Literature Review- Sickle Cell Anemia

Name of Student

Instructor's Name

[Institutional Affiliation(s)]

Abstract

It has been more than a century since the first case of Sickle Cell Anemia was identified. Has the progression of the illness from its discovery to the present day resulted in an improvement in the quality of life of those who are affected? In this review, I have addressed Sickle Cell Disease from its origins to current research, including gene therapies and informed consent, so that patients may have a better understanding of the risks involved with the treatments and what they can do to avoid those risks as much as possible.

Literature Review- Sickle Cell Anemia

The discovery of the molecular basis of sickle cell disease was a watershed moment in the history of molecular medicine. The use of cutting-edge methods in molecular and cellular biology has increased our understanding of the disease's pathophysiology and made it simpler to develop innovative therapies. "Sickle Cell Disease," often known as SCD, was first recognized in 1910 by a Chicago Cardiologist. The term "Sickle Cell Disease" is commonly abbreviated as SCD (Rees et al., 2010).

When the illness was initially discovered, it was given the name Herrick's Syndrome in honor of Dr. James B. Herrick, a Chicago physician who worked at Presbyterian Hospital. Dr. Herrick was also a faculty member at Rush Medical College in the city of Chicago, where he taught medical students and residents in the field of internal medicine. Ernest Irons, a dental student's intern, and Herrick were tasked with identifying the red blood cells of the dental student. Herrick used the term "sickled-shaped" to describe the patient's extraordinarily long and elongated red blood cells as a result of the patient's exceptionally long and elongated red blood cells. This phrase has remained with me (Stuart & Nagel, 2004).

An American scientist named Linus Carl Pauling and his colleagues did research in 1949 and discovered the mutation that causes sickle cell disease in the hemoglobin molecule. Known as hemoglobin, this protein present in red blood cells transports oxygen throughout the body's tissues and is important for the red blood cell's ability to carry oxygen. Hemoglobin is taken in by the lungs and then discharged into the surrounding tissue. Hemoglobin is also responsible for transporting carbon dioxide back to the lungs for removal. Hemoglobin is found in red blood cells, which is why they seem red in color. There are certain capillaries that are smaller than the diameter of a red cell, and the red cell must stretch in order to provide the oxygen that keeps it alive. For whatever reason, red blood cells may become ridged or hardened

and block capillaries, causing them to become clogged. The result is hemolysis depending on the spleen and red blood cell destruction (Rees et al., 2010). Red blood cells are produced by the bone marrow in an attempt to compensate for the loss of red blood cells. Sickle cell anemia is characterized by sickled red blood cells that only survive for 10–20 days. It is estimated that the typical lifetime of red blood cells is 90 to 120 days when they are in excellent health. Hemoglobin S deficiency, often known as Sickle Cell Anemia, is a kind of blood disease. In the hemoglobin gene, there is a genetic mutation that leads to the development of the illness. Because of the dominant, recessive nature of this gene anomaly, the defective gene must be handed down from both parents in order for it to be detected. The hemoglobin S gene is associated with hemoglobin that is faulty, while the hemoglobin A gene is associated with hemoglobin that is normal. Genetically, sickle cell trait patients inherit just one normal gene and one faulty gene, which is referred to be genotype AS. Sickle cell disease is characterized by the presence of the SS genotype.

As soon as a baby is born, sickle cell disease becomes visible and may be discovered by newborn screening. The screening for this ailment is required in all fifty states in the United States. In the majority of states, the isoelectric focusing (IEF) or high-performance liquid chromatography (HPLC) procedures are the most extensively utilized approaches (HPLC) (Rees et al., 2010). It is necessary to collect specimens prior to any blood transfusion in order to ensure that both tests are exceptionally effective. According to Ryan et al., the cellulose acetate electrophoresis (CAE) method is presented in widespread usage for variant detection and screening in genetic studies (2010). The quantity of testing required, the sample material (liquid blood or dried blood spots), the ease of handling, the repeatability, the availability and competency of local experts, and the cost will all influence the choice of testing and equipment to use. Furthermore, even if CAE materials are inexpensive, labor costs are expensive. As a consequence, it is possible that converting to HPLC will be more cost-effective. When there

are a high number of samples to be evaluated, this method is used. Hemoglobin electrophoresis is simple, reliable, and quick when performed using a cellulose acetate membrane at pH 84–86.

