABEs. They can convert a targeted C•G base pair to a T•A base pair using deamination. To convert cytosine (C) to uracil (U), a hydrolysis reaction must take place. The reaction between cytosine and water forms uracil and ammonia. BEs take components from CRISPR/Cas9 and modify them to create more precise edits. To ensure deamination only occurs on the target site, a deaminase enzyme that only acts on ssDNA is used. An impaired or dead Cas9 nuclease (dCas9) is fused with a base-modification enzyme; the deaminase enzyme that can only operate on single-stranded DNA (ssDNA). The deamination window on the ssDNA is limited to prevent off-target base deamination. The BE binds to the target locus on the ssDNA by matching base pairs between the DNA and gRNA. This forms an R-loop consisting of the gRNA and the ssDNA. Within the loop, the deaminase enzyme performs edits on the ssDNA. However, when the edit occurs, uracil N-glycosylase (UNG) initiates base excision repair (BER) to convert the U•G back to C•G. UNG is inhibited using a protein from a bacteriophage fused to a uracil DNA

[9] Ran, F Ann, Patrick D Hsu, Jason Wright, Vineeta Agarwala, David A Scott, and Feng Zhang. 2013. "Genome Engineering Using The CRISPR-Cas9 System". Nature Protocols 8 (11): 2281-2308. doi:10.1038/nprot.2013.143.

[10] Ran et al. 2013

glycosylase inhibitor. Additionally, the non-edited ssDNA with the guanine (G) from the original C•G pair is then nicked to initiate DNA repair. When DNA ligase repairs the strand, a U•A pair will be formed instead. Then when DNA replication occurs, the uracil will be replaced with thymine (T) to form the T•A base pair.

ABEs

ABEs convert targeted A•T base pairs to C•G base pairs. ABEs work akin to CBEs with a few variations. Similar to CBEs, deamination is performed on the target adenosine (A) on a ssDNA via hydrolysis. The deamination of adenosine results in inosine (I) rather than guanine. DNA polymerases read inosine as guanosine due to their similar properties and thus, guanine is assembled instead during DNA replication.[11]

BCL11A and Fetal Hemoglobin

BCL11A is the main transcription factor that controls the switch from fetal haemoglobin (HbF) to normal adult hemoglobin (HbA). HbF is found during the growth period of a fetus and has a slightly different structure than HbA. HbA is also composed of two α-globin subunits but instead of two β-globin subunits. Similarly, HbF is also composed of two α-globin subunits but has two γ-globin subunits instead of β-globin. As the fetus matures, fetal haemoglobin decreases and adult haemoglobin increases. Although HbF is usually designated for fetal development, it provides similar functions and is a better alternative to sickled Hb.

Methods

An initial Google search of "Sickle cell disease" was conducted to find general publications outlining basics of SCD and HBB mutation-related blood disorders. Non-research publications from the National Institutes of Health (NIH) and Harvard News were consulted to learn more about current trials being conducted. Further research was conducted on Google Scholar using key terms "gene therapy in sickle cell".

Results

Lentiviral Vector Transduced Human Hematopoietic Stem Cells

Lentiviral vectors (LV) provide means to transduce CD34^{+} hematopoietic stem cells (HSC) *ex-vivo* and open opportunities for autologous HSCT.

Comparison of β-AS3 LV and β-AS3 HS4 LV

Previous trials have investigated the stability of LVs in the transfer of the anti-sickling *HBB* transgene. The anti-sickling variants used were β^{T87Q} (*HBG*) and β^{AS3}. The transgene was placed under *HBB* promoter control and regulatory elements of the 16-kb β-locus control region (βLCR) which is important for *HBB* gene expression. Due to the LVs limited capacity, some trials have only included sections of three out of five of the LCR's hypersensitive sites - HS2, HS3, and HS4- approximately 0.2 to 0.4 kb each . However, the combination of sections of these sites has proven

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[11] Rees, Holly A., and David R. Liu. 2018. "Base Editing: Precision Chemistry On The Genome And Transcriptome Of Living Cells". Nature Reviews Genetics 19 (12): 770-788. doi:10.1038/s41576-018-0059-1.

to result in low *HBB* transgene expression and gene silencing.[12] Extended HS sites have yielded high *HBB* globin expression. Thus LVs containing the β-globin expressing genes require extended sections of HS2, HS3, and HS4. These LVs contain a total LCR of 2.6 to 3.4 kb, making them exceptionally large for a viral vector.[13] The large size required of the LVs is challenging to produce and poses issues in the efficiency of gene transfer in small hematopoietic and progenitor cells (HSPCs). Previous trials show that the choice and size of LCR regulatory elements play a critical role in *HBB* transgene expression and overall correction of the sickling mutation. High-titer LVs have also been shown to transduce a larger amount of HSPCs. The use of a high-titer LV with only the HS2 and HS3 βLCR regulatory elements has shown efficacy in the transduction of a large portion of HSPCs and *HBB* transgene expression for SCD therapy.[14]

