



VOLUME 02 ISSUE 02 (2023)

AMERICAN JOURNAL OF
**MEDICAL SCIENCE
AND INNOVATION**
(AJMSI)



PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

CRISPR-Cas9 Genomic Editing as an Innovation in the Management of Sickle Cell Disease: A Systematic Review

Solomon Musa¹, Aloysius Obinna Ikwuka^{2*}, Francis Chigozie Udeh², Abdullahi Adobanyi Musa³, Ugo Collins Chukwuezie⁴

Article Information

Received: June 16, 2023

Accepted: July 08, 2023

Published: July 30, 2023

Keywords

CRISPR-Cas9, Genomic Editing, Innovation, Management, Sickle Cell Disease, Gene Therapy

ABSTRACT

Genomic editing is a group of technologies that scientists have used to alter an organism's DNA. Of the several genomic editing techniques, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) is well known. The CRISPR-Cas9 system is faster, cheaper, more accurate, more efficient than other genomic editing methods, and it is an adaptation from bacteria's immune mechanism. Sickle cell diseases (SCDs) are a group of monogenic diseases, and despite their high prevalence and chronic debilitating nature, they continue to have few therapeutic options available. The aim of this study is to review existing literature and current clinical trials on CRISPR-Cas9 genomic editing as an innovation in the management of sickle cell disease (SCD), as well as the current state of treatment for SCD. For this systematic review, PubMed, Google Scholar, African Journals Online (AJOL), and Clinicaltrial.gov articles published up to 6th October, 2022 were searched. Searches for current clinical trials using CRISPR-Cas9 as intervention were conducted by using the search terms such as sickle cell disease, genomic editing, genetics, novel treatments, hematopoietic stem cell transplantation, gene therapy, and CRISPR-Ca9. Studies cited include meta-analyses, original research, prospective clinical trials, online abstracts, literature reviews, retrospective studies, case series, and scientific meetings. The primary search obtained 27,678 articles. Following a review of titles and abstracts, a total of 32 publications and 6 ongoing clinical trials were included in this systematic review based on the recent evidence-based management of SCD. CRISPR-Cas9 genomic editing stands out as a novel, innovative technology which has the potential to cure SCD in children and adults with minimal side effects. Six clinical trials are ongoing with a huge potential for scaling up to Phases 3 and 4.

INTRODUCTION

Genetic medicine is a newer terminology for medical genetics and incorporates areas such as gene therapy. Recent advances in medical genetics are revealing etiologies for morphologic, endocrine, cardiovascular, pulmonary, ophthalmologic, renal, psychiatric, and dermatologic conditions (Ikwuka, 2023a).

Sickle cell disease (SCD) encompasses a group of blood disorders resulting from inheriting two mutated copies of the β -globin gene (HBB) from both parents (Frangoul, 2021). HBB, located on chromosome 11p15.5, encodes the β -chain of hemoglobin (Onda, 2005). In normal circumstances, humans have three types of hemoglobin: hemoglobin A (consisting of 2α and 2β chains), hemoglobin A2 (made up of 2α and 2δ chains), and hemoglobin F (HbF) composed of 2α and 2γ chains (Hall, 2020). In sickle cell anemia, the most prevalent form of SCD, red blood cells (RBCs) contain an abnormal hemoglobin variant called hemoglobin S, wherein each of the two β -chains has a specific mutation (an amino acid valine with codon GTG substitutes glutamic acid with codon GAG at position 6) (Hall, 2020). Thus, the genetics of SCD (a hemoglobinopathy) is due to the substitution of valine for glutamic acid at position 6 of both β -globin polypeptide chains. Hemoglobin F persists until about 6 weeks of age. Thereafter, hemoglobin A persists throughout life.

The mode of inheritance of SCD is autosomal recessive (Ikwuka, 2023b).

Hemoglobin, a protein responsible for oxygen transport in red blood cells (RBCs), is synthesized during erythropoiesis (initiated in polychromatophil erythroblasts and continuing through the reticulocyte stage) (Hall, 2020). Erythropoiesis is stimulated and enhanced by erythropoietin, a substance synthesized by the kidneys. Results from different studies have shown that high levels of blood pressure, glucose and lipid metabolic disorders, asymptomatic hyperuricemia, activation of systemic immune inflammation and fibrogenesis, contribute to kidney damage (Ikwuka, 2015; Ikwuka, 2017a; Ikwuka, 2017c; Ikwuka, 2017d; Ikwuka, 2017e; Ikwuka, 2018d; Ikwuka, 2019a; Ikwuka, 2019c; Ikwuka, 2022; Ikwuka, 2023d; Ikwuka, 2023e; Virstyuk, 2016; Virstyuk, 2017a; Virstyuk, 2018a; Virstyuk, 2019; Virstyuk, 2021a; Virstyuk, 2021b), which contributes to anemia on account of disturbed erythropoietin production. However, Dapagliflozin which is a Sodium-Glucose Linked Transporter 2 (SGLT-2) inhibitor and Liraglutide which is a Glucagon-like Peptide 1 Receptor Agonist (GLP-1 RA) have been found to increase the effectiveness of treatment and improve the clinical course of disease in patients with such comorbidities (Ikwuka, 2017b; Ikwuka, 2018a; Ikwuka, 2018b; Ikwuka, 2018c; Ikwuka, 2019b; Ikwuka, 2021; Virstyuk, 2018b; Virstyuk, 2018c; Virstyuk, 2017b),

¹ Clinical Projects, Xcene Research, Ikeja, Nigeria

² College of Medicine and Health Sciences, American International University West Africa, Banjul, The Gambia

³ Department of Family Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria

⁴ Department of Community Medicine, University of Lagos, Surulere, Nigeria

* Corresponding author's e-mail: aloyssiussweet@yahoo.com

thereby reducing kidney damage, improve kidney functions, and reduce anemia. When hemoglobin S encounters low oxygen levels, it forms long crystals (sometimes 15 micrometers in length) within RBCs, impeding their flow through narrow capillaries. The pointed ends of these crystals can rupture the plasmalemma leading to sickle cell anemia (Hall, 2020). Major free radicals that are of physiological significance are superoxide anion, hydroxyl radical, and hydroperoxyl radical, while non-radical is hydrogen peroxide (Ikwuka, 2023c). *Rauwolfia vomitoria* has a neuroprotective ability as it elevates antioxidants and suppresses lipid peroxidation (Ekechi, 2023).

It is noteworthy that nearly two-thirds of infants worldwide with HbSS or SCD are born in Nigeria, the Republic of Congo, or India, where the childhood mortality rate associated with SCD remains alarmingly high (Piel, 2013). Symptoms and complications of SCD typically manifest around 5 to 6 months of age when fetal hemoglobin (HbF) synthesis significantly declines. These symptoms include severe anemia, episodes of pain (referred to as sickle cell crisis), swelling in the hands and feet, and potential complications such as bacterial infections and stroke (Frangoul, 2021; Hall, 2020). Long-term pain can develop as individuals grow older, and the average life expectancy in developed countries ranges from 40 to 60 years (National Heart, Lung, and Blood Institute, 2015).

