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Sickle Cell Disease and its Revolutionised Treatment: A Crispr/Cas9 Tale

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Abstract: ***Sickle cell disease (SCD) is a hereditary disorder characterized by the abnormal shape of red blood cells due to a mutation in the HBB gene on chromosome 11. This condition leads to various symptoms, including severe pain, fatigue, and complications such as vaso-occlusive crises. Traditional treatments have focused on symptom management, with recent advancements in therapies like hydroxyurea and monoclonal antibodies providing some relief but not addressing the root cause. The advent of CRISPR/Cas9 gene editing technology represents a significant breakthrough in the potential treatment of SCD, allowing for precise correction of genetic defects or reactivation of fetal hemoglobin production. Early clinical trials, particularly the CTX001 therapy, have shown promising results, leading to the first regulatory approval of a CRISPR-based treatment. However, challenges remain, including safety concerns, ethical implications, and accessibility issues, particularly in low-resource settings. Future advancements in gene editing techniques and delivery systems, alongside equitable healthcare policies, are crucial for the widespread adoption of CRISPR therapies in SCD management. This review highlights the transformative potential of CRISPR/Cas9 in addressing the genetic basis of SCD and the ongoing journey towards effective and accessible treatments.***

Keywords: CRISPR/Cas9, Sickle Cell Disease

1. Introduction

Sickle cell disease is an inherited, autosomal recessive group of disorders that gets its name from the crescent or sickle-like shape of red blood cells in patients, which was first described by Herrick. This disease includes sickle cell anemia (SCA), HbSC and HbSβ-thalassaemia, all of which are caused by a point mutation on Chromosome 11. Early symptoms include jaundice, extreme tiredness or fussiness, and painful swelling of hands and feet. Severe pain, fatigue, and fever, along with chest pain, coughing, shortness of breath, and sudden weakness or numbness on one side of the body, can also be observed as symptoms. Priapism is also a common symptom which could lead to erectile dysfunction. Despite the severity and constant research, a definitive cure for this disease was not developed until recently. Earlier treatments focused on minimizing symptoms and preventing complications. The emerging technique of CRISPR gene editing has somewhat revolutionized the treatment of diseases like SCD, allowing in vivo DNA editing as opposed to before, when extraction of tissue samples was required for genome editing. However, there remain challenges and limitations, including safety, ethical and regulatory issues.

Molecular Pathophysiology of SCD

Hemoglobin is a tetrameric protein which consists of two α-globin and two β-globin chains, each associated with a cofactor, **Haem**, which is capable of binding to oxygen. These globin chains are encoded by duplicate copies of the HBA and HBB genes, respectively. A single point mutation in the HBB gene present on chromosome 11 replaces the 17th nucleotide from thiamine to adenine, which in turn causes the addition of the hydrophobic amino acid valine at the sixth residue in place of the hydrophilic glutamic acid. This substitution results in the expression of the βs allele, which gives rise to mutant Hemoglobin S. The β1 chain of Hemoglobin S, in the presence of acidic or hypoxic conditions, binds with the β2 chain of another Hemoglobin S molecule, which leads to a polymer nucleus. The accumulation of these polymers in a red blood cell disrupts its architecture and dehydrates it causing physical and oxidative stress and thus leading the cell to lose its structural integrity and assuming a sickle-cell shape.

The aggregation of sickle erythrocytes with neutrophils, platelets and endothelial cells leads to stagnant blood flow, referred to as vaso-occlusion. Hemoglobin polymer bundles also cause lysis of erythrocytes which in turn leads to release of these polymers into the blood circulation. Oxygenated Hb promotes endothelial dysfunction by converting endothelial Nitric Oxide into nitrate and methanoglobin.