Two LVs were made carrying the anti-sickling *HBB* transgene (*HBBAS3*), *HBB* promoter and either two or three HSs from βLCR. β-AS3 LV contained HS2 and HS3 while β-AS3 HS4 LV contained HS2, HS3, and HS4. *HBBAS3* has three potentially beneficial point mutations; G16D, E22A, T87Q. D16 increases the affinity of the HBB-chain to HBA chains to maintain a tetramer Hb shape. A22 and Q87 impair contacts needed for the HbS polymers to form. Third-generations LVs, pseudotyped with virus glycoprotein G (VSV-G), were produced by standard transient transfection of HEK293T cells and concentrated via ultracentrifugation.[15]

Physical and Infectious Titer

The physical and infectious titers of the two LVs were measured and their ratio was used to determine the ability to establish infection. The physical titer of the two LVs was close as measured by the viral p24 protein, signifying a similar number of viral particles in both. The infectious titer of both LVs was measured by transducing HCT116 cells and human erythroid (HEL and K562) cell lines to find the viral copy number (VCN) per genome. The VCN of β-AS3 LV was higher than β-AS3 HS4 LV by approximately eight VPN. Due to its higher infectious titer, β-AS3 LV was flagged to have higher infectivity.[16] Human granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (mPB) HSPCs from healthy donors were transduced with increasing amounts for each vector *ex-vivo*.

To determine the factor that affects infectivity, HEK293T packaging cells were transduced with both vectors and viral packaging plasmids. A qRT-PCR revealed that more viral RNA with the ψ+ packaging signal was detected in cells transduced with β-AS3 LV. It was also revealed that the β-AS3 HS4 viral genome was shortened during transcription. The efficacy of the HS4 core vs the HS4 core and polyadenylation site was tested using the β-AS3 HS4 LV. Shortening of HS4 did not affect the infectious titer or overall infectivity of the LV, suggesting that the HS4 core is what was responsible for the lower VCN.[17]

Transgene Transfer Efficacy

To determine transgene transfer efficacy, SCD bone marrow-derived HSPCs samples were transduced in tests with an equal number of viral particles (MOI 4-360; n =2-6) of each LV.[18] The HSPCs were recognized in a colony-forming

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[12] Weber et al. 2018

[13] Weber et al. 2018

[14] Weber, Leslie, Valentina Poletti, Elisa Magrin, Chiara Antoniani, Samia Martin, Charles Bayard, and Hanem Sadek et al. 2018. "An Optimized Lentiviral Vector Efficiently Corrects The Human Sickle Cell Disease Phenotype". Molecular Therapy - Methods & Clinical Development 10: 268-280. doi:10.1016/j.omtm.2018.07.012.

[15] Weber et al. 2018

[16] Weber et al. 2018

[17] Weber et al. 2018

[18] Weber et al. 2018

cell (CFC) assay as either a "liquid culture toward the erythroid lineage or plated in a semi-solid medium"[19] with cytokines helping colony growth. The two pools determined were burst-forming unit-erythroid (BFU-E) and colony-forming unit-granulocyte-monocyte (CFU-GM) progenitors. Gene transfer via β-AS3 HS4 LV was negligible under an MOI of 36 while β-AS3 was able to achieve gene transfer with MOIs as low as 4 and 12. The indication of gene transfer was measured by the VCN in the various HSPCs groups. Within comparable MOIs, β-AS3 LV outperformed β-AS3 HS4 LV. A positive correlation was seen with the VCN and MOI for the β-AS3 LV.[20]

In a more precise measurement, the gene transfer was measured in individual BFU-E and CFU-GM from two donors. β-AS3 LV transduced 82% of BFU-E and 60% of CFU-GM while β-AS3 HS4 LV transduced less than 60% of CFCs.[21]