Newborn screening is the common diagnostic approach for identifying HbSS, and treatment options include penicillin (essential for children under five years with immature immune systems), folic acid supplementation, blood transfusions, vaccinations against encapsulated organisms, transcranial Doppler (TCD) screening to identify stroke risk in children (followed by blood transfusions, if necessary), pain management, hydroxyurea, and intensive hospital-based care (Adams, 1998; Gaston, 1986; Thornburg, 2012; WHO, 2011). Other diagnostic tests for SCD include sickling of the red blood cells on a blood film which is induced by the addition of sodium metabisulfite; and another test referred to as hemoglobin electrophoresis which detects abnormal hemoglobin forms (Ikwuka, 2023b). However, despite the significant need for effective treatment options for SCD patients, current treatments both traditional and newly developed, only ameliorate acute and chronic SCD manifestations without addressing the underlying cause. Hydroxyurea and long-term blood transfusions aim to prevent and treat complications associated with SCD. The recently approved crizanlizumab (Ataga, 2017) has shown a reduced incidence of cellular adhesion and vaso-occlusive crisis in SCD patients, but it does not target the root cause of the disease or fully alleviate its manifestations (Frangoul, 2021). Allogeneic hematopoietic stem cell transplantation (HSCT) remains the sole curative option for SCD, yet less than 20% of eligible patients have a suitable HLA-matched donor (Baronciani, 2016; Eapen, 2019; Gluckman, 2017). Further advances in the understanding of the pathophysiology of SCD contributed to the development of the exciting and novel “Clustered Regularly Interspaced

Short Palindromic Repeats (CRISPR)-associated protein 9 (CRISPR-Cas9)” genomic editing therapy to cure the disease and its complications. Bacteriophages have the ability to infect bacteria by implanting its genetic material into the bacterial genome (Chaudhary, 2020). Thereafter, bacteria have a natural defense mechanism against bacteriophages, whereby on the first exposure to a bacteriophage they produce CRISPR sequence as a form of genetic memory. CRISPR sequence is always found in association with the Cas9, a nuclease that can cleave the DNA. In subsequent exposure to a similar bacteriophage, the bacteria form guideRNA from the transcription of the CRISPR sequence. The guideRNA finds its target in the bacteriophage DNA, while the Cas9 cleaves the DNA (Chaudhary, 2020).

The advantage of this system is that once the CRISPR system has cleaved the DNA, a DNA template carrying the desired sequence can join the cleaved end, thereby facilitating recombination and replacement of the original sequence with the new version. The CRISPR-Cas9 nuclease system can be employed in cultured cells, including stem cells, as well as in fertilized eggs, enabling the generation of transgenic animals with targeted mutations. This genomic editing technique has been extensively studied in various organisms such as yeast, *Drosophila*, Zebrafish, plants, monkeys, and pigs, in addition to the bacteria from which the technique was originally derived from (Wen, 2017).

In the case of SCD, the CRISPR-Cas9 nuclease system is applied to hematopoietic stem and progenitor cells (HSPCs) at the erythroid-specific enhancer region of the BCL11A locus on chromosome 2 (Uda, 2008). Normally, BCL11A encodes a transcription factor that inhibits HbF synthesis. The CRISPR-Cas9 nuclease system effectively suppresses BCL11A expression in erythroid-lineage cells, thereby restoring γ -globin synthesis and reactivating HbF production (Canver, 2015; Wu, 2019). Unlike previous genomic editing methods, CRISPR-Cas9 has the capacity to target multiple genes simultaneously, enabling the treatment of not only diseases with point mutations but also those with polygenic mutations.

Researchers have recently realized that this system can be engineered to cleave DNA at precisely chosen loci, extending beyond viral DNA to any desired DNA sequence, simply by modifying the guideRNA to match the target (Chaudhary, 2020). In this systematic review, a comprehensive analysis of the current state of research on CRISPR-Cas9 for the treatment of sickle cell disease (SCD) was conducted. By gathering information from multiple sources, evaluation of the progress made with this innovative technology is determined, identified knowledge gaps for further research are checked, and the technology’s potential challenges and limitations are discussed.

METHODOLOGY

Search Strategy and Selection Criteria

This systematic review aimed to study all available literature on CRISPR-Cas9 genomic editing and its potential in the management of SCD. It also sought to shed more light on

the subject matter (considering the fact this technology is novel) and its stage of development is still in the clinical trials. A similar method of literature search as described by Suwito, *et al*, 2023 was used (Suwito, 2023). The literature search was done from the following databases over a period of 2 weeks: PubMed (mostly used), Google Scholar, and African Journals Online (AJOL) using the following terms: CRISPR-Cas9, genomic editing, gene editing, sickle cell disease, hemoglobinopathies, sickle cell anemia, genetic therapy, systematic review, new therapy/novel intervention for sickle cell disease cure/treatment.

Data Sources and Search Engines

A literature search was done from the following databases: PubMed (mostly used), Google Scholar, and African Journals Online (AJOL). While the clinical trials search was done on ClinicalTrials.gov.

Inclusion and Exclusion Criteria

Included in this study were studies that investigated the use of CRISPR-Cas9 genomic editing as a treatment for sickle cell disease, studies that included human participants or human cells/tissues, studies that provided data on the efficacy and/or safety of CRISPR-Cas9 genomic editing for sickle cell disease, and studies that were published in English language within the past 10 years on gene therapy use in SCD, specifically CRISPR-Cas9. Excluded articles were studies not related to CRISPR-Cas9 genomic editing or sickle cell disease, studies that used animal or plant models only, studies not published in English, studies

that did not provide data on the efficacy and/or safety of CRISPR-Cas9 genomic editing for sickle cell disease, and studies that had poor methodological quality or a high risk of bias.

Quality Assessment of Included Studies

The articles from the database search were reviewed to tailor them to the inclusion criteria. The abstracts of the articles that met the inclusion criteria were reviewed for relevant keywords. The abstracts and the free complete articles i.e. manuscripts for the selected articles were then read, reviewed, and the information on each of the key areas were summarized. This systematic review was carried out independently by four persons, to minimize errors. The summarized data were later compiled, reviewed, and discussed.

Data Extraction, Synthesis, and Results

The following keywords were used to extract articles from database searches:

- “CRISPR-Cas9”
- “Genomic editing”
- “Sickle cell disease, hemoglobinopathies, and sickle cell anemia”
- “Genetic therapy and gene editing”
- “Systematic literature review or systematic review”
- “New therapy/novel intervention for sickle cell”.

Study Selection and Characteristics

The search for articles and abstracts was done using

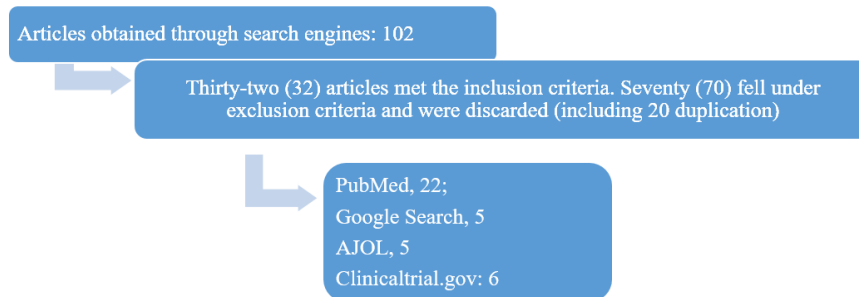


Figure 1: Steps of article selection

keywords on the three major search engines (PubMed, Google Scholar, and AJOL). However, the mostly used search engine was PubMed due to its advanced features and its large repository of articles. Study selection was based on articles, abstracts, or literature reviews which meet the inclusion criteria. Articles that were found under exclusion

criteria were discarded. The diagram below illustrates how articles were selected.