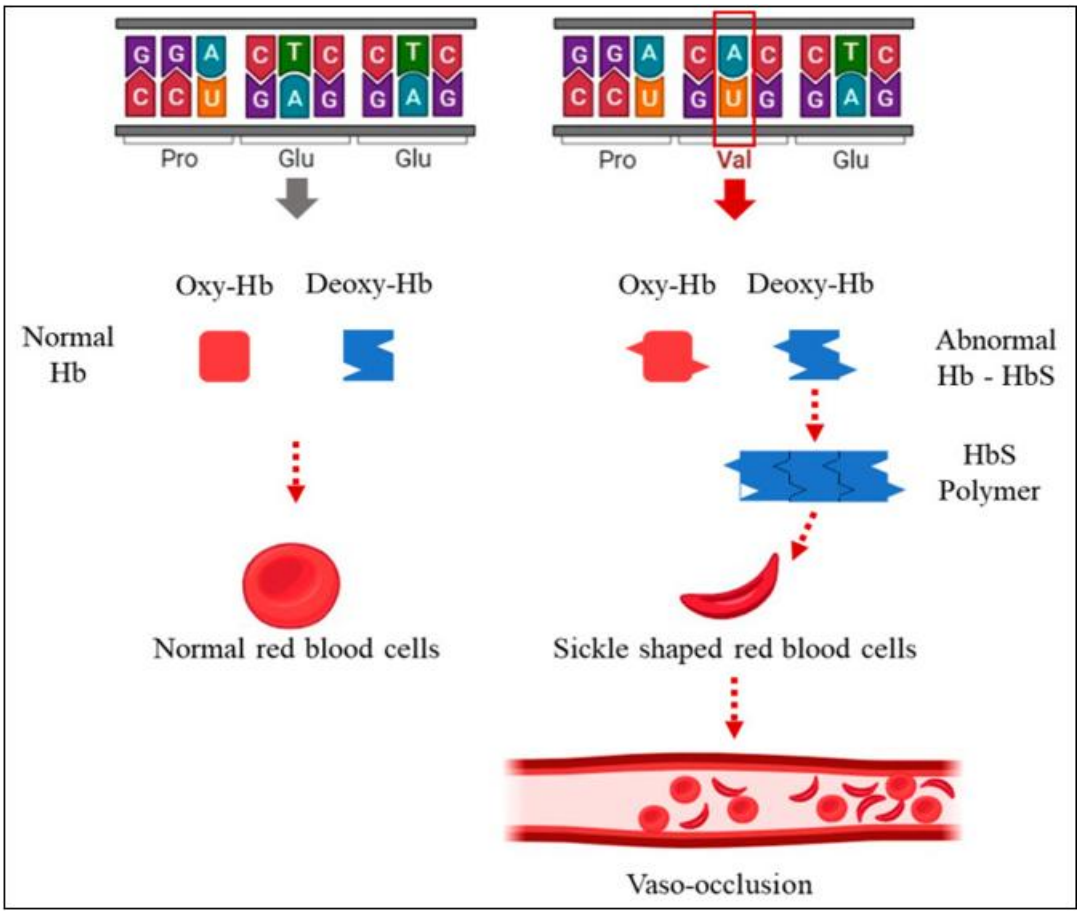

Figure 1

The most common clinical manifestation of sickle cell disease, a vaso-occlusive crisis (VOC) occurring when blood flow is blocked by sickled red blood cells (crescent-shaped) to the point that tissues and organs become deprived of oxygen, causing pain.

Reference: Bell V, Varzakas T, Psaltopoulou T, Fernandes T. Sickle Cell Disease Update: New Treatments and Challenging Nutritional Interventions. Nutrients. 2024 Jan 15;16(2):258. doi: 10.3390/nu16020258. PMID: 38257151; PMCID: PMC10820494

Treatment Strategies Applied in the Past

Various treatments have been used to prevent complications in SCD patients. Options primarily consisted of transfusion, pain management and hydroxyurea until therapies, including **luspatercept** and **crizanlizumab** which reduced vaso-occlusive episodes in SCD patients However, none of them came close to being a curative factor for this debilitating disease.