Long-Term Efficacy

To determine which LV had the greater efficacy in long-term repopulation of HSPCs, murine trials were conducted. Five mice per LV were injected with human blood marrow-derived HSPCs from a SCD patient that had been transduced with their respective LV with an MOI of 360. The mice were made NOD scid gamma (NSG) immunodeficient after a busulfan-based conditioning. Assessed *in vitro*, β-AS3 LV was more successful in producing greater VCNs in both BFU-E and CFU-GM pools than β-AS3 HS4 LV; "0.26 and 0.18 β-AS3 HS4 vector copies in BFU-E and CFU-GM; 2.03 and 0.66 β-AS3 vector copies in BFU-E and CFU-GM".[22] For the control group, five mice were transplanted with mock LV-transduced SCD HSPCs and five other mice were transplanted with adult cord blood (CB) HSPCs from human donors. It was found that engraftment of human cells was similar in both the control group and test groups. 15 weeks post injection the hematopoietic organs of the mice were analyzed for human cell engraftment. The total number of cells found in the blood marrow were isolated and put into a CFC-assay to determine the gene transfer efficiency in BFU-E and CFU-GM pools derived from HSPCs. The β-AS3 LV found to have more VCNs/cell (0.34 ± 0.12 in BFU-E and of 0.23 ± 0.08 in CFU-GM) than β-AS3 HS4 LV (0.04 ± 0.001 in BFU-E and 0.02 ± 0.001 in CFU-GM).[23]

SCD Phenotype Correction

To determine which LV was more successful in achieving transgene expression and correction of the SCD phenotype, *in vitro* LV-transduced HSPCs from two donors were matured into erythrocytes. The expression of the *HBBAS3* mRNA transgene was measured via qRT-PCR in erythroblasts derived from β-AS3 LV and β-AS3 HS4 LV transduced HSPCs. Both displayed the ability to achieve transgene expression and no significant difference was seen between the LVs. However, β-AS3 LV was shown to have a greater transduction efficiency and more effective transgene expression at a lower MOI than β-AS3 HS4 LV. The expression of the therapeutic β^{AS3} globin-chain (HBAS3) was found in mature erythrocytes along with a decrease in the β^S globin-chain.[24] A positive correlation was found in the VCN/cell in the β-AS3 LV transduced HSPCs and HBAS3 expression in mature erythrocytes.

BCH-BB694 LV Induction of HbF

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[19] Weber et al. 2018

[20] Weber et al. 2018

[21] Weber et al. 2018

[22] Weber et al. 2018

[23] Weber et al. 2018

[24] Weber et al. 2018

CD34^{+} HSPCs transduced with BCH-BB694 LV encoding for a "short hairpin RNA (shRNA) targeting BCL11A mRNA embedded in a microRNA (shmiR)"[25]allow for HbF expression in erythroid cells. BCH-BB694 LV is a third generation lentiviral vector. Second generation LV was modified using therapeutic shmiR controlled by regulatory elements from the β-globin locus. This resulted in a new, self-activating BCH-BB694 LV with a more optimized titer.

Eleven patients ranging from 7 to 29 years old with severe SCD genotypes HbSS, HbS/β[0], HbSD, or HbSO enrolled in the trial but only six had a 6 month follow-up by October 2020. Out of eleven, nine were transduced with BCH-BB694 shmiR-encoding LV. One patient dropped out of the trial and two patients received their follow-up prior to 6 months. Patients with HLA-MRD graft eligibility were excluded from the trial. The primary end-point was a neutrophil engraftment of at least "0.5×109 per liter for 3 sequential days during the 7 weeks after infusion".[26] Secondary endpoints were function of transgene in cells as well as laboratory and clinical features of SCD in the patients.

240 µg/kg of plexifor was used to mobilize CD34+ HSPCs with a target of 4 million CD34^{+} HSPCs/kg.[27] An additional 2 million non-transduced CD34^{+} HSPCs were stored via cryopreserve as a backup incase of engraftment failure. Patients had 4 days of myeloablative conditioning with IV busulfan with a target of 5500 µM/min. Infusions of transduced CD34+ HSPCs began 24 hours after the last busulfan dose. Adjustments were made to the patients' course of treatment based on their history. Patient 3 had previous neurovascular disease and high risk of stroke and thus was put on a transfusion regime post-infusion to maintain HbS levels less than their baseline.

There were no adverse events grade 3 or higher experienced by any patient associated with the trial procedure and infusion. Patient 1 had serious adverse effects caused by underlying complications from central venous access devices and/or myeloablative chemotherapy. Patient 8 developed type 1 diabetes mellitus two weeks after the infusion but presence of autoantibodies suggested that the patient was predisposed to the condition.