E. A. Beet (1947) examined the genetic evidence of sickle-cell Anemia in a particular African tribe. As a result of the sickle-cell trait (drepanocytosis), red blood cells in sealed wet preparations have a distinctive form. Sickle-cell Anemia, hemolytic Anemia, and the characters are all terms used to describe the same illness. It was conducted in the Serenje District of Northern Rhodesia, which is home to the Lala, a Bantu ethnic group. In the Serenje District, the only European inhabitants are government officials and their families, missionaries, and a single hotel proprietor. This is a rural environment, which makes it ideal for a tribal inquiry like this one. Other workers' methods were utilized to illustrate this trait: capillary blood was drawn from a finger prick and placed on a glass slide, which was then sealed with liquid paraben. All slides were stored at room temperature for at least 48 hours before being thrown away, if possible.

A four-generation family tree was created to illustrate how the characteristic arises in succeeding generations as a consequence of the research findings. A 50% likelihood of having drepanocytic offspring who are heterozygous (Xs) would be expected in mating between a heterozygous sickle-celled subject (8s) and a normal subject (ss) (Beet, 1947). There is a genetic defect in the erythron known as leptocytes, which is identical to Drepanocytosis of Africans and thalassemia of Mediterranean races, according to the research. Haemolytic Anemia, commonly known as Cooley's Anaemia, has been linked to thalassemia and may affect children who are homozygous for the condition.

In another study, Robert Brodsky's team at John Hopkins made substantial advances in the utilization of haploidentical bone marrow transplants. These findings were included in the

top 10 clinical research accomplishments of 2012, according to the Clinical Research Forum. Eleven out of 17 patients with sickle cell anemia who underwent bone marrow transplants from Brodsky's team were successful, according to the study. Half-matched donor marrow was used in eight of the successful bone marrow transplants, while completely matched marrow was used in three. A full-match donor was required for bone marrow transplants, which were seldom performed. It is common for patients' bodies to reject bone marrow that does not match their own. Researchers at Johns Hopkins have devised a process for preparing patients to receive organs from donors who are partly or fully matched. Low-dose immunosuppressive medicines are combined with low-toxicity – chemotherapy to eradicate the diseased cells from the body (Bolaños-Meade et al., 2012). The condition has come a long way since it was first discovered in the early 1900s, thanks to the efforts of scientists and medical professionals. Since then, gene therapy employing stem cells to treat sickle cell disease has progressed tremendously.

In discussing the possibility of stem cell treatment for sickle cell disease, Annalisa Lattanzi and her colleagues (2021), affirmed that stimulating human hematopoietic stem cells to repair the -globin gene may be an efficient therapy for Sickle Cell Anemia. The researchers are unclear why Sickle-Cell Anemia affects people throughout the globe, but this study hopes to find out. Autosomal recessive monogenic sickle cell disease is caused by a single point mutation (A>T) in the -globin gene's sixth codon (SCD). This missense mutation results in sickle-chain production by substituting Valine (V) for Glutamic acid (E) (HbS). Vaso-occlusive pain crises and acute chest crises (ACC) are two of the most prevalent SCD symptoms (ACS) (Lattanzi et al., 2021). To alleviate the symptoms of HgbS polymerization or to reduce the severity of pain crises or pain attacks, new drugs have been approved to treat the disease, such as hydroxyurea and blood transfusion. Hematologic stem cell transplantation with allogeneic HLA matching is the only approved treatment at this time (Allo-HSCT). A matched

sibling donor is only available in 10% to 15% of cases, and the use of haploidentical or unrelated donors is still deemed experimental in the majority of situations.