Blood transfusions help manage severe anemia and prevention of strokes, especially in children. However, long-term transfusion therapy can lead to iron overload and alloimmunization.

Elevated levels of **fetal Hemoglobin (HbF)** (comprising of 2 α and 2 γ-chains) inhibit the polymerization of sickle hemoglobin and are associated with improved morbidity and mortality in patients. **Hydroxuurea** (hydroxycarbamide), a ribonucleotide reductase inhibitor, remains the most commonly used treatment for increment in HbF levels, which also reduces the frequency of pain crises, acute chest syndrome, and the need for transfusions. However, there are concerns about its long term effect.

L-glutamine is believed to reduce oxidative stress in red blood cells, although its precise mechanisms are not fully understood and is therefore used to decrease the frequency of pain crises in patients above 5 years of age.

Voxelator, a small-molecule drug, approved by the FDA in 2019 and the EMA in 2022, reduces hemolytic anemia by stabilizing the oxygenated state of hemoglobin and inhibiting HbS polymerization.

Crizanlizumab, a monoclonal antibody targeting P-selectin, was approved to reduce the frequency of vaso-occlusive crises (VOCs). It works by preventing the adhesion of sickled cells to the vascular endothelium, which plays a central role in the inflammatory cascade that leads to VOCs. Administered monthly, crizanlizumab has been shown to significantly lower the rate of pain crises.

All these therapeutic treatments, however useful in reducing complications, do not touch the underlying cause of SCD. Looking at the global burden that SCD is, there seems an urgent need for curative and accessible therapies, particularly in resource–limited regions.

Potential Curative Strategies

Unlike symptomatic therapies, curative treatments aim to address the underlying cause of SCA and either eliminate

genetic defects or replace the defective hematopoietic system.

Allogenic **hematopoietic stem cell transplantation (HSCT)** is the most established curative option of all which comprises of transplantation of healthy hematopoietic stem cells from a compatible donor to the recipient, providing high long-term survival and disease-free rates. However, there is always a risk of graft rejection, leading to graft-versus-host disease and immune-suppression related complications. Further, the limited availability of suitable donors and the high costs make this treatment strategy relatively inaccessible.

This brought us the most recent and the most promising of all, gene-editing technologies, more specifically CRISPR/Cas9 based therapies. These approaches are aimed towards either correcting the defect in HBB gene or inducing expression of fetal Hemoglobin (HbF) by targeting regulatory genes like BCL11A. CRISPR technology is based on autologous transplant of hematopoietic stem cells which eliminates the risk of graft rejection. Early-phase clinical trials have attained positive results, and while still being under development, CRISPR/Cas9 technology could provide a cure for SCD once and for all.

The Rise of CRISPR/Cas9 Technology

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) provide a gene editing technology which allows researchers to manipulate genomic elements or turn on or off genes rather quickly, easily and cheaply. The origin of this technique comes from the ability of certain bacteria and archaea to immunize themselves from viral infections by storing fragments of that specific viral DNA into their own genome. These stored segments are known as CRISPR and they guide the Cas9 endonuclease to recognize and cut complementary sequences present in viral DNA during secondary infection.

Jennifer Doudna and Emmanuelle Charpentier, in 2012, proposed this technique as a potential alternate for **zinc-finger nucleases** and **transcription-activator–like effector nucleases** mechanisms. They described this now widely used genome-editing technique as **"efficient, versatile, and programmable by changing the DNA target-binding sequence in the guide chimeric RNA"**.

The CRISP-Cas9 system consists of two components:

1) **Cas9 enzyme:** It is an endonuclease enzyme which consists of two domains, each cleaving a single DNA strand at the specific site as guided by gRNA. The Cas9 protein thus leaves the target DNA with a site-specific double stranded break.
2) **Guide RNA (gRNA):** A single transcript that directs Cas9 protein to the specific site within the genome to be cleaved. The transcript includes a 20 nucleotide **spacer sequence** that is complementary to target DNA and a **scaffold** sequence that is the binding site of Cas9.