After induction of edited CD34^{+} HSPCs, the percent of Hb that was HbF increased and remained stable in all of the patients. Hb increased to a baseline range of 9.3-11.4g/dL for five of the patients.[28] Although hemolysis continued in all patients, it decreased for its baseline range. Patient 3 continued their RBC transfusions post-gene therapy but required less frequent transfusions to maintain HbS levels targeted pre-gene therapy. As of October 2020, the median HbF percentage in the five non transfused patients was 30.4% (21.6-40.0%) while the median HbF percentage in the six patients where HbF was induced was 30.5% (20.4-41.3%).[29] Patients 3, 4, 6, 7, and 8 had an HbF/F-cell range increase from 5.0-7.1pg/cell to 9.0-18.6pg/cell.[30] Patients 2, 3, and 7 were all receiving RBC transfusions before gene-therapy. Post-gene therapy, patients 2 and 7 had not received transfusions since neutrophil enfragment. The stability of HbF levels as well as decrease in hemolysis and RBC transfusions provides evidence that LV mediated *BCL11A* gene inhibition is effective in HbF induction for SCD treatment.[31]

CRISPR/Cas9 Editing of Human Hematopoietic and Progenitor Cells

CRISPR/Cas9 alteration of CD34^{+} HSPCs provides a more controlled gene editing technique and greater modified

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[25] Esrick, Erica B., Leslie E. Lehmann, Alessandra Biffi, Maureen Achebe, Christian Brendel, Marioara F. Ciuculescu, and Heather Daley et al. 2021. "Post-Transcriptional Genetic Silencing Of BCL11A To Treat Sickle Cell Disease". New England Journal Of Medicine 384 (3): 205-215. doi:10.1056/nejmoa2029392.

[26] Esrick et al. 2021

[27] Esrick et al. 2021

[28] Esrick et al. 2021

[29] Esrick et al. 2021

[30] Esrick et al. 2021

[31] Esrick et al. 2021

gene yield than other nuclease-based editors.[32] The system is also able to successfully inhibit the *BCL11A* transcription factor in CD34^{+} HSPCs of both SCD and β-thalassemia patients.

Benefits of CRISPR/Cas9 Editing Over Alternate Methods

Comparison between the ability of nucleases to lead site-specific gene editing of CD34^{+} HSPCs using a homologous donor template (HDR). TALENs and CRISPR/Cas9 gRNAs were co-delivered to K562 3.21 cells via electroporation along with a homologous donor template. The template was 1.1 kb of the *HBB* spanning the target site. Three different TALENs were tested alongside CRISPR/Cas9; TALEN 2 wild-type, TALEN 5 wild-type, and TALEN 5 ELD/KKR. TALENs produced an average gene modification of 8.2%-26.6% while CRISPR/Cas9 produced a greater range and maximum gene modification average of 4.2%-64.3%.[33] The gene modification average was analyzed via qPCR at the restriction fragment length polymorphisms (RFLP). CRISPR/CAS9 nuclease systems displayed more potential in gene modification of *HBB*.

Delivery of CRISPR/Cas9 reagents into CD34^{+} HSPCs was explored due to high cell toxicity resulting from plasmid delivery. Attempting to deliver gRNA and Cas9 without a donor template via electroporation resulted in a <5% disruption of alleles and low cleavage rates.[34] Thus, the lentiviral vector method of delivery was used. The LV contained gRNA with a U6 promoter and homologous donor template. CD34^{+} cells electroporated from the peripheral bloodstream with Cas9 mRNA and the transduction with LV containing the gRNA and HDR showed gene modification of 17.8%-18.7%.[35] The gene modification occurred without correlation to LV dose and resulted in minimal cell toxicity.

CTX001 Infusion Treatment

CRISPR/Cas9 targeting of the *BCL11A* zinc-finger containing transcription factor can provide therapeutic treatment for transfusion-dependent β-thalassemia (TDT) and SCD. CD34^{+} HSPCs are edited using CRISPR/Cas9 to modify the *BCL11A* to enable γ-globin expression and thus formation of HbF in erythroid cells. *BCL11A* contains single-nucleotide polymorphisms (SNPs) that are responsible for HbF expression in adults. The SNPs located in the *BCL11A* locus on chromosome 2 in particular reduce the severity of TDT and SCD.[36] LV delivered microRNA-adapted short hairpin RNA (shRNA) have shown to reactivate γ-globin expression in CD34^{+} HSPCs. Patients with TDT or SCD will often show symptoms later on in life when adult hemoglobin or Hb becomes the primary functional hemoglobin.[37] The alteration of adult HSPCs to later function as erythrocytes with HbF bypasses the use of adult hemoglobin with the mutated β-globin chain. CTX001 Trials

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[32] Hoban, Megan D, Dianne Lumaquin, Caroline Y Kuo, Zulema Romero, Joseph Long, Michelle Ho, and Courtney S Young et al. 2016. "CRISPR/Cas9-Mediated Correction Of The Sickle Mutation In Human CD34+ Cells". Molecular Therapy 24 (9): 1561-1569. doi:10.1038/mt.2016.148.

