The Sickle Cell-Malaria Connection and the Promise of CRISPR Technology

Hamsa Javagal[1] and Lawrence Tartaglia[#]

[1]Lehigh University, USA
[#]Advisor

ABSTRACT

Malaria is transmitted primarily by female Anopheles mosquitoes carrying *Plasmodium falciparum* and is a significant public health challenge in regions like Africa, South America, and Asia. Sickle Cell Disease (SCD), on the other hand, is a genetic disorder causing abnormal hemoglobin production (HbS) which poses severe health risks, especially in Malaria-endemic regions. This manuscript explores the relationship between Malaria and SCD, specifically the protective advantage that Sickle Cell Trait (SCT) can produce from having one copy of the HbS allele. Despite the many commonalities between Malaria and SCD, such as overlapping symptoms, the treatment for each differs significantly. Therefore, accurate diagnoses must be made before continuing with treatment. Current SCD treatments focus on symptom management and complication prevention, while Malaria treatments usually involve antimalarial medications. Recent innovations suggest a cure for SCD and Malaria, which is possible by changing an individual's SCD status to SCT to remedy the negative complications of SCD while maintaining the protective benefit against Malaria. The innovation being considered is the integration of gene editing technologies, such as CRISPR, in conjunction with bone marrow transplants (BMTs). The potential of these therapies, the ongoing research to ensure their safety, and the challenges of making these treatments accessible in low-resource settings are of the utmost importance.

Background Information

Malaria is a life-threatening disease that can be transmitted to humans by infected mosquitos and is mainly prevalent in regions of Africa, South America, and Asia. Specifically, the female *Anopheles (*genus*)* mosquitos are responsible for transmitting *Plasmodium falciparum* (*P. falciparum*), the causative agent of Malaria [1]. The transmission cycle begins when a mosquito feeds on a human host, contracting *P. falciparum* at a rate of about 7%, and then a potential transmission through a secondary bite on an uninfected human host [13]. After being bitten, humans can transmit the parasite to a mosquito, even if asymptomatic, as soon as gametocyte production begins, which takes about 10 days to occur [14]. Symptoms may start as early as a week or as late as over a year after infection [2]. This variability in symptom onset is due to *P. falciparum's* ability to stay dormant during times of low nutrition [15]. The most common symptoms include fever and flu-like illnesses, chills, headache, muscle aches, tiredness, nausea, vomiting, and diarrhea, which can also lead to more severe complications, such as anemia, jaundice, kidney failure, seizures, mental confusion, coma, and even death.

Some individuals may face such symptoms constantly while others face them periodically or recurringly. This is due to the life cycle of *P. falciparum*, specifically the reproductive stage (Figure 1). After entering the bloodstream from the salivary glands of the mosquito, the sporozoites travel to the liver [3]. There, they invade liver cells (hepatocytes) and perform multiple rounds of nuclear division within a single hepatocyte through asexual reproduction. Each nucleus then becomes encased in cytoplasm, forming a structure called the merozoite. Merozoites infect and multiply in the red blood cells (RBCs), using a 1- to 3-day developmental cycle [4]. As the parasite continues to reproduce the host may subsequently experience symptoms that could subside until the next reproductive cycle.

Most Malaria cases occur in tropical and subtropical regions of Africa, Central and South America, Asia, and Oceania [5]. Among these areas, the transmission rate and risk of infection vary widely, with over 90 percent of clinical Malaria infections and deaths occurring in sub-Saharan Africa alone [5]. Due to such high statistics, treatments for Malaria have been continuously evolving throughout the years. The most common antimalarial drugs include Chloroquine phosphate, Artemisinin-based combination therapies (ACTs), Atovaquone-proguanil, Quinine sulfate with doxycycline, and Primaquine phosphate, with ACTs currently recognized as the most effective treatment for *P. falciparum* Malaria [6]. Vector management measures, such as insecticides, environmental modification, and bed nets, are also imperative in slowing Malaria transmission [7].