RESULTS

The findings in this study are outlined in Tables 1 and 2.

Table 1: Summary of Articles in the Literature Search

S/N	Paper Title	Abstract summary	Study type	Outcome measured/ Summary of conclusion
1	CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β -Thalassemia (Frangoul, 2021)	CRISPR-Cas9-targeting erythroid-specific enhancers modified 80% of the alleles at this locus in healthy donors.	Phase 1/2	<ul style="list-style-type: none"> • Allelic editing in bone marrow and blood • \uparrow in HbF • Transfusion independence • Elimination of vaso-occlusive episodes in the patient with SCD

2	A Review of the Therapeutic Potential, Prospects, and Challenges of CRISPR-Cas9 Genome Editing in the Treatment of Sickle Cell Disease (SCD) (Chaudhary, 2020)	CRISPR-Cas9 is a potential therapeutic tool in the management of SCD.	Review	<ul style="list-style-type: none"> • Through this review paper, the scope and possibilities of CRISPR-Cas9 as a potential therapeutic tool in the management of SCD was analyzed
3	Therapeutic CRISPR-Cas9 Genome Editing for Treating Sickle Cell Disease (Park, 2016)	Optimized CRISPR-Cas9 systems for genomic editing can be achieved in CD34+ cells.	Review	<ul style="list-style-type: none"> • Rates of Non-Homologous End Joining (NHEJ) events • Rates of Homology Directed Repair (HDR) events • Genome editing frequencies at both DNA and mRNA levels • Expression of globin and other erythroid markers • Number and type of colonies following induction of differentiation • Genotype of edited cells • Translation of edited β-globin protein and formation of HbS
4	CRISPR-Cas9 Mediated Correction of the Sickle Mutation in Human CD34+ cells (Hoban, 2016a)	Targeted genomic editing technology can correct the SCD mutation of the β -globin gene in hematopoietic stem cells.	Experimental	<ul style="list-style-type: none"> • Gene modification rate • Production of wild type hemoglobin.
5	CRISPR-Cas9 to Induce Fetal Hemoglobin for the Treatment of Sickle Cell Disease (Demirci, 2021)	Genomic editing is potentially a curative technique available to all individuals with β -hemoglobinopathies, including SCD.	Review	<ul style="list-style-type: none"> • Fetal Hemoglobin (HbF) induction • Editing of transcriptional HbF silencers • Modulating epigenetic intermediates that govern HbF expression
6	CRISPR-Cas9 for Sickle Cell Disease: Applications, Future Possibilities, and Challenges (Demirci, 2019)	Genomic editing approach has proven valuable as a curative option.	Review	<ul style="list-style-type: none"> • Disease severity • Mortality • Morbidity
7	CRISPR-Cas9 Gene Editing for Curing Sickle Cell Disease (Park, 2021a)	Ex vivo engineering of autologous HSPCs followed by transplantation of genetically modified cells potentially provides a permanent cure applicable to all patients regardless of the availability of suitable donors and graft-vs-host reaction.	Review	<ul style="list-style-type: none"> • Severity of pain • End organ damage • Early mortality
8	CRISPR-Cas9 Genomic Engineering: Trends in Medicine and Health (Zaib, 2022)	CRISPR-Cas9 technology offers the simplest, fastest, most versatile, reliable and precise method of genetic manipulation.	Review	<ul style="list-style-type: none"> • Genetic manipulation • Removing sections of the DNA sequence • Adding sections of the DNA sequence • Altering sections of the DNA sequence

				<ul style="list-style-type: none"> • Treatment of various medical conditions including cancer, hepatitis B, cardiovascular diseases or even high cholesterol • Minimization of the off-target effects of gene editing and incomplete matches between single guideRNA and genomic DNA by Cas9
9	CRISPR-Cas9: ANew and Promising Player in Gene Therapy (Xiao-Jie, 2015)	CRISPR-Cas9 can be applied for therapeutic purposes in cell lines or animal models.	Review	<ul style="list-style-type: none"> • Gene therapy outcomes • Correction of causal mutations in monogenic disorders • Rescue of disease phenotypes • Engineering of pathogen genome for therapeutic purposes • Induction of protective or therapeutic mutations in host tissues • Deactivation of oncogenic virus • Induction of onco-suppressor expressions
10	Emerging Genetic Therapy for Sickle Cell Disease (Orkin, 2019)	Transcript factors that mediate silencing of the γ -like fetal globin gene after birth have been identified and demonstrated to act as the β -globin promoters.	Review	<ul style="list-style-type: none"> • Efficacy of genetic strategies to cure SCD • Safety of genetic approaches to cure SCD
11	Era of Genomic Medicine: A Narrative Review on CRISPR Technology as a Potential Therapeutic Tool for Human Diseases (Kotagama, 2019)	The guideRNA can be modified to match a DNA sequence of interest in the cell.	Review	<ul style="list-style-type: none"> • An insight with relation to a few of the many diseases that are being tackled with the aid of the CRISPR-Cas9 mechanism and the trends, successes, and challenges of this application as a gene therapy are discussed in this review
12	CRISPR-Cas9 Genome Editing in Human Hematopoietic Stem Cells (HSCs) (Bak, 2018)	Genomic editing via homologous recombination (HR) in human HSCs has the power to reveal gene-function relationships and potentially transform curative hematological gene and cell therapies.	Experimental	<ul style="list-style-type: none"> • Production of HR targeted HSCs • Enrichment of HR targeted HSCs • In vitro analysis of HR targeted HSCs • In vivo analysis of HR targeted HSCs • Manipulation of genes for investigation of gene functions during hematopoiesis • Correction of genetic mutations in HSC transplantation-based therapies for diseases such as SCD, β-Thalassemia, and Primary Immunodeficiencies
13	Efficacy of CRISPR-Based Gene Editing in a Sickle Cell Disease Patient as Measured through the Eye (Pinhas, 2022)	Optical coherence tomography angiography can detect and measure micro-occlusive events within the retinal capillary bed before and after RBC exchange transfusion and following CRISPR-based gene editing.	Case report	<ul style="list-style-type: none"> • Micro occlusive events within the retinal capillary bed