The CRISPR/Cas9 technique holds several advantages over the previously developed gene editing mechanisms such as being easy to design, cost effective and high-throughput.

Since its discovery, CRISPR/Cas9 has emerged as a powerful tool in medicine, agriculture and biotechnology. It is a considered as a potential tool for curing genetic diseases, developing healthier crops and eliminating infectious diseases.

Majority of the total known 6,000 genetic diseases do not have a definite cure and gene therapy is the latest advancement in biotechnology that aims to find the antidote for these genetic diseases. Between 1998 and August 2019, 22 mechanisms of gene therapy have been approved for the treatment of human diseases, one of which is CRISPR/Cas9.

CRISPR in Sickle Cell Disease

The application of CRISPR/Cas9 technology in SCD can be viewed as a revolution in precision medicine. The technology directly targets the underlying root of the genetic disease. There can be two approaches of CRISPR towards treatment of SCD in a patient. Since, SCD arises from a single-point mutation (Glu6Val) in the β-globin gene (*HBB*), the first one is the precise correction of the mutant HBB allele using homology-directed repair i.e., correction of mutation in patient derived hematopoietic stem cells (HSCs) and subsequent transplantation. However, this approach still faces challenges in efficiency. The other approach focuses on reactivation of the silenced fetal hemoglobin (HbF) gene. This is done by knocking out regulatory elements like the BL11A erythroid-specific enhancer using CRISPR/Cas9 to increase expression of HbF which compensated for the defective adult hemoglobin and therefore prevents sickling. The edited HSCs are then reinfused into the patient after myeloablation, offering a potentially curative, one time treatment. The basis of this strategy lies in the ex vivo therapy **CTX001** which was developed by CRISPR Therapeutics and Vertex Pharmaceuticals which has shown positive response in clinical trial with treated patients independent of transfusion and significantly low frequency of vaso-occlusive crises.

Beyond these two approaches, other gene targets like ZBTB7A, LCR (locus control region), and other base editing techniques are also being developed to ensure efficacy and safety. Overall, CRISPR-Cas9 is a transformative approach for the treatment of SCD, that focuses on a curative approach rather than limiting to symptomatic control.

Clinical trials utilizing CRISPR/Cas9 for the treatment of CSD have shown remarkable results and granted hope for the permanent resolution for this disease. The most advanced and publicized trial is **CLIMB-121,** for the ex vivo therapy **CTX001.** This therapy was approved by the U.S. FDA under the name **Casgevy™** in late 2023, making it the first CRISPR-based therapy to receive regulatory approval. Other clinical trials such as the homology-based repair and base editing are undergoing exploration with high hopes. Various studies are being led by institutions like **NIH**, **Boston Children's Hospital**, and **UCSF** for the aspects that still require improvement. However, the results of clinical trials depict the immense potential that this technique possesses.

Safety and Ethical Considerations

Despite the immense potential of this technique, it poses new challenges because it is different from conventional

gene therapy. For instance, the knocking out methodology may cause adverse complications by **off-target effects** i.e., generating unintended mutations or cleavage at non-targeted sites. This may disrupt essential genes or mechanisms leading to consequences, such as severe as oncogenesis. Another key limitation of CRISPR/Cas9 therapy is a need for myeloablading conditioning which uses high dose chemotherapy and is essential before transplantation of edited stem cells, but also can lead to risks of infection, organ toxicity, and infertility, which makes it unsuitable for many patients. Safer, non-toxic alternatives of myeloablation are being explored, such as antibody based conditioning.

Moreover, **insertion-deletion** mutations (INDELS) and large genomic arrangements that can be generated from double stranded breaks may harm genomic stability, especially in hematopoietic stem cells.

This technique may raise concerns over **equity and justice** in healthcare due to currently being available only in clinical trials or in high-resource environments. The high cost of ex vivo gene manipulation may restrict access of this technique towards low- and middle-income areas where the problem of SCD impacts the most. While there are no **ethical issues** involving alterations in somatic cells, any kind of manipulation in germ cells, even if unintentional leads to a bigger debate concerning ethics in genetic engineering.