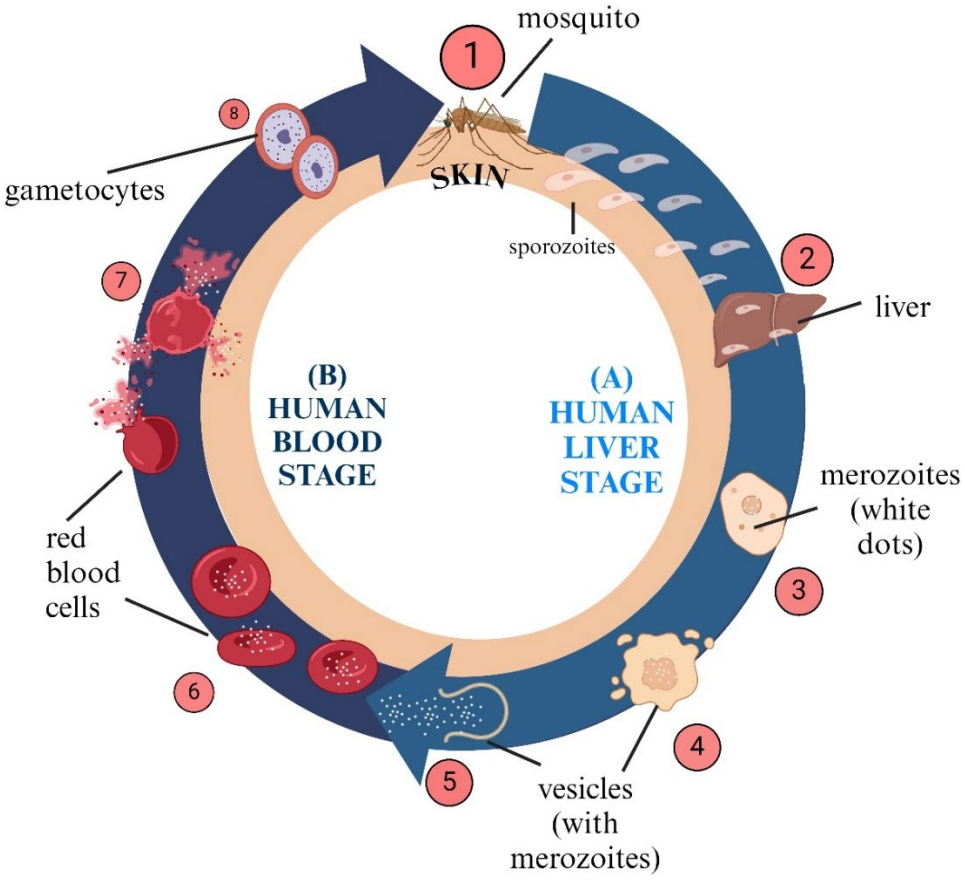

Figure 1. The Life Cycle of Plasmodium falciparum in Humans. (A) The human liver-stage comprises multiple events: (1) Mosquito bites uninfected humans and transfers sporozoites. Then, (2) sporozoites travel to the liver where they invade hepatocytes and mature. (3) Matured sporozoites multiply and transform into merozoites. The (4) vesicles containing merozoites burst, releasing merozoites into the bloodstream. (5) Free merozoites invade RBCs and begin to multiply within them. After this, the (B) Human Blood Stage follows where (6) multiplication of merozoites inside RBCs causes the cells to burst, releasing the merozoites to infect additional RBCs. (7) Some merozoites develop into gametocytes, which are necessary for transmission and replication in mosquitoes. (8) When another mosquito bites the infected host, it ingests the gametocytes, continuing the entire cycle in the new mosquito.

Despite Malaria's negative impacts on human health, some individuals are 'immune' to Malaria's pathogenic effects through a unique genetic disorder. For example, Sickle Cell Disease (SCD) is a genetic disorder that causes a mutation in hemoglobin, a protein complex that transmits oxygen in RBCs [8]. This mutation occurs at chromosome 11p15.5 and results in the production of abnormal hemoglobin (HbS), which differs from normal adult hemoglobin

(HbA) [16]. SCD has an autosomal recessive inheritance pattern, meaning both parents must carry a copy of the recessive allele inheritance from parent to offspring [8]. When an individual receives two recessive alleles, they are recognized to have SCD.

As depicted on the left side of Figure 2, RBCs are naturally round and circular. They flow through the many blood vessels in the human body to deliver oxygen to body tissues. However, in those with SCD blood cells become hard, sticky, and C-shaped, also known as a sickled form. Sickled hemoglobin has a greater risk of aggregating in blood vessels and arteries, which can lead to blockages, a phenomenon demonstrated in Figure 2B.

Such blockages are also known as vaso-occlusion and can lead to serious and often fatal complications if not treated promptly. The most common and notable symptom is vaso-occlusive crises, which cause constant and unbearable body pain in the areas where blood flow has been obstructed from aggregated sickled RBCs [9]. Furthermore, there are additional common symptoms of SCD including fever, tiredness, jaundice, swelling, anemia, organ damage, chest pain, stroke, shortness of breath, mental confusion, and even death [10]. SCD is also known to severely weaken the immune system leaving the body prone to contracting other infections, such as pneumonia, meningitis, and septicemia [17].