14	Current Sickle Cell Disease Gene Therapy Treatments: Literature Review (Ranadive, 2022)	The base editor shows promise in its ability to surpass many issues faced with both viral vectors and CRISPR-Cas9 in human trials.	Literature review	<ul style="list-style-type: none"> • Success of lentiviral vectors in genotype correction and HbF induction • Success of CRISPR-Cas9 in genotype correction and HbF induction • Success of base editors in genotype correction and HbF induction
15	CRISPR-Cas9 Editing Induces High Rates of Unintended Large Gene Modifications in HSPCs from Patients with Sickle Cell Disease (Park, 2021b)	Unintended on-target large deletions occur at high rates in gene-edited SCD HSPCs.	Experimental	<ul style="list-style-type: none"> • Unintended gene modifications due to Cas9 induced Double Stranded Breaks (DSBs) in SCD HSPCs, including large deletions, insertions, and complex chromosomal arrangements
16	Automated Good Manufacturing Practice-Compatible CRISPR-Cas9 Editing of Hematopoietic Stem and Progenitor Cells for Clinical Treatment of β -Hemoglobinopathies (Urena-Bailen, 2023)	The enhancer of the BCL11A gene is a CRISPR target in ongoing clinical trials for β -thalassemia and SCD treatment.	Experimental	<ul style="list-style-type: none"> • Editing efficiency • HbF resurgence
17	In vivo Selection for Corrected β -globin Alleles after CRISPR-Cas9 Editing in Human Sickle Hematopoietic Stem Cells (HSCs) Enhances Therapeutic Potential (Magis, 2018)	Cas9-mediated gene editing in long-term engrafting human HSCs yields more than 20% correction of the sickle mutation in long-term engrafting human HSCs.	Experimental	<ul style="list-style-type: none"> • Percentage of correction of the sickle mutation in long term engrafting human HSCs • RNA sequence data to find cells carrying corrected SS globin alleles • Efficiency of editing with almost no off target events
18	Multiplex CRISPR-Cas9 Genomic Editing in Hematopoietic Stem Cells for Fetal Hemoglobin Reinduction Generates Chromosomal Translocations (Samuelson, 2021)	Genomic editing therapies targeting either the BCL11A erythroid enhancer or the HBG promoter are already proving successful in reinducing HbF.	Experimental	<ul style="list-style-type: none"> • HbF reinduction • Engraftment • Lineage differentiation potential of edited cells post xenotransplantation • Chromosomal rearrangement events
19	CRISPR-Cas9: A Preclinical and Clinical Perspective for the Treatment of Human Diseases (Sharma, 2021)	CRISPR-Cas9 is a promising genome-editing tool that has therapeutic potential against incurable genetic disorders by modifying their DNA sequences.	Experimental	<ul style="list-style-type: none"> • Modulation of predefined gene expression (upregulation or downregulation)
20	Genomic editing: A Perspective on the Application of CRISPR-Cas9 to Study Human Diseases (Review) (Rodriguez-Rodriguez, 2019)	The CRISPR-Cas9 system can repair the damage caused to DNA.	Review	<ul style="list-style-type: none"> • The basic principles of the CRISPR-Cas9 system are reviewed, as well as the strategies and modifications of the enzyme Cas9 to eliminate the off-target cuts, and the different applications of CRISPR-Cas9 as a system for visualization and gene expression activation or suppression

21	Use of Genomic Editing Tools to Treat Sickle Cell Disease (Tasan, 2016)	The only existing curative treatment for SCD is based on allogeneic stem cell transplantation from healthy donors.	Experimental	<ul style="list-style-type: none"> • Discussion of the three programmable nucleases that are commonly used for genomic editing purposes: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and CRISPR-Cas9
22	CRISPR-Cas9 β -globin Gene Targeting in Human Hematopoietic Stem Cells (Dever, 2016)	Ex vivo gene correction in patient-derived HSCs followed by autologous transplantation could be used to cure hemoglobinopathies.	Preclinical trial	<ul style="list-style-type: none"> • Homologous recombination at the HBB Gene in HSCs • Efficiency of correction of the Glu6Val mutation responsible for SCD • Expression of adult β-globin (HbA) messenger RNA after differentiation into erythrocytes
23	A Systematic Review of Gene Editing Clinical Trials (Eshka, 2022)	There are promising phase-I and phase-II trials testing the safety and feasibility of gene editing in different clinical settings.	Literature review	<ul style="list-style-type: none"> • Gene editing clinical trials • Genetically engineered T-Cell therapies for cancer • Virus infections • Monogenic diseases
24	Genetic Treatment of a Molecular Disorder: Gene Therapy Approaches to Sickle Cell Disease (Hoban, 2016b)	The initial-retroviral vectors, next-generation lentiviral vectors, and novel genomic engineering and gene regulation approaches share the goal of preventing erythrocyte sickling.	Review	<ul style="list-style-type: none"> • Effective medical management for SCD • Preventing erythrocyte sickling • Clinical success
25	Efficient Ablation of Genes in Human Hematopoietic Stem and Effector Cells using CRISPR-Cas9 (Mandal, 2014)	CRISPR-Cas9 can efficiently ablate genes in HSPCs.	Experimental	<ul style="list-style-type: none"> • Efficacy of CRISPR-Cas9-mediated genomic editing in primary human CD4+ T Cells and CD34+ HSPCs • Gene deletion efficacy in CD4+ T Cells and CD34+ HSPCs • Multilineage potential of HSPCs that had undergone genome editing with CRISPR-Cas9 • Predicted on and off target mutations via target capture sequencing in HSPCs • Levels of off target mutagen
26	Selection-Free Genomic Editing of the Sickle Mutation in Human Adult Hematopoietic Stem/Progenitor Cells (DeWitt, 2016)	A Cas9 RNP can mediate efficient hematopoietic stem cell genomic editing in human hematopoietic stem cells from sickle cell disease patients.	Experimental	<ul style="list-style-type: none"> • Production of normal hemoglobin • Production of HbS RNA and protein • Production of wild type hemoglobin
27	Genomic Editing for Sickle Cell Disease: A Little BCL11A Goes a Long Way (Hossain, 2017)	CRISPR-Cas9 or ZFNs are useful tools to delete or replace sequences involved in the production of hemoglobin.	Review	<ul style="list-style-type: none"> • Feasibility of genomic editing in HSPCs • Ability of CRISPR-Cas9 or ZFNs to delete or replace sequences involved in the production of hemoglobin

28	Highly Efficient Editing of the β -globin Gene in Patient-Derived Hematopoietic Stem and Progenitor Cells to Treat Sickle Cell Disease (Park, 2019)	Gene-corrected sickle-cell HSPCs engrafted in vivo.	Experimental	<ul style="list-style-type: none"> • Efficiency of correcting the sickle mutation in the β-globin gene in HSPCs • Reduction of sickle cells in erythrocytes derived from gene edited cells • Level of normal adult hemoglobin (HbA) in erythrocytes derived from gene edited cells • Engraftment of gene-edited SCD HSPCs in Non-Obese Diabetic (NOD) SCID Gamma (NSG) mice
29	Cas9 Protein Delivery Non-Integrating Lentiviral Vectors for Gene Correction in Sickle Cell Disease (Uchida, 2021)	The Cas9 protein delivery non-integrating lentiviral all-in-one system efficiently corrected the SCD mutation in the endogenous S-globin gene without electroporation.	Experimental	<ul style="list-style-type: none"> • Efficiency of correction of the SCD mutation in the endogenous β-globin genes • Protein level of the corrected β-globin genes
30	Application of CRISPR-Cas9 Genomic Editing in Genetic Disorders: A Systematic Review Up to Date (Pandey, 2017)	CRISPR-Cas9 system has been used from last few years in the field of biomedical research.	Review	<ul style="list-style-type: none"> • Genomic editing technologies over the past few years is providing fast and effective tool to precisely manipulate the genome at specific locations.
31	Development of β -globin Gene Correction in Human Hematopoietic Stem Cells as a Potential Durable Treatment for Sickle Cell Disease (Lattanzi, 2021)	Ex vivo β -globin gene correction in autologous patient-derived HSPCs may potentially provide a curative treatment for SCD.	Phase 1/2	<ul style="list-style-type: none"> • Gene correction • Genotoxicity • Tumorigenicity • Multilineage engraftment • Abnormal hematopoiesis • Toxicology
32	Combination of Lentiviral and Genomic Editing Technologies for the Treatment of Sickle Cell Disease (Ramadier, 2022)	Transduced cells from sickle cell patients were transduced with lentiviral vectors expressing AS3 and a guideRNA either targeting the endogenous β -globin gene or regions involved in HbF silencing.	Experimental	<ul style="list-style-type: none"> • Clinical benefit in SCD patients • Vector Copy Number (VCN) • Anti-sickling hemoglobins • Rescue of the SCD phenotype • Genotoxicity risk

Table 2: Current Clinical Trials on CRISPR-Cas 9 (from Clinicaltrial.gov, assessed on April 5th, 2023)

S/N	Study Title	Conditions	Interventions	Locations (First 3)
1	A Safety and Efficacy Study Evaluating CTX001 in Subjects with Severe Sickle Cell Disease	<ul style="list-style-type: none"> • Sickle cell disease • Hematological diseases • Hemoglobinopathies 	<ul style="list-style-type: none"> • Biological: CTX001 • Phase 1/2/3 study • 45 estimated participants 	<ol style="list-style-type: none"> 1. Lucille Packard Children's Hospital of Stanford University, Palo Alto, California, United States. 2. Ann & Robert Lurie Children's Hospital of Chicago, Chicago, Illinois, United States. 3. University of Illinois at Chicago Hospitals and Health Systems, Chicago, Illinois, United States.