Sickle cell illness has recently seen a flurry of gene therapy research, including the use of ex vivo lentiviral transduction of autologous hematopoietic stem and progenitor cells (SCD). There is just one cause of sickle cell disease (SCD) in humans, and that is a single-point mutation in codon six of the HBB gene. Stem and progenitor cells from SCD patients were used in the study, which focused on the premise that HSPCs may be an ideal therapeutic (Lattanzi et al., 2021). SCD-causing mutations may be fixed in HSPCs employing high-fidelity Cas9 complexed with chemically modified guide RNAs prior to this procedure. Using plerixafor-mobilized CD34+ cells from healthy and SCD patient donors, the preclinical study demonstrated that HBB gene repair was possible, effective, and safe (Drug Product-gcHBB-SCD). To make matters even more astonishing in clinical-scale production of HBB allele correction in immunodeficient NSG mice, the researchers were able to achieve 60% HBB allele correction, with 20% multi-lineage allele gene repair (Lattanzi et al., 2021).

There were no symptoms of abnormal hematopoiesis, genotoxicity, or tumorigenicity in the long-term safety and toxicological studies of the engrafted SCD Drug Product. The safety, efficacy, and repeatability of our preclinical data imply that the researchers can commence a Phase I/II clinical investigation for SCD patients using a gene repair approach. Based on preclinical efficacy and safety data, a clinical trial including -globin gene repair in patient-derived hemopoietic stem cells to treat sickle cell disease is required.

Expanding on the gene therapy treatment via the use of stem cells, Selami Demirici, Noya Uchida, and John D. Tisdale (2018) are focused on treading Sickle cell disease anamoly in individuals using gene therapy. Millions of people worldwide have sickle cell disease (SCD), one of the most common and most dangerous monogenic illnesses. Scientists have noticed that

there are several treatment methods available to address the illness. Since SCD was originally diagnosed more than a century ago [6], blood transfusions, preventive drugs such as penicillin prophylaxis and pneumococcal vaccination, and hydroxyurea treatment have all been utilized in clinics. Allogenic hematopoietic stem cell transplantation is now the sole therapy for this illness, despite its high success rate, limited availability of matched sibling donors, and significant risk of transplant-related side effects. A race to develop new theories has ensued among scientists (Demirci et al., 2018).

Understanding erythropoiesis regulation and better transplantation processes have boosted confidence in finding a one-time therapy for SCD. Gene addition tests have previously shown that anti-sickling -globin cures the disease's repercussions (NEJM article) (Demirci et al., 2018). If these positive results are proven correct in the near future, the researchers believe that potentially curative medicines for people with SCD will be widely available in the near future.

This method has been extensively researched and is currently being tested in clinical trials, with promising outcomes. When human stem cells (HSCs) are grown in vitro, they lose their ability to regenerate. Transplantation of a target gene must be done in a way that avoids any potential safety difficulties, such as an immunogenic reaction or oncogenesis due to insertional mutations. Recent breakthroughs in our understanding of the molecular mechanisms underlying mammalian erythropoiesis and hemoglobin synthesis have made new and fascinating therapeutic options possible. Genome editing technologies such as CRISPR/Cas9 may be used to repair cellular stem and progenitor cells generated from patients and iPSCs (HSPCs) (Demirci et al., 2018). In order to be employed in clinical practice, these promising techniques need to address a variety of safety and efficacy issues.

Scientists have made the SCD dream come true in patient-derived iPSCs and blood progenitor stem cells with CRISPR/Cas9 and other genome editing methods described in Science Translational Medicine. Some issues remain, including offering these tools without diminishing their ability to graft, enabling high-efficiency correction, and reducing off-target editing. If more rapid advancements in hemoglobinopathies treatment are made, newer procedures should lead to more widely available remedies for hemoglobinopathies.