Table 1
Investigational gene-edited autologous cell therapy products for sickle cell disease

Product	Sponsor	Technology	Effect	Activation	Status
CTX001	Vertex Pharmaceuticals Incorporated	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* erythroid enhancer (antisickling)	2018	Submitted BLA spring 2023
BIVV003 (now called SAR445136)	Sangamo Therapeutics	CRISPR/Cas9 editing	ZFN/*BCL11A* erythroid enhancer (antisickling)	2019	Further development discontinued
OTQ923/HIX763	Novartis Pharmaceuticals	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* (antisickling)	2020	Further development discontinued
GPH101	Graphite Bio, Inc.	CRISPR/Cas9 HDR	CRISPR-Cas9/$b^S > b^A$ (nonsickling)	2021	Further development discontinued
EDIT-301	Editas Medicine, Inc.	CRISPR/Cas912a editing	CRISPR-Cas9/HGB1/2 (antisickling)	2021	Active, data not reported
CRISPR_SCD001	UCSF Benioff Children's Hospital Oakland	CRISPR/Cas9 HDR	CRISPR-Cas9/$b^S > b^A$ (nonsickling)	2021	Not yet recruiting
BEAM-101	Beam Therapeutics	Base Editing	Base editing *BCL11A* erythroid enhancer (antisickling)	2022	Not yet recruiting

Reference: Leonard A, Tisdale JF. Gene therapy for sickle cell disease. Hematology Am Soc Hematol Educ Program. 2023 Dec 8;2023(1):542-547. doi: 10.1182/hematology.2023000487. PMID: 38066927; PMCID: PMC10727030.

Although, early clinical trials have shown promising results in terms of safety and efficacy, the **long-term effects** of CRISPR-Cas9 treatment in SCD patients remain largely unknown**.** There could be potential underlying risks that are not yet understood such as delayed adverse effects, secondary malignancies, or overtime loss of therapeutic effects. This highlights the need for long-term surveillance of SCD patients treated using CRISPR/Cas9 technology.

Future Aspects

The future of CRISPR-Cas9 technique holds immense potential with the vigorous research that is going on to improve and master the technique. Improvement in gene editing platforms, such as base editing and prime editing might provide safer options without causing double standard breaks in DNA and therefore **minimizing off target effects. In vivo delivery systems** are being worked upon such as lipid nanoparticles or viral vectors, which negate the need for hematopoietic stem cell transplantation. Moreover, the search for alternatives for Myeloablation is also in progress. With the increase in availability of gene editing techniques, it is expected to become more common and therefore more cost effective which would ultimately make CRISPR-Cas9 far more accessible to a normal person. **AI driven Technologies** could be used for generation of guide RNA or for automated editing platforms which could lead to technical advancement of the technique.

However, achieving these future possibilities will depend not only on continued technical innovation but also on the establishment of strong regulatory systems, thorough long-term safety surveillance, and fair access to ensure that CRISPR-based therapies reach all individuals affected by SCD worldwide.

2. Conclusion

Despite being a well-characterized genetic disorder, Sickle Cell Disease continues to impose a substantial burden due to limited curative options and resource constraints in affected populations. While past treatments have improved patient outcomes, they do not address the root genetic cause. The development of CRISPR/Cas9 has revolutionized the field of gene therapy by offering precise, efficient, and potentially curative solutions. With promising results from early clinical trials, CRISPR-based therapies have the potential to redefine SCD management. However, widespread adoption will depend on overcoming technical challenges, ensuring long-term safety, and addressing ethical, regulatory, and accessibility concerns. Continued research and equitable

healthcare policies are essential to realize the full impact of these innovations on global SCD care. In conclusion, this marks only the beginning of what is going to be a long journey for CRISPR-Cas9 technology, given its tremendous promise for the future of genetic medicine.