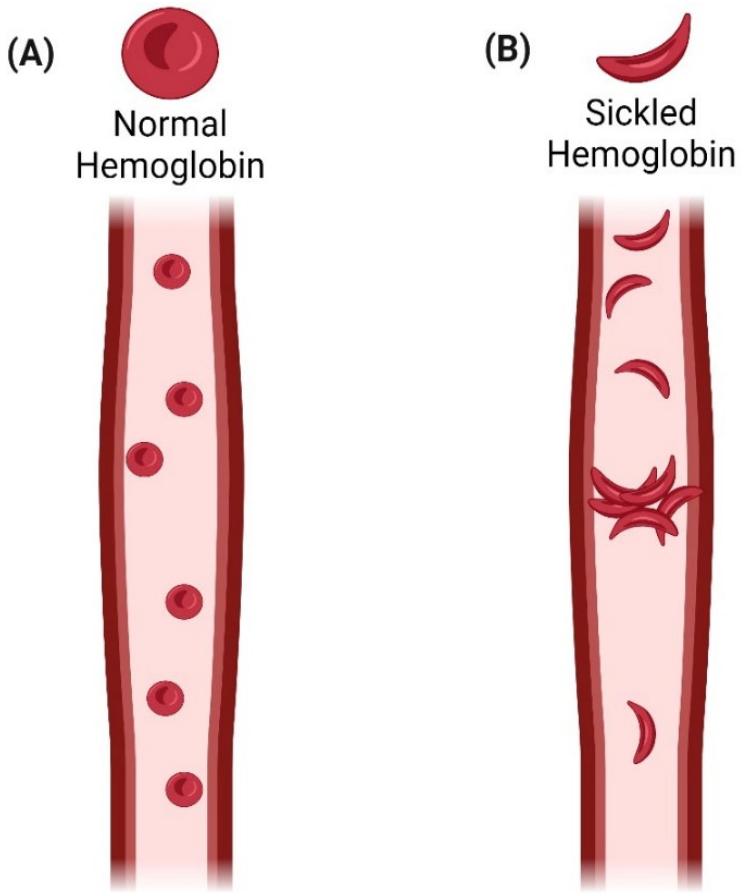

Figure 2. Normal Hemoglobin Compared to Sickled Hemoglobin. (A) Normal hemoglobin is defined by circular-shaped red blood cells, whereas (B) sickled hemoglobin is defined by crescent moon-shaped red blood cells.

Most SCD cases occur in people of African descent, Hispanic Americans from Central and South America, and Middle Eastern, Asian, Indian, and Mediterranean descent, with the global mortality approximately being 4.8 to 5.1 deaths per 100,000 people [18]. However, of these regions, Africa faces the greatest burden with an estimate of 50%

to 90% of children with SCD in this country dying before the age of five [11]. There are many medications available to help prevent and manage the onset of pain crises. Over-the-counter medications, such as acetaminophen or ibuprofen, can treat mild or moderate pain; however, for more severe pain, hospital visits may be necessary, and doctors may treat patients with stronger pain medications such as opioids. Medications to treat vaso-occlusive crises and prevent sickling of RBCs are also available, including Hydroxyurea, Voxelotor, L-glutamine, and Crizanlizumab-tmca [12]. Additionally, Penicillin is often prescribed to address the increased risk of infection that SCD causes, and transfusions may be necessary to bring the patient's RBC count back to normal [12]. Regarding cures for this disease, bone marrow transplants (BMTs) were the only viable option until recently, as the FDA approved two new genetic therapies – one that adds a gene to the body and the other makes changes to a gene already in the body [12].

Relationship Between SCD and Malaria

Despite their differences, SCD and Malaria have commonalities. For example, the symptoms between the two are almost identical, such as fever, tiredness, jaundice, anemia, mental confusion, and death. More importantly, the regional locations of the diseases overlap. This overlap is significant because it raises the question of why it exists and whether the explanation is causative or independent of one another. The answer is that it is causative, specifically regions with a high prevalence of Malaria are also seeing a notable increase in individuals testing positive for SCD [17]. The prevailing theory for this correlation is due to a concept called the heterozygous advantage, or the advantage of having the Sickle Cell Trait (SCT).

The Heterozygous Advantage

Individuals with SCT have the genotype of HbAS, meaning they received a copy of the sickled hemoglobin allele (HbS) from one parent and a copy of the normal hemoglobin allele (HbA) from the other [21]. The Punnett Square shown in Figure 3 depicts the genetic basis for the HbAS genotype. The HbAS genotype can provide many benefits; however, to explore these advantages, it is important to understand the specific distinctions between SCD and SCT phenotypically, as well.