[33] Hoban et al. 2016

[34] Hoban et al. 2016

[35] Hoban et al. 2016

[36] Frangoul et al. 2021

[37] Frangoul, Haydar, David Altshuler, M. Domenica Cappellini, Yi-Shan Chen, Jennifer Domm, Brenda K. Eustace, and Juergen Foell et al. 2021. "CRISPR-Cas9 Gene Editing For Sickle Cell Disease And B-Thalassemia". New England Journal Of Medicine 384 (3): 252-260. doi:10.1056/nejmoa2031054.

The CTX001 treatment is the process of infusing patients with CD34^{+} HSPCs edited with CRISPR/Cas9 to inhibit *BCL11A* from preventing γ-globin expression. One trial investigated the outcome of the CTX001 treatment in two patients, one with TDT and one with SCD. The trials names were CLIMB THAL-111 and CLIMB SCD-121 respectively. In CLIMB THAL-111 and in CLIMB SCD-121, the patient was given an intravenous infusion of CTX001. Both trials required the patient to be between 18-25 years old. CLIMB THAL-111 required a patient with a β-thalas

semia with the hemoglobin E phenotype and either homozygous or compound heterozygous for the mutation. The patient had to also have received minimum transfusions of 100 ml/kg of body weight or 10 units of packed red blood cells (RBC) within the two previous years. CLIMB SCD-121 required that the patient had a recorded homozygous β[S] genotype or heterozygous β[S] genotype of β[S]/ β[0]. The patient also had to have a history of vaso-occlusive crises in the previous two years to be considered.[38]

CD34^{+} HSPCs from the bloodstream were collected from the patients via apheresis. In CLIMB THAL-111, the patient was given filgrastim for infection prevention and plerixafor as an immunostimulant to mobilize the HSPCs. In CLIMB SCD-121, the patient was given plerixafor. Apheresis was done after eight weeks of packaged red blood cell transfusions to achieve a sickled Hb level of <30%. Both patients received adjusted busulfan myeloablation before the CTX001 infusion. CLIMB THAL-111 lasted 21.5 months with a study visit after 18 months. CLIMB SCD-121 lasted 16.6 months with a study visit after 15 months.[39]

The CLIMB THAL-111 patient, a 19-year old female, had received RBC transfusions since birth and suffered from "iron overload, inactive hepatitis C, splenomegaly, and osteonecrosis of the skull". [40] The patient experienced an increase in HbF levels from a start of 0.3g/dL to 8.4g/dL at the third month, 12.4g/dL at the sixth month, and 13.1g/dL at the twelfth month. Erythrocytes with fetal hemoglobin (F-cell) expression increased from a start of 10.1% to 99.7% after 6 months and were maintained up until the 18 month study visit. The patient's final stable hemoglobin level was 12.1g/dL. The patient experienced 32 adverse effects from the infusion. Most were categorized as grade 1 or grade 2 events; pneumonia and veno-occlusive liver disease with sinusoidal obstruction syndrome (VOD-SOS). Both grade 2 events began on day 13. Pneumonia resolved on day 28. The VOD-SOS reached grade 3 severity despite treatment with defibrotide but eventually resolved on day 39.[41]

The CLIMB SCD-121 patient, a 33-year old female, had an average of seven vaso-occlusive crises per year, 3.5 SDC-related hospitalizations per year, and five RBCs per year. The patient suffered from "chronic pain, cholelithiasis, increased lactic dehydrogenase levels, and decreased haptoglobin levels".[42] The patient experienced an increase in hemoglobin levels from a start of 7.2g/dL to 10.1g/dL at the third month and 12 g/dL at the fifteenth month. HbF levels started at 9.1% and sickle Hb levels started at 74.1%. By the 15 month study visit, HbF levels had increased to 43.2% and sickle Hb levels had dropped to 52.3%.[43] The patient's F-cell expression was 99.9% at the fifth month and maintained at approximately 100% by the 15 month study visit.[44] The patient experienced 114 adverse effects from the infusion. Three events were categorized as serious; sepsis on day 16, cholelithiasis on day 49, and abdominal pain on day 56. All three events were resolved with treatment. Intermittent, non serious lymphopenia was also observed due to delay in T-cell recovery following the CTX001 infusion but was resolved by day 351. The patient did not have an vaso-occlusive crisis during the duration of the study.

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[38] Frangoul et al. 2021

[39] Frangoul et al. 2021

[40] Frangoul et al. 2021

[41] Frangoul et al. 2021

[42] Frangoul et al. 2021

[43] Frangoul et al. 2021

[44] Frangoul et al. 2021

Although adverse effects were reported in both patients during the study, they were all resolved with treatment. The CTX001 infusion was successful in increasing HbF levels, relieving some major symptoms of TDT or SCD, and reducing the need for RBC transfusions.[45] Not only did HbF levels increase consistently in both studies, but stability was achieved at the end for the studies' duration.