2	Evaluation of Efficacy and Safety of a Single Dose of CTX001 in Participants with Transfusion-Dependent Beta Thalassemia and Severe Sickle Cell Disease	<ul style="list-style-type: none"> • β-Thalassemia • Thalassemia • Hematologic disease 	<ul style="list-style-type: none"> • Biological: CTX001 • Phase 3 • 12 estimated participants 	<ol style="list-style-type: none"> 1. Columbia University Medical Center, New York, United States. 2. Atrium Health Levine Children's Hospital, Charlotte, North Carolina, United States. 3. SCRI at the Children's Hospital at TriStar Centennial, Nashville, Tennessee, United States.
3	Evaluation of Safety and Efficacy of CTX001 in Pediatric Participants with severe SCD	<ul style="list-style-type: none"> • SCD • Hydroxyurea failure • Hydroxyurea intolerance 	<ul style="list-style-type: none"> • Biological: CTX001 • Phase 3 • 12 estimated participants 	<ol style="list-style-type: none"> 1. Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States. 2. St. Jude Children's Research Hospital, Memphis, Tennessee, United States. 3. The Children's Hospital at TriStar Centennial Medical Center/ Sarah Cannon Center for Blood Cancers.
4	Transplantation of CRISPR- Modified Hematopoietic Progenitor Stem Cells (CRISPR-SCD 001) in Patients with Severe Sickle Cell Disease	<ul style="list-style-type: none"> • SCD 	<ul style="list-style-type: none"> • Drug: CRISPR_ SCD001 • Phase 1/2 • 9 estimated participants 	<ol style="list-style-type: none"> 1. University of California, Los Angeles, California, United States. 2. UCSF Benioff Children's Hospital, Oakland, California, United States.
5	Gene Correction in autologous CD34+ Hematopoietic stem cells (HbS to HbA) to treat severe SCD	<ul style="list-style-type: none"> • SCD 	<ul style="list-style-type: none"> • Genetic: GPH101 Drug Product • Phase 1/2 • 15 estimated participants 	<ol style="list-style-type: none"> 1. University of Alabama, Birmingham, Alabama, United States. 2. Lucile Packard Children's Hospital, Palo Alto, California, United States. 3. Washington University, Saint Louis, Missouri, United States.
6	A Long-Term Follow-up Study in Patients Who Received CTX001	<ul style="list-style-type: none"> • β-Thalassemia • Thalassemia • SCD 	<ul style="list-style-type: none"> • Biological: CTX001 • Cohort Study • 114 estimated participants 	<ol style="list-style-type: none"> 1. Columbia University Medical Center (21+ years), New York, United States. 2. Columbia University Medical Center, New York, United States. 3. Children's Hospital of Philadelphia, Pennsylvania, United States.

DISCUSSION

This systematic literature review provides an overview of CRISPR-Cas9 genomic editing and its application to sickle cell disease (SCD), while also addressing the ethical implications associated with this technology in SCD management. Despite the wide use of CRISPR-Cas9 as a mature genomic editing tool, therapeutic applications still face challenges such as off-target effects, complex in vivo Cas9 protein delivery, low gene editing efficiency, and packaging issues. To become an ideal delivery method for therapeutics, CRISPR-Cas9 strategies should exhibit high delivery efficiency, precise targeting ability, and ease of mass production. However, current approaches in this field are far from achieving this desired level of performance (Guo, 2022). While there is significant literature on why SCD is a suitable candidate for

CRISPR-Cas9, less attention has been given to the ethical implications of including SCD in CRISPR-Cas9 research. In addition, the implications of CRISPR-Cas9 for sickle cell disease have significant consequences for clinical practice and policy. The following points highlight some of the potential implications:

Improved Outcomes

CRISPR-Cas9 holds the potential to cure SCD by correcting the underlying genetic mutation. This breakthrough could lead to improved outcomes for patients, including reduced pain, enhanced quality of life, and increased lifespan.

Reduced Healthcare Costs

SCD treatment can be expensive, and the use of CRISPR-

Cas9 may reduce healthcare costs by offering a curative approach rather than merely managing symptoms.

Ethical Considerations

The use of CRISPR-Cas9 in humans raises ethical concerns regarding safety and the possibility of unintended consequences. The development of policies is necessary to ensure the ethical and reliable application of CRISPR-Cas9.

Access to Treatment

Issues related to access to CRISPR-Cas9 treatment for SCD patients may arise, particularly in low- and middle-income countries. Policies should be developed to ensure equitable access to the benefits of this technology for all patients in need.

CONCLUSION

After analyzing the available evidence on the use of CRISPR-Cas9 for the management of SCD, it can be concluded that this technology is novel and shows promise as a potential therapeutic option for the condition. Studies have demonstrated the successful correction of the genetic mutation responsible for SCD in clinical settings. One of the main challenges of this technology is the delivery of the CRISPR-Cas9 system to the bone marrow, where the hematopoietic stem cells reside. The off-target effects of the CRISPR-Cas9 system also need to be further studied and minimized to ensure the safety of the treatment.

Despite these challenges, the potential benefits of CRISPR-Cas9 for SCD cannot be neglected. The ability to correct the genetic mutation responsible for the condition offers a potentially curative approach to the disease. Overall, further research and clinical trials are necessary to fully evaluate the safety and efficacy of CRISPR-Cas9 as a therapeutic option for SCD. Nevertheless, the current evidence suggests that CRISPR-Cas9 has the potential to revolutionize the treatment of this debilitating disease by offering a curative option.

Conflict of Interest

The authors hereby declare no conflict of interest in conducting this research, and in publishing this manuscript.

REFERENCES

Adams, R. J., McKie, V. C., Hsu, L., Files, B., Vichinsky, E., Pegelow, C. & *et al.* (1998). Prevention of a first stroke by transfusions in children with sickle cell anaemia and abnormal results on transcranial Doppler ultrasonography. *The New England Journal of Medicine*, 339(1), 5-11. <https://doi.org/10.1056/NEJM199807023390102>.

Ataga, K. I., Kutlar, A., Kanter, J., Liles, D., Cancado, R., Friedrisch, J. & *et al.* (2017). Crizanlizumab for the prevention of pain crises in sickle cell disease. *The New England Journal of Medicine*, 376, 429-439.

Bak, R. O., Dever, D. P. & Porteus, M. H. (2018). CRISPR-Cas9 genome editing in human hematopoietic stem

cells. *Nature Protocols*, 13(2), 358–376.