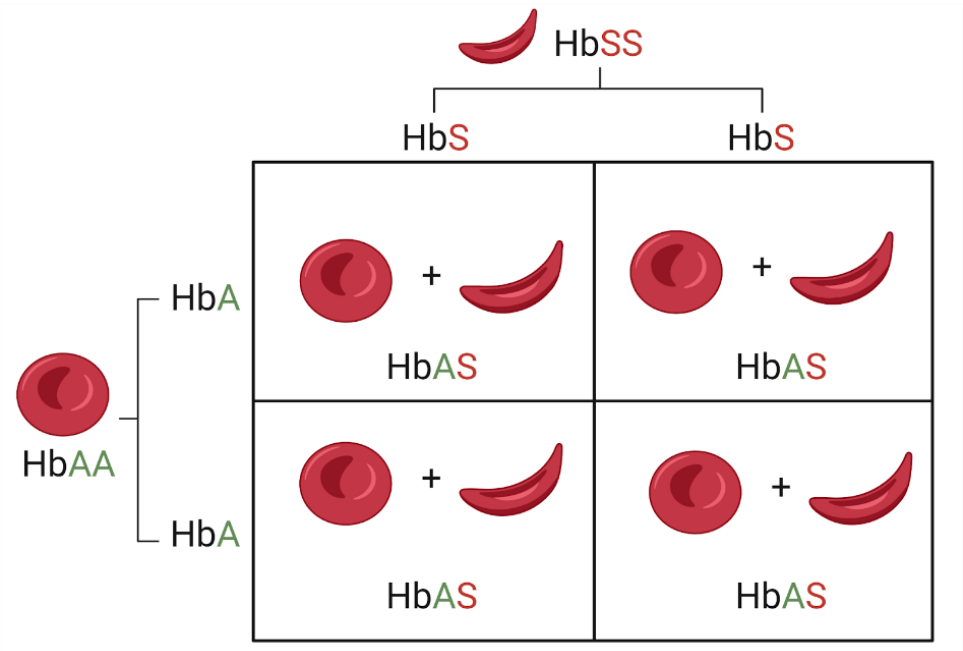

Figure 3. Punnett Square of Normal vs. Sickled Hemoglobin Genotypes. HbAA represents normal hemoglobin, HbSS represents sickled hemoglobin, and HbAS indicates the genotype for the Sickle Cell Trait, which produces both normal and sickled hemoglobin.

A few notable differences exist between SCD and SCT (Figure 4). Individuals with SCT are heterozygous for the disease, allowing them to produce both normal and sickle-shaped hemoglobin. In contrast, individuals with SCD, who are homozygous recessive, carry two copies of the HbS allele only, resulting in exclusive production of sickle RBCs. Therefore, those with SCD are often subject to severe symptoms due to sickled RBCs obstructing blood flow whereas, those with SCT may only face mild symptoms, if any, due to their possession of the HbA allele. Furthermore, although those with SCD have abundant sickle RBCs in their bloodstream, those with SCT have predominantly normal-shaped RBCs, with a small percentage exhibiting sickling under low oxygen conditions.

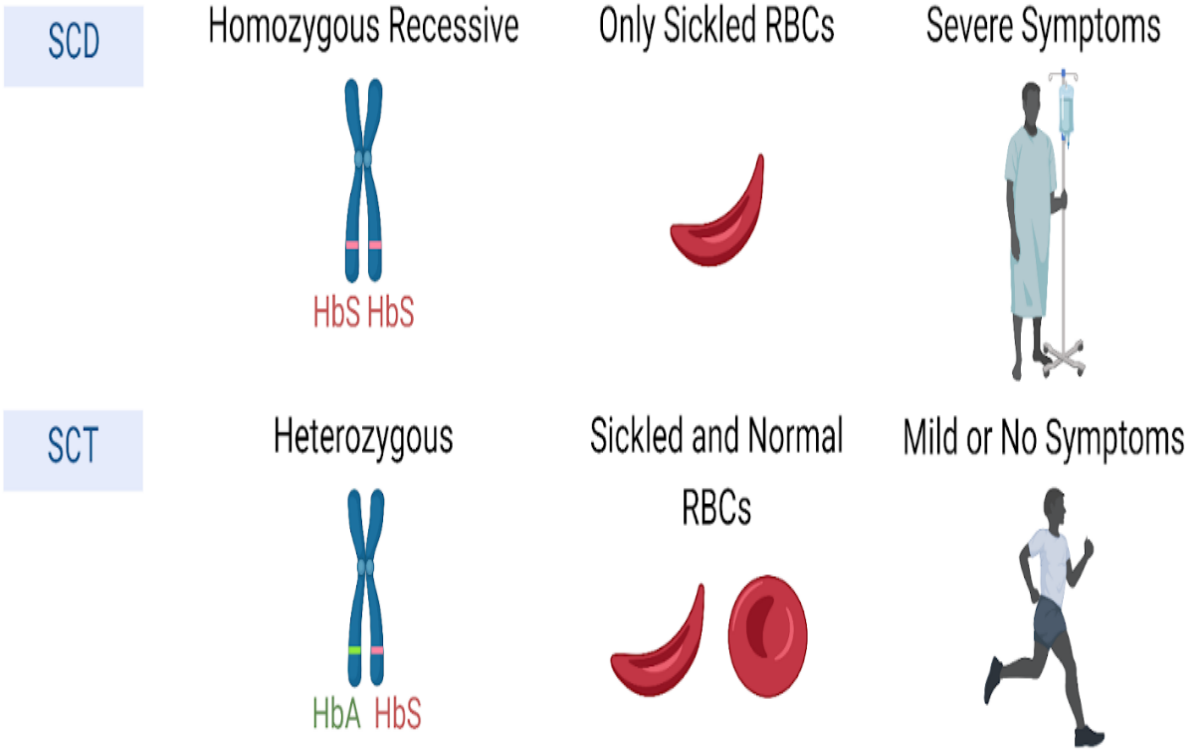

Figure 4. Comparing SCT and SCD.

As Malaria is a bloodborne pathogen, knowing one's Sickle Cell status is particularly relevant in Malaria-endemic regions since SCT is known to have certain survival benefits, hence the term Heterozygous Advantage. To be exact, the small number of sickled RBCs in those with SCT is the factor helping to reduce Malaria's reproduction in the human body and, in turn, its transmission [22]. Although the SCT individual can still be bitten, deliver the sporozoites to the liver, and create merozoites to be transported through the bloodstream, the cycle often gets halted at the Human Blood Stage (Figure 1). This is because the merozoites rely on RBCs for many reasons: to infect other RBCs, multiply within RBCs, and then turn into gametocytes, which is a necessary step for transmission to mosquitoes. However, in individuals with SCT, the infected RBCs are more likely to sickle, making them prone to hemolysis, the process of being destroyed by the body's immune system and removed by the spleen.