Base Editing of Hematopoietic Stem Cells in Mice

Base editing of CD34+ HSPCs provides a method for single edits and decreases the occurrence of off-target edits or genetic disruptions for NHEJ repairs. The ssDNA breaks and repair forgo the need of NHEJ repairs as needed in gene editing methods that edit via dsDNA breaks (Cas9 nuclease).

β^S to Makassar β-globin

An ABE (ABE8e-NRCH) was used to convert β^S to a non-pathogenic variant, Makassar β-globin. ABE8e-NRCH was able to convert 80% of Val (GUG) to alanine or Ala (GCG) in an *ex vivo* delivery into human SCD HSPCs.[46] After base editing, human HSPCs were transplanted into immunodeficient mice for 15 weeks and observed. Post-observation period, Makassar β-globin frequency was 68% and sickling of bone marrow reticulocytes had decreased five fold.[47] To further test the physiological effects of the base edited cells, edited human HSPCs were transplanted from one mouse into irradiated mice. After 16 weeks, Makassar β-globin made up 79% of β-globin in the blood and sickling was reduced three fold.[48] The second transplant revealed that a 20% conversion of β^S to Makassar β-globin was sufficient to consider the SCD phenotype corrected.[49] Mice transplanted with edited cells showed close to normal haematological parameters and reduced splenic infection compared to the control group. Base editing avoided activation and mutation of p53 (can cause cancerous tumours) and dsDNA breaks seen with Cas9 editing.[50]

HbF Induction via Base Editor

CBE edited human peripheral blood (PB) derived CD34+ HSPCs at the BCL11A +58 erythroid enhancer was found to successfully produce on-target single cytosine base edits with few indels (insertion or deletion of base in genome). The ability to induce HbF expression in erythroid cells was similar in both the use of the CBE and other nuclease editing methods.

In a study conducted by a collaboration of multiple research institutions in Boston and Shanghai, a CBE was successful in reducing sickling and sickled β-globin chains in HSPCs from both SCD and β-thalassemia.[51] Base edits produced multilineage and self-repopulating HSCs with HbF expression induced in vitro in animal models.

[45] Frangoul et al. 2021

[46] Newby, Gregory A., Jonathan S. Yen, Kaitly J. Woodard, Thiyagaraj Mayuranathan, Cicera R. Lazzarotto, Yichao Li, and Heather Sheppard-Tillman et al. 2021. "Base Editing Of Haematopoietic Stem Cells Rescues Sickle Cell Disease In Mice". Nature 595 (7866): 295-302. doi:10.1038/s41586-021-03609-w.

[47] Newby et al. 2021

[48] Newby et al. 2021

[49] Newby et al. 2021

[50] Newby et al. 2021

[51] Zeng, Jing, Yuxuan Wu, Chunyan Ren, Jasmine Bonanno, Anne H. Shen, Devlin Shea, and Jason M. Gehrke et al. 2020. "Therapeutic Base Editing Of Human Hematopoietic Stem Cells". Nature Medicine 26 (4): 535-541. doi:10.1038/s41591-020-0790-y.

A purified A3A (N57Q)-BE3 BE electroplated with five modified synthetic sgRNAs as ribonucleoprotein (RNP) complexes was analyzed in its ability to target the BCL11A +58 erythroid enhancer in CD34+ HSPCs. When editing with sgRNA-1620, C was converted to T (at 38.2%), G (at 21.2%), and A (at 4.2%) at C6 (position 6).[52] The use of sgRNA-1620 was more successful in base edits on-target than sgRNA-1619 (43.0% at C7). sgRNA-1617 produced 50.6% edits at C5 but not within the target binding motif (half E-box/GATA).[53] CD34+ HSPCs were electroplated with A3A (N57Q)-BE3 BE paired with sgRNA-1620 at concentrations ranging from 10–50 μM. It was found that that 50 μM produced 81.7% of base edits at C6.[54] Base edits at C6 had a strong positive correlation with HbF expression.

For HbF induction via A3A (N57Q)-BE3 RNP editing of HSPCs in SCD and β-thalassemia, a trial was conducted to study the optimal number of electroprotain cycles for the greatest base editing frequency. In HSPCs of healthy donors, base editing frequency was 70.3% after one cycle of electroporation and 92.5% after two cycles separated by 24 hours.[55] However, HSPCs viability decreased from 83% to 47%.[56] In mobilized peripheral blood CD34+ HSPCs from two SCD donors, base editing at C6 was recorded after two electroporations was 91.2% and 86.3%.[57] HbF expression in the majority of erythroid cells was increased to 32.2% from 5.0% and 27.9% from 6.4%.[58] In non-mobilized peripheral blood CD34+ HSPCs from two β-thalassemia donors, base editing frequencies were 93.3% and 90.6% following electroporation.[59] Both donor cells displayed increased production in γ-globin chains and HbF expression.