Similar to Demirci, a study titled "Editing the Sickle Cell Disease Mutation in Human Hematopoietic Stem Cells" by Zulema Romero and colleagues (2019) found that treating hereditary blood cell illnesses using point mutation correction in autologous stem and progenitor cells (HSPCs) is achievable. Autologous hematopoietic stem and progenitor cells (HSPCs) may be able to cure hereditary blood cell disorders, such as hemoglobinopathies and primary immunodeficiencies, by correcting the defective gene that produces a monogenic disease (Romero et al., 2019).

As a means of delivering the homologous donor template, the researchers tested the effectiveness and cytotoxicity of CRISPR/Cas9 in conjunction with viral (AAV6 and IDLV), as well as non-viral techniques (Ad5/35 serotype) in an SCD disease model with a sickle mutation at the HBB gene. The following in vitro tests were performed: viability (by trypan blue exclusion and Annexin V), editing results at the sickle mutation site by high-throughput sequencing (HTS) to determine the frequencies of HDR and NHEJ-mediated indels, hematopoietic progenitor potential (by colony-forming unit [CFU] assay), effects on cell cycle status, and (5) differential gene expression (Romero et al., 2019). After conducting in vitro research, the researchers needed to compare the short-term results with long-term results obtained when the researchers transplanted changed HSPCs into immunodeficient mice to see how different editing agents influenced HSPC survival and function and the quality of the transplanted cells (HDR and NHEJ).

Most NHEJ/NHEJ genotypes were found in the RNP + sdODN group (far more often than in the RNP + AAV6 treatments), suggesting that the majority of NHEJ-indicating alleles are bi-allelic disrupted. After transplantation of cells with an altered population of cells, it is anticipated that some degree of erythropoiesis will be impaired, although the physiologic consequences of this remain unknown. Results from short-term in vitro culture experiments and long-term in vivo evaluations of HSPC reconstitution in NSG mice xenograft were very different (Romero et al., 2019). To compare in vivo gene-editing assessments using AAV6 or ssODN donors in the long-term HSPCs engrafted in NSG mice, HDR and NHEJ rates were similar. The acute cytotoxicity of AAV6 vectors was shown to be insignificant after 24 h post-EP cell counts and viability investigations.

Before moving on to testing reagents suitable for clinical usage, the researchers evaluated some of the most often used endonucleases and donor templates for gene editing in vitro and in vivo. These include employing chemically produced (and base modified) gRNAs, more precise endonucleases such Cas9 proteins, and cell processing scale-up using electroporator systems that can handle bigger cell doses, as well as using electroporation systems that can handle larger cell doses (Romero et al., 2019). Hereditary blood cell defects may now be treated using site-specific gene corrections in HSCs, allowing for the generation of monogenetic diseases in patients.

"CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells" by Megan D. Hoban and colleagues (2016) also studied how targeted genome editing technology may treat sickle cell disease mutations in hematopoietic stem cells. Research shows that TALENs and CRISPR/Cas9 may be used to target particular DNA areas around the sickle-cell mutation in the -globin gene, resulting in precise cleavage and repair when a homologous donor template is supplied, enabling precision repair (Hoban et al., 2016). TALE binding sites in pairs may be specified by TALENs, which comprise several repeat-variable residue domains

that can be used to identify DNA binding sequences. Each TALE is broken apart by FokI endonucleases dimerization when the 12 to 20 base pair spacer regions between each TALE are cleaved by FokI. A ZFN or TALEN heterodimer is generally required for the FokI nuclease to function. Genome editing approaches such as ZFN, TALENs, and CRISPR/Cas9, which are powerful genome editing tools, may help treat SCD and other genetic disorders. FokI nuclease homodimerization, on the other hand, results in off-target cleavage. Recently constructed FokI backbone, ELD/KKR, has been proven to increase selectivity but may compromise enzymatic activity (Hoban et al., 2016).