Hemolysis significantly disrupts the parasite's development and ability to reach the gametocyte stage, resulting in individuals with SCT experiencing milder forms of Malaria. Specifically, a study by Thomas N. Williams, et al. observed SCT to be 90% protective against severe or complicated Malaria because these individuals have lower parasite densities in their bloodstream [19]. Compared with people with normal hemoglobin, individuals with SCT have about a 50 - 90% reduction in parasite density, leading to fewer complications and symptoms if infected with Malaria [24]. This protective effect explains why SCT is more prevalent in areas where Malaria thrives since these individuals have a higher chance of surviving and passing down a copy of the HbS gene to future generations.

With this finding, it might be compelling to assume that since SCT poses an advantage against Malaria, SCD should as well due to its ability to produce sickled RBCs; however, this is not exactly the case. Those with SCD often face many adverse effects when contracting Malaria compared to those with SCT (Figure 5) [23]. One of the reasons for this is due to the side effects of having SCD: increased risk of infection. With the damage to the spleen, the main organ for producing red and white blood cells, the body's immune system becomes compromised and finds it harder to protect itself from other bacteria and viruses, such as Malaria. Therefore, not only is Malaria able to survive in SCD individuals, but it is also able to thrive, reproducing abundantly and causing a higher risk of death to these human hosts.

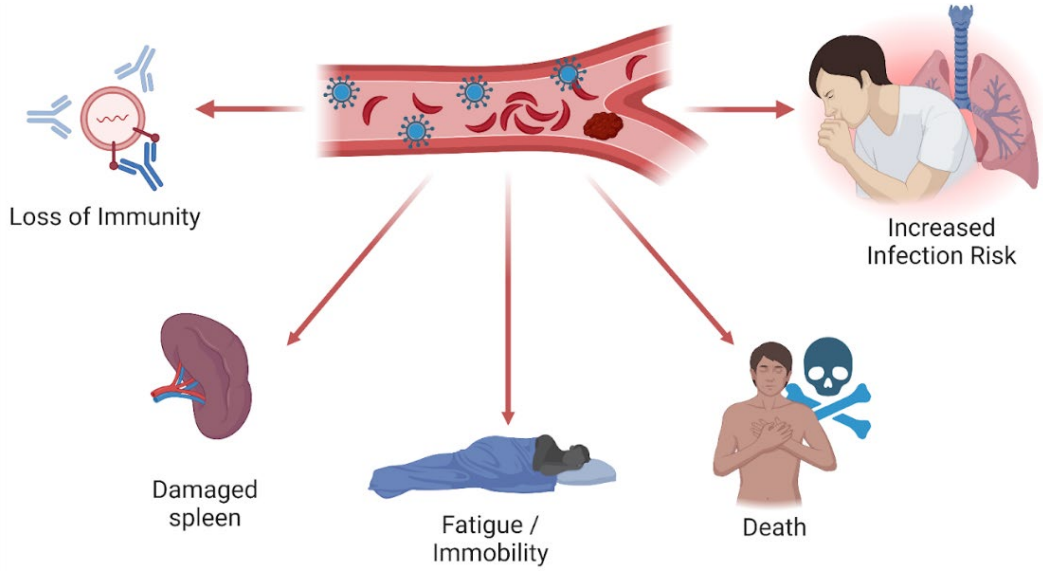

Figure 5. Complications in those with SCD and Malaria.

Additionally, those with SCD often face increased fatigue and exhaustion that comes with Sickle Cell, causing many to be on bed rest and immobile [25]. This immobility makes them more prone to coming into contact with a Malaria-infected mosquito that is looking for its next blood meal. Not having adequate energy can also cause individuals to fail to take precautionary measures, such as setting up bed nets or swatting at mosquitos. Such reasons contribute to increased Malaria-induced death rates among individuals with SCD and those with SCT.

However, it has been proven that those with SCD may face some survival advantages against Malaria compared to those who have only normal hemoglobin and no sickle cell allele. For example, the overall mortality in patients with SCD was noted to be 3% while the mortality rate of the non-disease population was much higher at 9% [20]. Additionally, Malaria accounted for approximately 35.4% of deaths in the non-SCD population while in the SCD population, it accounted for slightly less, at 20.4%. These statistics show that although Malaria-induced SCD mortality continues to be higher than in those with SCT, having two copies of the HbS allele might still provide some benefit compared to not having any abnormal hemoglobin at all.

Treatment Options and Prevention Strategies

Differentiating between Malaria and SCD can often be challenging due to their overlapping symptoms, especially in underdeveloped regions such as Africa since access to proper diagnostic equipment is limited. This often leads to misdiagnoses, resulting in improper treatment and prolonged patient suffering. Although the symptoms of both illnesses overlap, the treatment options vary significantly, making accurate diagnosis crucial before initiating any treatment. For example, Malaria treatment typically involves antimalarial medications, with Artemisinin-based combination therapies (ACTs) being at the frontline of treatment [2]. Chloroquine, which was once the standard treatment for Malaria, is now not as effective due to the widespread resistance in P. falciparum. Other antimalarials, such as quinine, mefloquine, and atovaquone-proguanil, are used in specific cases, particularly for severe Malaria or when resistance to the other drugs is detected.