Discussion

Gene therapy for SCD and similar blood disorders caused by a mutation of the *HBB* gene provide long-lasting curative treatments for the majority of those affected. The current curative allogeneic HSCT treatment for SCD is unavailable for 85% of SCD patients due to the requirement of an HLA-MRD graft.[60] Editing the *HBB* gene in HSPCs to correct the mutation or bypassing the use of the gene altogether through various gene editing technologies has proven to provide relief to both SCD and β-thalassemia patients. The three techniques discussed, Lentiviral Vectors, CRISPR/Cas9, and Base Editors, are able to perform edits on patients' HSPCs without the need for additional grafts. Although gene therapy methods are currently still in clinical trials, they show potential for becoming the primary course of treatment for severe SCD.

This literature review was limited in the types and number of papers included. Papers limited to English publications, to prevent miscommunication or translation errors. However, two clinical trials of HbF induction were international collaborations between departments from two different countries (American and Chinese researchers). Selection of papers that were focused on was further limited by a 2 month time period to write the review as well as the current knowledge of the author on specific terminology and practices. The research papers discussed are a small representation of literature currently available in gene therapy for SCD.

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[52] Zeng et al. 2020

[53] Zeng et al. 2020

[54] Zeng et al. 2020

[55] Zeng et al. 2020

[56] Zeng et al. 2020

[57] Zeng et al. 2020

[58] Zeng et al. 2020

[59] Zeng et al. 2020

[60] Kato, Gregory J., Frédéric B. Piel, Clarice D. Reid, Marilyn H. Gaston, Kwaku Ohene-Frempong, Lakshmanan Krishnamurti, and Wally R. Smith et al. 2018. "Sickle Cell Disease". Nature Reviews Disease Primers 4 (1). doi:10.1038/nrdp.2018.10.

Table 1**.** Gene Therapy Methods' Progression

Method	SCD Phenotype Correction	Human Trials	HbF Induction	Human Trials
LentiviralVector (LV)	✓ β-AS3 LV	X	✓ BCH-BB694 LV	✓
CRISPR/Cas9	✓	on going (no data)	✓ CTX001	✓
Base Editor	✓ ABE8e-NRCH	X	✓ A3A (N57Q)-BE3 RNP	X

All three gene therapy methods were able to achieve SCD phenotype correction *in vivo* of human and murine SCD CD34+ HSPCs as seen in Table 1. Comparison between β-AS3 LV and β-AS3 HS4 LV included physical and infectious titer, transgene transfer efficacy, long-term efficacy and overall SCD phenotype correction in mice trials. The overall SCD phenotype correction by β-AS3 LV was measured by the number of VCN in CFC assays and VCN/cell. β-AS3 LV was able to transduce 82% of BFU-E and 60% of CFU-GM in the CFC assay and successfully correct the overall SCD phenotype using the HBBAS3 mRNA transgene.[61] Since LVs are inert viruses, they have the added risk of cell toxicity or infection recurrence and thus must be extensively tested prior to transduction of cells. When the CRISPR/Cas9 system was tested against other nuclease based editing methods (TALENs), it outperformed multiple TALEN wild-type in gene modification of human SCD CD34+ HSPCs with a maximum modification rate of 64.3%.[62] CRISPR/Cas9 was delivered into cells via electroporation which led to high cell toxicity. Thus, the LV method was explored which decreased cell toxicity but also decreased the maximum modification rate to 18.7%.[63] The ABE8e-NRCH BE used to edit human SCD CD34+ HSPCs was able to correct the SCD phenotype by converting β^S to Makassar β-globin. Base pair inversion to convert Val and Ala was successful in converting 79% of β-globin

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[61] Weber et al. 2018

[62] Hoban et al. 2016

[63] Hoban et al. 2016

protein to Makassar β-globin after 16 weeks.[64] This was well above the minimum 20% required for SCD phenotype correction to be considered.[65] Makassar β-globin is an extremely rare β-globin type with only a handful of reported cases in history. Little is known about the implications of increasing Makassar β-globin through gene editing and its long-term implications to the host body post edited cell transplant. While CRISPR/Cas9 and BEs reduce the chance of infection (which is an issue with viral vectors), delivery via electroporation into cells can cause cell toxicity and lower cell viability.