Baronciani, D., Angelucci, E., Potschger, U., Gaziev, J., Yesilipek, A., Zecca, M. & *et al.* (2016). Haemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Haemoglobinopathy Registry, 2000-2010. *Bone Marrow Transplant*, 51, 536-541.

Canver, M. C., Smith, E. C., Sher, F., Pinello, L., Sanjana, N. E., Shalem, O. & *et al.* (2015). BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature*, 527, 192-197. <https://doi.org/10.1038/nature15521>.

Chaudhary, A., Kumari, B., Choudhury, M., Singh, S. & Kaur, 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>.

Demirci, S., Leonard, A., Essawi, K. & Tisdale, J. F. (2021). CRISPR-Cas9 to induce fetal hemoglobin for the treatment of sickle cell disease. *Molecular Therapy, Methods & Clinical Development*, 23, 276–285.

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*, 1144, 37-52. https://doi.org/10.1007/5584_2018_331.

Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E. & *et al.* (2016). CRISPR/Cas9 β -globin gene targeting in human hematopoietic stem cells. *Nature*, 539(7629), 384–389.

DeWitt, M. A., Magis, W., Bray, N. L., Wang, T., Berman, J. R., Urbinati, F. & *et al.* (2016). Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Science Translational Medicine*, 8(360), 360ra134. <https://doi.org/10.1126/scitranslmed.aaf9336>.

Eapen, M., Brazauskas, R., Walters, M. C., Bernaudin, F., Bo-Subait, K., Fitzhugh, C. D. & *et al.* (2019). Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: a retrospective multicentre, cohort study. *The Lancet Haematology*, 6(11), e585-e596. [https://doi.org/10.1016/S2352-3026\(19\)30154-1](https://doi.org/10.1016/S2352-3026(19)30154-1).

Ekechi, H. O., Ikwuka, A. O., Udeh, F. C. & Abraham, J. C. (2023). Effects of Ethanol Extract of Rauwolfia vomitoria Leaf on Lipid Profile and Cerebellar Histology in Cisplatin-induced Oxidative Stress. *British Journal of Medical and Health Research*, 10(5), 16-39. <https://doi.org/10.5281/zenodo.8042521>.

Eshka, S. F. A., Bahador, M., Gordan, M. M., Karbasi, S., Tabar, Z. M. & Basiri, M. (2022). A systematic review of gene editing clinical trials. medRxiv, <https://doi.org/10.1101/2022.11.24.22282599>.

Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y. S., Domm, J., Eustace, B. K. & *et al.* (2021). CRISPR-Cas9 gene editing for sickle cell disease and β -thalassemia.

- The New England Journal of Medicine*, 384(3), 252–260.
- Gaston, M. H. & *et al.* (1986). Prophylaxis with oral penicillin in children with sickle cell anaemia: a randomized trial. *The New England Journal of Medicine*, 1593(52), 314–52.
- Gluckman, E., Cappelli, B., Bernaudin, F., Labopin, M., Volt, F., Carreras, J. & *et al.* (2017). Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood*, 129, 1548-1556.
- Guo, N., Liu, J. B., Li, W., Ma, Y. S. & Fu D. (2022). The power and the promise of CRISPR/Cas9 genome editing for clinical application with gene therapy. *Journal of Advanced Research*, 40, 135–152. <https://doi.org/10.1016/j.jare.2021.11.018>.
- Hall, J. E. & Hall, M. E. (2020). Guyton and Hall Textbook of Medical Physiology. 14th Edition. Elsevier, p. 441-446.
- Hoban, M. D., Lumaquin, D., Kuo, C. Y., Romero, Z., Long, J., Ho, M. & *et al.* (2016)a. CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. *Molecular Therapy*, 24(9), 1561–1569.
- Hoban, M. D., Orkin, S. H. & Bauer, D. E. (2016)b. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. *Blood*, 127(7), 839–848.
- Hossain, M. A. & Bungert, J. (2017). Genome editing for sickle cell disease: a little BCL11A goes a long way. *Molecular Therapy*, 25(3), 561– 562. <https://doi.org/10.1016/j.ymthe.2017.02.003>.
- Ikwuka, A. O. (2015). Risk factors for the pathogenesis of diabetes mellitus type 2. Materials of 84th Scientific and Practical Conference of Students and Young Scientists with International Participation “Innovations in medicine”, p. 19. Available online: http://www.ifnmu.edu.ua/images/snt/files/konferenciya/Tezu_2015.pdf
- Ikwuka, A. O. (2017)a. Dyslipidemia risk severity in patients with diabetes mellitus type 2 and essential hypertension. Journal of the 21st International Medical Congress of Students and Young Scientists, p. 59.
- Ikwuka, A. O. (2017)b. Effectiveness of dapagliflozin in patients with diabetes mellitus type 2 and essential hypertension. Book of abstracts of the 7th International Students’ Scientific Conference of Young Medical Researchers, p. 102. Available online: http://www.stn.umed.wroc.pl/files/lm/Accepted_papers.16113.pdf
- Ikwuka, A. O. (2017)c. Influence of dyslipidemia in patients with diabetes mellitus type 2 and essential hypertension. *The Pharma Innovation Journal*, 6(3), 101-103. Available online: <http://www.thepharmajournal.com/archives/?year=2017&vol=6&issue=3&part=B>
- Ikwuka, A. O. & Haman, I. O. (2017)d. Features of kidney damage in patients with diabetes mellitus type 2 and essential hypertension. *Journal of 86th Scientific and Practical Conference of Students and Young Scientists with International Participation “Innovations in medicine”*, p. 144. Available online: [http://www.ifnmu.edu.ua/images/snt/86-konf-tezi%20\(1\).pdf](http://www.ifnmu.edu.ua/images/snt/86-konf-tezi%20(1).pdf)
- Ikwuka, A. O., Virstyuk, N. G. & Luchko, O. R. (2017)e. Features of the functional state of kidneys in patients with diabetes mellitus type 2 and essential arterial hypertension. *Materials of scientific-practical conference with international participation “Babenkivski reading”*, p. 48.
- Ikwuka, A. O. (2018)a. Clinical dynamics in patients with diabetes mellitus type 2 and concomitant essential hypertensive disease treated with dapagliflozin. *Journal of the 22nd International Medical Congress of Students and Young Scientists*, p. 32.
- Ikwuka, A. O. (2018)b. Clinical effectiveness of SGLT-2 inhibitors in patients with diabetes mellitus type 2 and essential hypertensive disease. *Endocrine Practice*, 24(1), 74. [https://doi.org/10.1016/S1530-891X\(20\)47129-0](https://doi.org/10.1016/S1530-891X(20)47129-0).
- Ikwuka, A. O. (2018)c. Features of kidney damage in patients with arterial hypertension and type 2 diabetes mellitus and optimization of treatment. *Specialized Academic Council IFNMU*, Available online: http://www.ifnmu.edu.ua/images/zagalna_informacia/spec_vcheni_radi/2017-2019/%D0%9420.601.01/Ikwuka/Avtoreferat.pdf
- Ikwuka, A. O. & Paliy, Yu. (2018)d. Structural changes of the left ventricular myocardium in patients with essential arterial hypertension and diabetes mellitus type 2. *Abstracts of the 87th Scientific Conference of Students and Young Scientists with International Participation “Innovations in medicine”*, p. 25-26. Available online: https://www.ifnmu.edu.ua/images/snt/zaproshehennia_eng.pdf
- Ikwuka, A. O. (2019)a. Clinical dynamics of nephropathy in patients with diabetes mellitus type 2 and concomitant essential hypertensive disease. *Clinical Medicine*, 19(2), s39. <https://doi.org/10.7861/clinmedicine.19-2-s39>.
- Ikwuka, A. O. (2019)b. Clinical effectiveness of GLP-1 RAs in patients with metabolic syndrome diseases. *Endocrine Practice*, 25(1), 104-105. [https://doi.org/10.1016/S1530-891X\(20\)46611-X](https://doi.org/10.1016/S1530-891X(20)46611-X).
- Ikwuka, A. O. & Virstyuk, N. G. (2019)c. Pattern of cardiac remodelling of the left ventricle in patients with essential hypertensive disease and concomitant type 2 diabetes mellitus. *Clinical Medicine*, 19(3), s92. <https://doi.org/10.7861/clinmedicine.19-3-s92>.
- Ikwuka, A. O. & Virstyuk, N. G. (2021). Influence of SGLT2 inhibitor and A2RB (AT1) on fibrogenesis and heart failure in patients with essential hypertensive disease combined with diabetes mellitus type 2. *E-Poster No. 143 of the 44th & 45th Annual General and Scientific Meeting of the West African College of Physicians (WACP)*, 1 - 3 November, 2021. <https://doi.org/10.13140/RG.2.2.26912.87047>.
- Ikwuka, A. O. & Virstyuk, N. (2022). Prognostic markers of nephropathy in patients with dual metabolic syndrome diseases (essential hypertensive disease and concomitant type 2 diabetes mellitus). *Endocrine Practice*, 28(5), S65-S66. <https://doi.org/10.1016/j.eprac.2022.03.164>.
- Ikwuka, A. O. (2023)a. *Dr. Aloy's Core Essential Series (DACES) Medical Genetics*. 1st Edition. Science and Education Publishing, USA, p. 1.