On the other hand, SCD treatment strategies primarily focus on managing symptoms, preventing complications, and reducing the frequency of pain crises [10]. Hydroxyurea is a common medication SCD patients use as it increases fetal hemoglobin (HbF) production. Increasing the amount of HbF reduces the frequency of pain crises as it has a higher affinity for oxygen than even HbA. Regular blood transfusions are also employed to manage severe anemia by increasing the number of healthy red blood cells in circulation, which is especially important for those with severe SCD complications. Additionally, the use of over-the-counter pain relievers for mild pain, prescription opioids for severe pain, and physical therapy are critical. Alongside medication, preventive measures like vaccines and antibiotics, are needed to combat the high risk of infection that usually comes along with those with SCD. Lastly, it is important to encourage prenatal and newborn screening to know one's SCD status and also emphasize family planning for those who already know their status so that preventative measures can be taken from day one.

Bone Marrow Transplants

Weighing the benefits of having SCT and the negative complications of SCD in individuals battling Malaria, there have been many theories for what the best treatment strategies are for those who have both conditions. One of these medical advancements is known as bone marrow transplants (BMTs) [27]. BMTs replace the SCD patient's bone marrow with that from a compatible donor capable of producing HbA. While this procedure may come with many benefits, such as the potential to cure SCD, it also has significant risks, the common ones including infections and the need for lifelong immunosuppression. A more serious side effect includes graft-versus-host disease, an immune response that recognizes the recipient's tissue as foreign and attacks it. Moreover, one of the biggest hurdles to cross remains to be finding a suitable donor, especially in regions with limited healthcare access and resources. Research is still ongoing in this field and is focused on refining this procedure to make it safe, accessible, and reliable in the long run.

CRISPR

However, researchers have recently found a new solution that integrates BMTs with a new gene editing technology called CRISPR [26]. The general concept of this approach is depicted in Figure 6, where CRISPR technology converts the SCD genotype to SCT by editing the β-globin gene to replace one of the two HbS alleles with an HbF allele. The modified hematopoietic stem cells are then engrafted into the bone marrow so they can begin to produce RBCs that express both HbS and HbF. Using BMTs and CRISPR alongside one another has the potential to offer a long-lasting solution to the pain people with SCD face while also providing the benefits of the Heterozygous Advantage against Malaria. It also increases the possibility of using the patient's own cells by editing them to correct the genetic defect and reintroducing them back into the body. This reduces the risks associated with donor compatibility and immune rejection while decreasing the ethical and practical issues of finding a suitable donor.

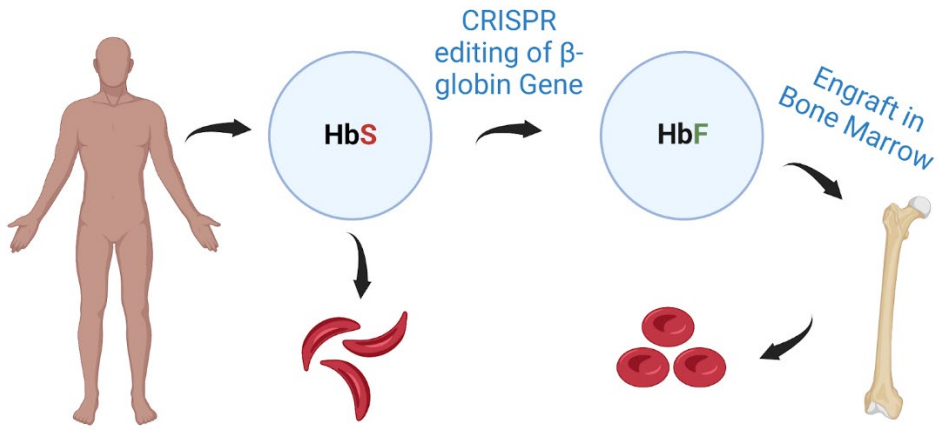

Figure 6. CRISPR Technology Modifying HbS to HbF. The HbS alleles in the human body, which produce Sickled RBCs, are modified through CRISPR editing of the β-globin Gene to turn into HbF, which is fetal hemoglobin and produces circular RBCs. This is then engrafted into the bone marrow, which allows for the future production of circular RBCs.

To understand the CRISPR/Cas9 mechanism more specifically, it is pertinent to see how it is applied at the molecular level (Figure 7). CRISPR/Cas9 targets and edits the HBB gene, introducing double-stranded breaks at specific mutation sites. These breaks can be repaired through Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR). This approach has demonstrated the potential to convert the SCD genotype to SCT, increasing HbF levels and reducing the number of sickle RBCs. The main advantage is that it retains the natural protective effect against malaria, similar to SCT, which could benefit those living in malaria-endemic regions. However, the safety and efficacy of gene editing are still being tested to ensure that negative side effects are minimal and that the long-term benefits are worth the risks.