The study concluded that find out how many cleavages happened on and off-target, a variety of TALEN and CRISPR guide RNA combinations were examined. When CRISPR/Cas9 components were supplied to CD34+ cells in vitro, roughly 18% of the genes were altered. Research shows that sickle cell disease mutations may be repaired in the CD34+ stem and progenitor cells of sickle cell disease patients, leading to the production of normal hemoglobin in the blood (Hoban et al., 2016). The sickle mutation in patient-derived CD34+ cells was repaired using CRISPR/Cas9 technology.

When it comes to who is at stake while exploring the genetic base as well as the possible genetic therapy for the treatment of sickle cell disease, Brittany M. Hollister and colleagues (2019) focused their research on questioning the stakeholders in the disease. Using CRISPR technology to modify the DNA of future generations has sparked an international controversy (HGE). Researchers throughout the globe are increasingly debating whether or not to undertake human genome editing (HGE) research.

As part of a mixed-methods study that comprised 15 focus groups: six groups of SCD patients, six parent groups, and three groups of SCD doctors, the research team prepared CRISPR genome editing training movies and conducted a post-video survey. Twenty, for this

study, the researchers only looked at the 12 focus groups with SCD patients and their parents. Participants have to be at least eighteen years of age in order to qualify. Participants from the Mid-Atlantic and Southern regions of the United States gathered between April and December of 2017.

According to the results of the research, SCD stakeholders exhibited varying degrees of support for SGE and HGE. Figure 1 shows that most SCD stakeholders were either indifferent or agreed that both the use of stem cell embryogenesis and the use of human germline embryos are morally acceptable (v2 = 7.39, p = 0.02) (Hollister et al., 2019). Compared to the identical question concerning SGE, more stakeholders felt that HGE was not morally acceptable. When there were no other options, more SCD stakeholders opposed HGE than opposed SGE in the same circumstance.

The study by Persaud, Blizinsky, and Bonham (2019) also highlighted that SCD stakeholders and genetics professionals were shown to be just as favorable of HGE as the general American public. In spite of their growing optimism, SCD stakeholders are more worried than genetics professionals about the dangers of HGE. Rather than implying a lack of capacity to appreciate HGE's complexity, SCD stakeholders' optimistic perspective for the future is based on the assumption that the burden of sickness may outweigh moral concerns.

Despite the huge promise of genome editing, there is also a great deal of risk. Those with Sickle Cell Disease (SCD), the most common hereditary blood disorder, will be the first to reap the benefits of this new diagnostic technique. In contrast, the patient community is unfamiliar with this new technology. In order to acquire a better understanding of what patients, parents, and physicians think about future CRISPR-mediated somatic genome editing clinical trials, this research aims to gather information from these groups.

Our study made use of an educational video tool, a two-part survey, and 15 moderated, audio-recorded focus groups held across seven American cities. SCD patients may now look forward to a less painful, stigmatized, and ignored future owing to improvements in gene editing research. The SCD group is medically disqualified, so this optimism is tinged with anxiety (Persaud et al., 2019). When conducting clinical trials, present and future research findings are critical, particularly when it comes to patient involvement and consent. As a starting point for future research, the perspectives of SCD stakeholders may help provide light on the goals of new patient groups and those who are presently being considered for CRISPR applications

However, before the patients are subjected to clinical trials, informed consent the patients is important. According to Stacy Desine and colleagues (2020) in their paper "The Meaning of Informed Consent: Genome Editing Clinical Studies for Sickle Cell Disease," sickle cell anemia participants should have the option to express their informed consent in clinical trials. Somatic genome editing has already been used to treat sickle cell disease (SCD), which affects more than a quarter-million people in the United States (SGE). SGE developments rely heavily on patient participation in first-in-human clinical studies. People who are ill may overestimate the benefits of early research while underestimating the risks. SGE clinical trial participants must be aware of the study's risks and benefits before agreeing to participate (Desine et al., 2020). A mixed-methods study was conducted on adults with SCD, as well as their parents and clinicians. Participants were asked to watch an educational video on genome editing, complete a two-part survey, and engage in focus group discussions. Discussions in focus groups included clinical trials, gene editing ethics, and what the researchers still don't know about gene editing. All of the focus groups were analyzed using traditional content analysis methods.