- Ikwuka, A. O. (2023)b. *Dr. Aloy's Core Essential Series (DACES) Medical Genetics*. 1st Edition. Science and Education Publishing, USA, p. 20-27.
- Ikwuka, A. O. (2023)c. *Dr. Aloy's Core Essential Series (DACES) Immunology*. 1st Edition. Science and Education Publishing, USA, p. 31.
- Ikwuka, A. O. & Virstyuk, N. (2023)d. Patterns and Influence of Cardio-Metabolic Insufficiency in Patients with Essential Hypertensive Disease and Concomitant Type 2 Diabetes Mellitus. *Endocrine Practice*, 29(5), S32-S33. <https://doi.org/10.1016/j.eprac.2023.03.076>.
- Ikwuka, A. O., Virstyuk, N. G., Luchko, O. R. & Kobitovych, I. (2023)e. Heterogeneity Of Renal Pathogenicity On The Background Of Asymptomatic Hyperuricemia In Patients With Dual Metabolic Syndrome Diseases (Essential Hypertensive Disease and Type 2 Diabetes Mellitus). *British Journal of Medical and Health Research*, 10(2), 1-9. <https://doi.org/10.5281/zenodo.7690636>.
- Kotagama, O. W., Jayasinghe, C. D. & Abeysinghe, T. (2019). Era of genomic medicine: a narrative review on CRISPR technology as a potential therapeutic tool for human diseases. *Biomed Research International*, 2019, 1–15. <https://doi.org/10.1155/2019/1369682>.
- Lattanzi, A., Camarena, J., Lahiri, P., Segal, H., Srifa, W., Vakulskas, C. A. & et al. (2021). Development of β -globin gene correction in human hematopoietic stem cells as a potential durable treatment for sickle cell disease. *Science Translational Medicine*, 3(598), eabf2444. <https://doi.org/10.1126/scitranslmed.abf2444>.
- Magis, W., DeWitt, M. A., Wyman, S. K., Vu, J. T., Heo, S., Shao, S. J. & et al. (2018). In vivo selection for corrected β -globin alleles after CRISPR/Cas9 editing in human sickle hematopoietic stem cells enhances therapeutic potential. *BioRxiv*; <https://doi.org/10.1101/432716>.
- Mandal, P. K., Ferreira, L. M., Collins, R., Meissner, T. B., Boutwell, C. L., Friesen, M. & et al. (2014). Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. *Stem Cell*, 15(5), 643-652. <https://doi.org/10.1016/j.stem.2014.10.004>.
- National Heart, Lung, and Blood Institute. (2015). "What is sickle cell disease?"; Available online: <https://www.nhlbi.nih.gov/health/sickle-cell-disease>
- Onda, M., Akaishi, J., Asaka, S., Okamoto, J., Miyamoto, S., Mizutani, K. & et al. (2005). Decrease expression of haemoglobin beta (HBB) gene in anaplastic thyroid cancer and recovery of its expression inhibits cell growth. *British Journal of Cancer*, 92, 2216-2224. <https://doi.org/10.1038/sj/bjc.6602634>.
- Orkin, S. H. & Bauer, D. E. (2019). Emerging genetic therapy for sickle cell disease. *Annual Review of Medicine*, 70(1), 257–271.
- Pandey, V. K., Tripathi, A., Bhushan, R., Ali, A. & Dubey, P. K. (2017). Application of CRISPR/Cas9 genome editing in genetic disorders: a systematic review up to date. *Journal of Genetic Syndromes & Gene Therapy*, 8(2), 321-331. <https://doi.org/10.4172/2157-7412.1000321>.
- Park, S. H., Lee, C. M., Deshmukh, H. & Bao, G. (2016). Therapeutic CRISPR/Cas9 genome editing for treating sickle cell disease. *Blood*, 128(22), 4703–4703.
- Park, S. H., Lee, C. M., Dever, D. P., Davis, T. H., Camarena, J., Srifa, W. & et al. (2019). Highly efficient editing of the β -globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. *Nucleic Acids Research*, 47(15), 7955-7972. <https://doi.org/10.1093/nar/gkz475>.
- Park, S. H. & Bao, G. (2021)a. 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>.
- Park, S. H. J. P., Cao, M., Zhang, Y., Sheehan, V. A. & Bao, G. (2021)b. CRISPR/cas9 editing induces high rates of unintended large gene modifications in HSPCs from patients with sickle cell disease. *Blood*, 138(Suppl.1), 3969–3969.
- Piel, F. B. (2013). Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. *PLoS Medicine*, 10(7), e1001484. <https://doi.org/10.1371/journal.pmed.1001484>.
- Pinhas, A., Zhou, D. B., Otero-Marquez, O., CastanosToral, M. V., Migacz, J. V., Glassberg, J. & et al. (2022). Efficacy of CRISPR-based gene editing in a sickle cell disease patient as measured through the eye. *Case Reports in Hematology*, 2022, 6079631. <https://doi.org/10.1155/2022/6079631>.
- Ramadier, S., Chalumeau, A., Felix, T., Othman, N., Aknoun, S., Casini, A. & et al. (2022). Combination of lentiviral and genome editing technologies for the treatment of sickle cell disease. *Molecular Therapy*, 30(1), 145–163. <https://doi.org/10.1016/j.ymthe.2021.08.019>.
- Ranadive, A. & Linga-Easwaran, J. (2022). Current sickle cell disease gene therapy treatments: literature review. *Journal of Student Research*, 1(1). <https://doi.org/10.47611/jsr.v1i1.1534>.
- Rodriguez Rodriguez, D., Ramirez Solis, R., Garza Elizondo, M., Garza Rodriguez, M. & Barrera Saldaia, H. (2019). Genome editing: a perspective on the application of CRISPR/Cas9 to study human diseases (Review). *International Journal of Molecular Medicine*, 43(4), 1559-1574. <https://doi.org/10.3892/ijmm.2019.4112>.
- Samuelson, C., Radtke, S., Zhu, H., Llewellyn, M., Fields, E., Cook, S. & et al. (2021). Multiplex CRISPR/Cas9 genome editing in hematopoietic stem cells for fetal haemoglobin reinduction generates chromosomal translocations. *Molecular Therapy, Methods and Clinical Development*, 23, 507–523. <https://doi.org/10.1016/j.omtm.2021.10.008>.
- Sharma, G., Sharma, A. R., Bhattacharya, M., Lee, S. S. & Chakraborty, C. (2021). CRISPR-Cas9: a preclinical and clinical perspective for the treatment of human diseases. *Molecular Therapy*, 29(2), 571–586.
- Suwito, B. E., Adji, A. S., Widjaja, J. S., Angel, S. C. S., Al Hajiri, A. Z. Z. & Salamy, N. F. W. (2023). A review of CRISPR Cas9 for SCA: treatment strategies and could target β -globin gene and BCL11A gene using CRISPR