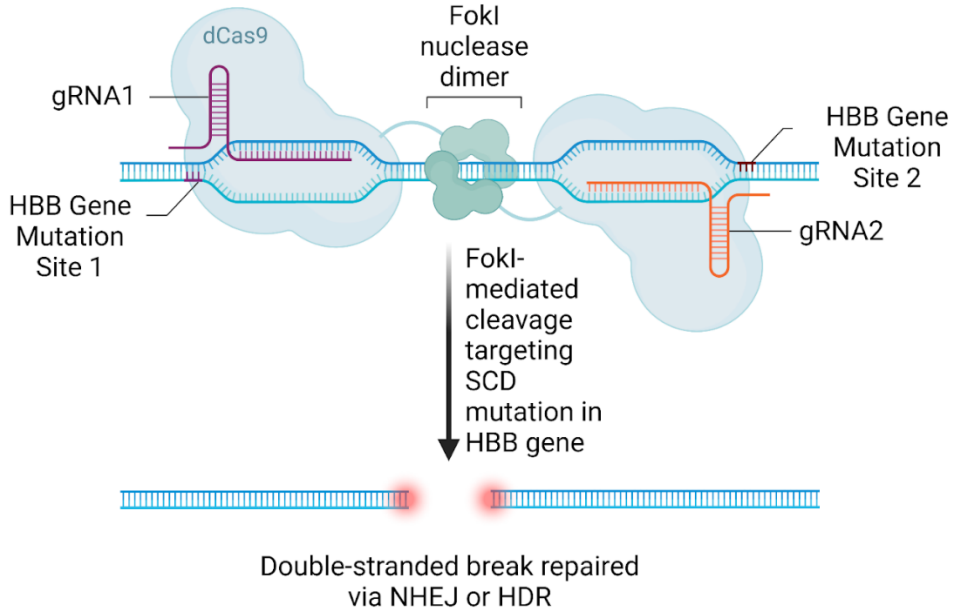

Figure 7. CRISPR/Cas9 Gene Editing for Sickle Cell Disease. CRISPR/Cas9 technology targets and edits the mutation in the HBB gene, which causes the production of the abnormal hemoglobin variant HbS in SCD patients. Guide

RNAs (gRNAs) direct the Cas9-FokI nuclease complex to specific mutation sites within the HBB gene, where FokI-mediated cleavage introduces double-stranded breaks. These breaks can be repaired through Non-Homologous End Joining (NHEJ), which may disrupt the BCL11A gene to increase fetal hemoglobin production or Homology-Directed Repair (HDR), which can precisely correct the HbS mutation.

Conclusion and Future Directions

In summary, addressing the challenges posed by SCD and Malaria in regions where they are both prevalent requires solutions that are feasible in the long run and viable in low-resource settings. One potential solution is the integration of gene editing technologies, such as CRISPR, with BMTs. This combination could reduce the severe complications that SCD individuals face while maintaining the protective advantage that having a copy of the HbS allele can offer. However, this innovation is still being tested further to assess its long-term effects on patients, the possibility for unforeseen off-target genetic changes, and how edited cells will behave once reintroduced into the body. Additionally, the immune response to gene-edited cells and its durability over a patient's lifetime remain active research areas. Furthermore, the availability of suitable donors and the accessibility of these therapies in underdeveloped regions are still yet to be fully explored. As research in this field continues to expand, such strategies may be able to completely revolutionize treatment options for those battling both SCD and Malaria, ultimately improving patient outcomes and quality of life.

References

[1]Shaw WR, Marcenac P, Catteruccia F. Plasmodium development in Anopheles: a tale of shared resources. *Trends Parasitol.* 2022;38(2):124-35. doi: 10.1016/j.pt.2021.08.009.

[2]Centers for Disease Control and Prevention. *Symptoms of malaria.* Atlanta: Centers for Disease Control and Prevention; 2024 Mar 12 [cited 2024 Jul 29]. Available from: https://www.cdc.gov/malaria/symptoms/index.html

[3]*Malaria: MedlinePlus Medical Encyclopedia* [Internet]. Bethesda: National Library of Medicine; [cited 2024 Jul 29]. Available from: https://medlineplus.gov/ency/article/000621.htm.

[4]Buck E, Finnigan NA. Malaria. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan– [updated 2023 Jul 31; cited 2024 Jul 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551711/.

[5]Bloland PB, Williams HA; National Research Council (US) Committee on Population; Program on Forced Migration and Health at the Mailman School of Public Health, Columbia University. *Malaria control during mass population movements and natural disasters*. Washington (DC): National Academies Press (US); 2002. Chapter 3, Epidemiology of malaria. Available from: https://www.ncbi.nlm.nih.gov/books/NBK221152/.

[6]Mayo Clinic Health Library. *Malaria - diagnosis & treatment* [Internet]. Rochester (MN): Mayo Clinic; 2023 Feb 9 [cited 2024 Jul 29]. Available from: https://www.mayoclinic.org/diseases-conditions/malaria/diagnosis-treatment/drc-20351190.