References

[1] Abdulfatah Alayoubi, Khawaji, Z. Y., Mohammed, M. A., & Mercier, F. (2023). CRISPR-Cas9 system: a novel and promising era of genotherapy for beta-hemoglobinopathies, hematological malignancy, and hemophilia. *Annals of Hematology*, *103*(6). https://doi.org/10.1007/s00277-023-05457-2

[2] Adashi, E. Y., Gruppuso, P. A., & Cohen, I. G. (2024). CRISPR Therapy of Sickle Cell Disease: The Dawning of the Gene Editing Era. *The American Journal of Medicine*, *137*(5). https://doi.org/10.1016/j.amjmed.2023.12.018

[3] Asmamaw, M., & Zawdie, B. (2021). Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. *Biologics : Targets & Therapy*, *15*(1), 353–361. https://doi.org/10.2147/BTT.S326422

[4] Ballantine, J., & Tisdale, J. F. (2024). Gene Therapy for Sickle Cell Disease: Recent Advances, Clinical Trials, and Future directions. *Cytotherapy*, *27*(7). https://doi.org/10.1016/j.jcyt.2024.11.006

[5] Bell, V., Varzakas, T., Psaltopoulou, T., & Fernandes, T. (2024). Sickle Cell Disease Update: New Treatments and Challenging Nutritional Interventions. *Nutrients*, *16*(2), 258–258. https://doi.org/10.3390/nu16020258

[6] Carroll, D. (2016). A Perspective on the State of Genome Editing. *Molecular Therapy*, *24*(3), 412–413. https://doi.org/10.1038/mt.2016.28

[7] Chaudhary, A., Kumari, B., Choudhury, M., Singh, S., & Kaur, Dr. R. (2020). A review of the therapeutic potential, prospects, and challenges of CRISPR/Cas9 genome editing in the treatment of Sickle Cell Disease (SCD). *International Journal of Scientific and Research Publications (IJSRP)*, *11*(1), 670–676. https://doi.org/10.29322/ijsrp.11.01.2021.p10983

[8] Demirci, S., Leonard, A., Haro-Mora, J. J., Uchida, N., & Tisdale, J. F. (2019). CRISPR/Cas9 for Sickle Cell Disease: Applications, Future Possibilities, and Challenges. *Advances in Experimental Medicine and Biology*, *5*. https://doi.org/10.1007/5584_2018_331

[9] Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E., … Porteus, M. H. (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature*, *539*(7629), 384–389. https://doi.org/10.1038/nature20134

[10] Dimitrievska, M., Bansal, D., Vitale, M., Strouboulis, J., Miccio, A., Nicolaides, K. H., … Jacków-Malinowska, J. (2024). Revolutionising healing: Gene Editing's breakthrough against sickle cell disease. *Blood Reviews*, *65*(101185), 101185. https://doi.org/10.1016/j.blre.2024.101185

[11] Doudna, J. A., & Charpentier, E. (2014). The New Frontier of Genome Engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096–1258096. https://doi.org/10.1126/science.1258096

[12] Frangoul, H. (2020). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *New England Journal of Medicine*, *384*(3). https://doi.org/10.1056/nejmoa2031054

[13] Frati, G., Brusson, M., Sartre, G., Mlayah, B., Felix, T., Chalumeau, A., … Miccio, A. (2024). Safety and efficacy studies of CRISPR-Cas9 treatment of sickle cell disease highlights disease-specific responses. *Molecular Therapy*, *32*(12). https://doi.org/10.1016/j.ymthe.2024.07.015

[14] Gulei, D., & Berindan-Neagoe, I. (2017). CRISPR/Cas9: A Potential Life-Saving Tool. What's next? *Molecular Therapy - Nucleic Acids*, *9*, 333–336. https://doi.org/10.1016/j.omtn.2017.10.013

[15] Hoban, M. D., Lumaquin, D., Kuo, C. Y., Romero, Z., Long, J., Ho, M., … Kohn, D. B. (2016). CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells. *Molecular Therapy*, *24*(9), 1561–1569. https://doi.org/10.1038/mt.2016.148