- Cas9 prevent the patient from sickle cell anaemia? *Macedonian Journal of Medical Sciences*, 11(F), 95-106. <https://doi.org/10.3889/oamjms.2023.11435>.
- Tasan, I., Jain, S. & Zhao, H. (2016). Use of genome-editing tools to treat sickle cell disease. *Human Genetics*, 135(9), 1011–1028. <https://doi.org/10.1007/s00439-016-1688-0>.
- Thornburg, C. D., Files, B. A., Luo, Z., Miller, S.T., Kalpathi, R., Iyer, R. & *et al.* (2012). Impact of hydroxyurea on clinical events in the BABY HUG trial. *Blood*, 120(22), 4304-4310. <https://doi.org/10.1182/blood-2012-03-419879>.
- Uchida, N., Drysdale, C. M., Nassehi, T., Gamer, J., Yapundich, M., DiNicola, J. & *et al.* (2021). Cas9 protein delivery non-integrating lentiviral vectors for gene correction in sickle cell disease. *Molecular Therapy, Methods and Clinical Development*, 21, 121–32. <https://doi.org/10.1016/j.omtm.2021.02.022>.
- Uda, M., Galanello, R., Sanna, S., Lettre, G., Sankaran, V. G., Chen, W. & *et al.* (2008). Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of β -thalassemia. *Proceedings of the National Academy of Science of the United States of America*, 105, 1620-1625.
- Ureña-Bailén, G., Block, M., Grandi, T., Aivazidou, F., Quednau, J., Krenz, D. & *et al.* (2023). Automated good manufacturing practice-compatible CRISPR-Cas9 editing of hematopoietic stem and progenitor cells for clinical treatment of β -hemoglobinopathies. *The CRISPR Journal*, 6(1), 5–16.
- Virstyuk, N. G., Ikwuka, A. O., Haman, I. O. & Adebomi, M. S. (2016). Diabetes mellitus type 2, arterial hypertension and dyslipidemia. *Materials of 2nd International Scientific and Practical Conference “Therapeutic readings: modern aspects of diagnosis and treatment of diseases of internal organs”*, p. 46-47.
- Virstyuk, N. G. & Ikwuka, A. O. (2017)a. Diagnostic and prognostic markers of the diabetes mellitus type 2 course in connection with essential arterial hypertension taking into account the kidney function. *Precarpathian Journal Pulse*, 8(44), 53-62.
- Virstyuk, N. H., Ikwuka, A. O., Losyuk, L. V., Kobrynska, O. Ya. & Markiv, H. D. (2017)b. Dapagliflozin utility in patients with diabetes mellitus type 2 and essential hypertensive disease. *Actual Problems of Modern Medicine*, 4(60)1, 76-79. Available online: http://www.umsa.edu.ua/journal2stat4_2017_eng.html
- Virstyuk, N. G. & Ikwuka, A. O. (2018)a. Features of asymptomatic hyperuricemia in patients with diabetes mellitus type 2 and concomitant essential arterial hypertension. *Clinical and Experimental Pathology*, 1(63), 22-26. <https://doi.org/10.24061/1727-4338.XVII.1.63.2018.5>.
- Virstyuk, N. G., Ikwuka, A. O. & Didushko, O. M. (2018)b. Effect of dapagliflozin on the level of uric acid during asymptomatic hyperuricemia in patients with diabetes mellitus type 2 and concomitant arterial hypertension. *Art of Medicine*, 1(5), 21-26. Available online: <https://art-of-medicine.ifnmu.edu.ua/index.php/aom/article/view/179/150>
- Virstyuk, N. H. & Ikwuka, A. O. (2018)c. Dapagliflozin influence on the clinical course of diabetes mellitus type 2 and essential hypertension in patients. *Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions*, p. 2007-2008. https://doi.org/10.1007/978-3-319-70548-4_582.
- Virstyuk, N. G. & Ikwuka, A. O. (2019). Nephropathic characteristics in patients with diabetes mellitus type 2 and essential hypertensive disease. *Art of Medicine*, 1(5), 44-47. <https://doi.org/10.21802/artm.2019.1.9.44>.
- Virstyuk, N. G. & Ikwuka, A. O. (2021)a. Asymptomatic hyperuricemia and functional state of the kidneys in patients with essential arterial hypertension and concomitant diabetes mellitus type 2. *European Journal of Clinical Medicine*, 2(3), 100-104. <https://doi.org/10.24018/clinicmed.2021.2.3.65>.
- Virstyuk, N. H., Ikwuka, A. O., Luchko, O. R. & Kocherzhat, O. I. (2021)b. Peculiarities of renal insufficiency in patients with diabetes mellitus type 2 and arterial hypertension. *Materials of scientific-practical conference with international participation “Achievements and prospects of experimental and clinical endocrinology” Twentieth Danilevsky readings*, p. 86-87.
- Wen, J., Tao, W., Hao, S. & Zu, Y. (2017). Cellular function reinstatement of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing. *Journal of Hematology & Oncology*, 10(1), 119. <https://doi.org/10.1186/s13045-017-0489-9>.
- World Health Organization. (2011). Sickle-cell disease and other haemoglobin disorders. Fact sheet No. 308. Available online: <http://www.who.int/mediacentre/factsheets/fs308/en/>
- Wu, Y., Zeng, J., Roscoe, B. P., Liu, P., Yao, Q., Lazzarotto, C. R. & *et al.* (2019). Highly efficient therapeutic gene editing of human hematopoietic stem cells. *Nature Medicine*, 25, 776-783. <https://doi.org/10.1038/s41591-019-0401-y>.
- Xiao-Jie, L., Hui-Ying, X., Zun-Ping, K., Jin-Lian, C. & Li-Juan, J. (2015). CRISPR-Cas9: a new and promising player in gene therapy. *Journal of Medical Genetics*, 52(5), 289–296. <https://doi.org/10.1136/jmedgenet-2014-102968>.
- Zaib, S., Saleem, M. A. & Khan, I. (2022). CRISPR-Cas9 genome engineering: trends in medicine and health. *Mini-Reviews in Medicinal Chemistry*, 22(3), 410–421.