[7]National Institute of Allergy and Infectious Diseases. *Malaria prevention, treatment, and control strategies* [Internet]. Bethesda: National Institute of Allergy and Infectious Diseases; 2011 [cited 2024 Jul 29]. Available from: https://www.niaid.nih.gov/diseases-conditions/malaria-strategies.

[8]National Heart, Lung, and Blood Institute. *Sickle cell disease: causes and risk factors* [Internet]. Bethesda: National Heart, Lung, and Blood Institute; [cited 2024 Jul 29]. Available from: https://www.nhlbi.nih.gov/health/sickle-cell-disease/causes.

[9]Elendu C, Amaechi DC, Alakwe-Ojimba CE, et al. Understanding sickle cell disease: causes, symptoms, and treatment options. *Medicine (Baltimore)*. 2023;102(38). doi: 10.1097/MD.0000000000035237.

[10]National Heart, Lung, and Blood Institute. *Symptoms of sickle cell disease* [Internet]. Bethesda: National Heart, Lung, and Blood Institute; [cited 2024 Jul 29]. Available from: https://www.nhlbi.nih.gov/health/sickle-cell-disease/symptoms.

[11]Oron AP, Chao DL, Ezeanolue EE, et al. Caring for Africa's sickle cell children: will we rise to the challenge? *BMC Med*. 2020;18(1):92. doi: 10.1186/s12916-020-01557-2.

[12]National Heart, Lung, and Blood Institute. *Sickle cell disease treatment* [Internet]. Bethesda: National Heart, Lung, and Blood Institute; [cited 2024 Jul 29]. Available from: https://www.nhlbi.nih.gov/health/sickle-cell-disease/treatment.

[13]Miura K, Crompton PD. What goes around comes around: modeling malaria transmission from humans back to mosquitos. *J Clin Invest*. 2018 Mar 12;128(4):1264-6. doi: 10.1172/JCI120260.

[14]Institute of Medicine (US) Committee for the Study on Malaria Prevention and Control; Oaks SC Jr, Mitchell VS, Pearson GW, et al., editors. *Malaria: obstacles and opportunities*. Washington (DC): National Academies Press (US); 1991. Chapter 6, Parasite biology. Available from: https://www.ncbi.nlm.nih.gov/books/NBK234327/.

[15]Habtewold T, Sharma AA, Wyer CAS, Masters EKG, Windbichler N, Christophides GK. Plasmodium oocysts respond with dormancy to crowding and nutritional stress. *Sci Rep*. 2021 Feb 4;11(1). Available from: https://www.nature.com/articles/s41598-021-81574-0.

[16]National Center for Biotechnology Information (US). *Genes and disease* [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 1998-. Anemia, sickle cell. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22238/.

[17]Indian J, Hematol. Sickle cell disease and malaria. *Blood Transfus*. 23(3-4):70-2. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3453113/pdf/12288_2008_Article_1.pdf.

[18]GBD 2021 Sickle Cell Disease Collaborators. Global, regional, and national prevalence and mortality burden of sickle cell disease, 2000-2021: a systematic analysis from the Global Burden of Disease Study 2021. 2023. Available from: https://doi.org/10.1016/S2352-3026(23)00118-7.

[19]Williams TN, Mwangi TW, Wambua S, et al. Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. *J Infect Dis*. 2005;192(1):178-86. doi: 10.1086/430744.

[20]Eleonore NLE, Cumber SN, Charlotte EE, et al. Malaria in patients with sickle cell anemia: burden, risk factors and outcome at the Laquintinie hospital, Cameroon. *BMC Infect Dis*. 2020;20(1):40. Doi: 10.1186/s12879-019-4757-x.

[21]Ashorobi D, Ramsey A, Killeen RB, et al. Sickle cell trait. [Updated 2024 Feb 25]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537130/.

[22]National Center for Biotechnology Information (US). *A guide to principles and practices in scientific research* [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); [cited 2024 Jul 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK588130/pdf/Bookshelf_NBK588130.pdf.

[23]McAuley CF, Webb C, Makani J, et al. High mortality from Plasmodium falciparum malaria in children living with sickle cell anemia on the coast of Kenya. *Blood*. 2010;116(10):1663-8. doi: 10.1182/blood-2010-01-265249.

[24]Archer NM, Petersen N, Clark MA, Buckee CO, Childs LM, Duraisingh MT. Resistance to Plasmodium falciparum in sickle cell trait erythrocytes is driven by oxygen-dependent growth inhibition. *Proc Natl Acad Sci U S A*. 2018;115(28):7350-5. doi: 10.1073/pnas.1804388115.

[25]Keidan AJ, Stuart J. Rheological effects of bed rest in sickle cell disease. *J Clin Pathol*. 1987;40(10):1187-8. doi: 10.1136/jcp.40.10.1187.

[26]Park, S. H., & Bao, G. (2021). CRISPR/Cas9 gene editing for curing sickle cell disease. Transfusion and apheresis science: official journal of the World Apheresis Association: official journal of the European Society for Haemapheresis, 60(1), 103060. https://doi.org/10.1016/j.transci.2021.103060.

[27]Ashorobi D, Naha K, Bhatt R. Hematopoietic stem cell transplantation in sickle cell disease. [Updated 2023 Jul 19]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538515/.