[16] Hu, X. (2016). CRISPR/Cas9 system and its applications in human hematopoietic cells. *Blood Cells, Molecules, and Diseases*, *62*, 6–12. https://doi.org/10.1016/j.bcmd.2016.09.003

[17] Ikwuka, A. O., Musa, S., Udeh, F. C., Musa, A. A., & Chukwuezie, U. C. (2023). CRISPR-Cas9 Genomic Editing as an Innovation in the Management of Sickle Cell Disease: A Systematic Review. *American Journal of Medical Science and Innovation*, *2*(2), 44–56. https://doi.org/10.54536/ajmsi.v2i2.1760

[18] Ilham Youssry, & Ayad, N. (2023). Sickle cell disease: combination new therapies vs. CRISPR-Cas9 potential and challenges — review article. *Annals of Hematology*, *103*(8). https://doi.org/10.1007/s00277-023-05510-0

[19] Jiang, W., & Marraffini, L. A. (2015). CRISPR-Cas: New Tools for Genetic Manipulations from Bacterial Immunity Systems. *Annual Review of Microbiology*, *69*(1), 209–228. https://doi.org/10.1146/annurev-micro-091014-104441

[20] Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science*, *337*(6096), 816–821. https://doi.org/10.1126/science.1225829

[21] Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., … Vichinsky, E. P. (2018). Sickle Cell Disease. *Nature Reviews Disease Primers*, *4*(1). https://doi.org/10.1038/nrdp.2018.10

[22] Katta, V., O'Keefe, K., Li, Y., Thiyagaraj Mayurathan, Lazzarotto, C. R., Wood, R. K., … Lockey, T. (2024). Development and IND-enabling studies of a novel Cas9 genome-edited autologous CD34+ cell therapy to induce fetal hemoglobin for sickle cell disease. *Molecular Therapy*, *32*(10). https://doi.org/10.1016/j.ymthe.2024.07.022

[23] Khan, S. H. (2019). Genome editing technologies: Concept, pros and cons of various genome editing techniques and bioethical concerns for clinical application. *Molecular Therapy - Nucleic Acids*, *16*(16), 326–334. https://doi.org/10.1016/j.omtn.2019.02.027

[24] Lattanzi, A., Camarena, J., Lahiri, P., Segal, H., Srifa, W., Vakulskas, C. A., … Bhatia, 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. https://doi.org/10.1126/scitranslmed.abf2444

[25] Leonard, A., & Tisdale, J. F. (2023). Gene therapy for sickle cell disease. *Hematology*, *2023*(1), 542–547. https://doi.org/10.1182/hematology.2023000487

[26] Liu, H., Ding, Y., Zhou, Y., Jin, W., Xie, K., & Chen, L.-L. (2017). CRISPR-P 2.0: An Improved CRISPR-Cas9 Tool for Genome Editing in Plants. *Molecular Plant*, *10*(3), 530–532. https://doi.org/10.1016/j.molp.2017.01.003

[27] Ma, L., Yang, S., Peng, Q., Zhang, J., & Zhang, J. (2023). CRISPR/Cas9-based gene-editing technology for sickle cell disease. *Gene*, *874*(147480), 147480. https://doi.org/10.1016/j.gene.2023.147480

[28] McManus, M., Haydar Frangoul, & Steinberg, M. H. (2024). Crispr-based gene therapy for the induction of fetal hemoglobin in sickle cell disease. *Expert Review of Hematology*, *17*(12). https://doi.org/10.1080/17474086.2024.2429605

[29] Mehta, J. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *New England Journal of Medicine*, *384*(23), e91. https://doi.org/10.1056/nejmc2103481

[30] Park, S. H. (2021). CRISPR/Cas9 gene editing for curing sickle cell disease. *Transfusion and Apheresis Science*, *60*(1), 103060. https://doi.org/10.1016/j.transci.2021.103060