For this study, the researchers polled the views of SCD stakeholders on genome editing in order to find out what information they wanted in order to make an informed decision about taking part in a clinical trial using SGE. Concerns concerning SGE's potential adverse effects, method of action, and impact on quality of life were raised by a variety of interested parties. More genetic literacy was found in SCD patients than clinicians had expected, raising concerns about the patients' grasp of SGE-related concepts.

The SCD community must be involved in culturally appropriate ways in order to create ethically acceptable genome-editing clinical trials so that people may make informed decisions about participating in research.

All the studies discussed in the review expand on the possibility of the treatment of SCD using gene therapy. While most of the articles are focused on highlighting the potential that gene therapy holds for treating SCD, two of the discussed studies also shed light on the stakes that the treatment holds and the need for the patients to be aware of the limitations of the treatment. In addition, one study also highlights the need for informed consent for patients participating in the trial studies, so no ethical dilemma or concerns arise in the medical community concerning the experimentation studies of the treatment.

References

Beet, E. A. (1947). The genetics of the sickle-cell trait in a Bantu tribe. *Annals of Eugenics*, *14*(1), 279–284.

Bolaños-Meade, J., Fuchs, E. J., Luznik, L., Lanzkron, S. M., Gamper, C. J., Jones, R. J., & Brodsky, R. A. (2012). HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood, The Journal of the American Society of Hematology*, *120*(22), 4285–4291.

Demirci, S., Uchida, N., & Tisdale, J. F. (2018). Gene therapy for sickle cell disease: An update. *Cytotherapy*, *20*(7), 899–910.

Desine, S., Hollister, B. M., Abdallah, K. E., Persaud, A., Hull, S. C., & Bonham, V. L. (2020). The meaning of informed consent: Genome editing clinical trials for sickle cell disease. *AJOB Empirical Bioethics*, *11*(4), 195–207.

Hoban, M. D., Lumaquin, D., Kuo, C. Y., Romero, Z., Long, J., Ho, M., Young, C. S., Mojadidi, M., Fitz-Gibbon, S., & Cooper, A. R. (2016). CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. *Molecular Therapy*, *24*(9), 1561–1569.

Hollister, B. M., Gatter, M. C., Abdallah, K. E., Armsby, A. J., Buscetta, A. J., Byeon, Y. J. J., Cooper, K. E., Desine, S., Persaud, A., & Ormond, K. E. (2019). Perspectives of sickle cell disease stakeholders on heritable genome editing. *The CRISPR Journal*, *2*(6), 441–449.

Lattanzi, A., Camarena, J., Lahiri, P., Segal, H., Srifa, W., Vakulskas, C. A., Frock, R. L., Kenrick, J., Lee, C., & Talbott, N. (2021). Development of β-globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. *Science Translational Medicine*, *13*(598), eabf2444.

Persaud, A., Desine, S., Blizinsky, K., & Bonham, V. L. (2019). A CRISPR focus on attitudes and beliefs toward somatic genome editing from stakeholders within the sickle cell disease community. *Genetics in Medicine*, *21*(8), 1726–1734.

Rees, D. C., Williams, T. N., & Gladwin, M. T. (2010). Sickle-cell disease. *The Lancet*, *376*(9757), 2018–2031.

Romero, Z., Lomova, A., Said, S., Miggelbrink, A., Kuo, C. Y., Campo-Fernandez, B., Hoban, M. D., Masiuk, K. E., Clark, D. N., & Long, J. (2019). Editing the sickle cell disease mutation in human hematopoietic stem cells: Comparison of endonucleases and homologous donor templates. *Molecular Therapy*, *27*(8), 1389–1406.

Stuart, M. J., & Nagel, R. L. (2004). Sickle-cell disease. *The Lancet*, *364*(9442), 1343–1360.