Research on human trials for autologous HSCT with gene editing of the *HBB* mutation directly has not been documented and published yet. One ongoing trial that started during the summer of 2021 is a joint initiative between UC Berkeley and UCSF utilizing CRISPR/Cas9 to edit CB and marrow SCD CD34+ HSPCs for autologous HSCTs[66]. The three gene editing methods were also all able to induce HbF via *BCL11A* transcription factor inhibition.

Table 2. HbF Human Trial Comparison (*BCL11A* Inhibition)

Trial	# Patients	Max HbF level	Max Adverse Event	Patient Variety	Follow Up
BCH-BB694 LV[67]	6 (+ 5 drop outs)	41.3%	Grade 3	SCD	6 mo (avg)
CTX001[68]	2	43.2% (SCD) 13.1g/dL (TDT)	Grade 3 (SCD and TDT)	SCD, β-thalasse-mia (TDT)	15 mo (SDC) 18 mo (TDT)
A3A (N57Q)-BE3 RNP[69]	N/A	32.2%	N/A	SCD, β-thalasse-mia	N/A

As seen in Table 2, both LV and CRISPR/Cas9 methods have reached and shown some success in human trials. The BCH-BB694 shmiR-encoding LV was successful in inhibition of *BCL11A* transcription factor in human trials in 6 patients with a variety of different SCD genotypes. Out of the three patients within the test pool who had previously been receiving RBC transfusions, two were able to maintain lower HbS levels without transfusions post-gene therapy. Out of 11 consented participants, only 6 completed the trial and received a 6 month follow-up. Furthermore, the maximum HbF level achieved was 41.3% at the 6 month follow up and remained stable.[70] The CRISPR/Cas9 CTX001 trial had two participants, one with SCD and one with β-thalassemia. Both patients experienced adverse events (grade 1, 2, and 3) but were able to have them resolved before trial ended. The maximum HbF levels achieved

[64] Newby et al. 2021

[65] Newby et al. 2021

[66] Fernandes, Lorna. 2021. "UC Consortium Launches First Clinical Trial Using CRISPR To Correct Gene Defect That Causes Sickle Cell Disease". UC Consortium Launches First Clinical Trial Using CRISPR To Correct Gene Defect That Causes Sickle Cell Disease | UC San Francisco. https://www.ucsf.edu/news/2021/03/420137/uc-consortium-launches-first-clinical-trial-using-crispr-correct-gene-defect.

[67] Esrick et al. 2021

[68] Frangoul et al. 2021

[69] Zeng et al. 2020

[70] Esrick et al. 2021

were 43.2% (after 15 month follow-up) and 13.2g/dL (after 18 month follow-up) in the SCD and β-thalassemia respectively.[71] Both patients experienced F-cell rise to >99% following CTX001 infusion treatment.[72] The CBE (A3A (N57Q)-BE3 RNP) was able to achieve HbF expression in two SCD donor CD34+ HSPCs and two β-thalassemia donor CD34+ HSPCs which high base editing frequencies following two cycles of electroporation 24 hours apart.[73] The base editor was able to forgo the dsDNA off-target breaks and p53 mutation sometimes caused by CRISPR/Cas9 as well as decrease the number of indels.[74] Although the CBE (A3A (N57Q)-BE3 RNP) HbF induction method currently remains in preliminary trials (*in vitro* animal trials), its results and benefits show promise for further investigation.

While both the CTX001 and LV trials investigated the impact of HbF induction in a variety of different SCD phenotypes (LV trial) or blood disorders (CTX001), ages (both), and gender (LV trial), neither trial specified diversity in ethnicity, health care accessibility and country of origin. In order to ensure a thorough investigation of all possible adverse events from treatments, future trials would need to be larger with a greater diversity in patients. For instance, male participants in the CTX001 trial would investigate the occurrence of priapism following treatment. Diversity in patients would allow for in depth analysis on how the treatments affect different groups of people and allow treatments to become accessible to a larger number of those affected.

Currently CTX001 and LV induction of HbF provide substantial evidence in human trials as possible methods of autologous HSCTs for individuals who do not have access to HLA-MRD grafts for allogeneic HSCTs. Perfection and advancement of these methods in mutation induced blood disorders can open opportunities for correction of other similar major diseases caused by mutations. This can already be seen with SCD trials being coupled with β-thalassemia trials. Long-term follow up reports on both human trials as well as progression of BEs to human trials will be required in order to implement a definite autologous HSCT via edited CD34+ HSPCs.

Limitations

Literature chosen was limited to primary research published within the past 10 years on gene therapy use in SCD, specifically via lentiviral vectors, CRISPR/Cas9, and base editors.

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[71] Frangoul et al. 2021

[72] Frangoul et al. 2021

[73] Zeng et al. 2020

[74] Zeng et al. 2020

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