[31] Parums, D. V. (2024). Editorial: First Regulatory Approvals for CRISPR-Cas9 Therapeutic Gene Editing for Sickle Cell Disease and Transfusion-Dependent β-Thalassemia. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, *30*, e944204. https://doi.org/10.12659/MSM.944204

[32] Rees, D. C., Williams, T. N., & Gladwin, M. T. (2010). Sickle-cell Disease. *The Lancet*, *376*(9757), 2018–2031. https://doi.org/10.1016/s0140-6736(10)61029-x

[33] Scaramellini, N., Panzieri, D. L., & Cappellini, M. D. (2025). Gene therapy for sickle cell disease and thalassemia. *Current Opinion in Hematology*, *32*(3). https://doi.org/10.1097/moh.0000000000000867

[34] Sharma, A., Jaap Jan Boelens, Cancio, M., Hankins, J. S., Bhad, P. A., Marjohn Azizy, … Stevenson, S. C. (2023). CRISPR-Cas9 Editing of the HBG1 and HBG2 Promoters to Treat Sickle Cell Disease. *The New England Journal of Medicine*, *389*(9), 820–832. https://doi.org/10.1056/nejmoa2215643

[35] Singh, M., Singh, D., Suyal, D. C., Mehra, A., Tripathi, V., Bhasin, S., … Ilyas, A. (2021). A Review on CRISPR/ Cas9: A Molecular Approach to Genome Editing. *Invertis Journal of Renewable Energy*, *11*(1), 17–22. https://doi.org/10.5958/2454-7611.2021.00003.5

[36] Singh, V., Braddick, D., & Dhar, P. K. (2017). Exploring the potential of genome editing CRISPR-Cas9 technology. *Gene*, *599*, 1–18. https://doi.org/10.1016/j.gene.2016.11.008

[37] Sundd, P., Gladwin, M. T., & Novelli, E. M. (2019). Pathophysiology of Sickle Cell Disease. *Annual Review of Pathology: Mechanisms of Disease*, *14*(1), 263–292. https://doi.org/10.1146/annurev-pathmechdis-012418-012838

[38] Tariq, H., Khurshid, F., Muhammad Hamza Khan, Aamna Dilshad, Zain, A., Rasool, W., … Akbar, A. (2024). CRISPR/Cas9 in the treatment of sickle cell disease (SCD) and its comparison with traditional treatment approaches: a review. *Annals of Medicine and Surgery*, *86*(10), 5938–5946. https://doi.org/10.1097/ms9.0000000000002478

[39] Testa, U., Leone, G., & Cappellini, M. D. (2024). Therapeutic Gene Editing for Hemoglobinopathies. *Mediterranean Journal of Hematology and Infectious Diseases*, *16*(1), e2024068. https://doi.org/10.4084/MJHID.2024.068

[40] Wang, D., Zhang, F., & Gao, G. (2020). CRISPR-Based Therapeutic Genome Editing: Strategies and In Vivo Delivery by AAV Vectors. *Cell*, *181*(1), 136–150. https://doi.org/10.1016/j.cell.2020.03.023

[41] Wang, H., La Russa, M., & Qi, L. S. (2016). CRISPR/Cas9 in Genome Editing and Beyond. *Annual Review of Biochemistry*, *85*(1), 227–264. https://doi.org/10.1146/annurev-biochem-060815-014607

[42] You, L., Tong, R., Li, M., Liu, Y., Xue, J., & Lu, Y. (2019). Advancements and Obstacles of CRISPR-Cas9 Technology in Translational Research. *Molecular Therapy - Methods & Clinical Development*, *13*, 359–370. https://doi.org/10.1016/j.omtm.2019.02.008

[43] Zhang, F., Wen, Y., & Guo, X. (2014). CRISPR/Cas9 for genome editing: progress, implications and challenges. *Human Molecular Genetics*, *23*(R1), R40–R46. https://doi.org/10.1093/hmg/ddu125