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Review Article

THERAPEUTIC CRISPR/CAS9 GENOME EDITING TOOL FOR TREATING SICKLE CELL DISEASE

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Abstract: *Sickle cell disease (SCD) is a serious inherited condition that leads to high rates of illness and mortality, and a complete cure is not yet available. Recent advances suggest that correcting the genetic mutations in hematopoietic stem/progenitor cells (HSPCs) or boosting fetal hemoglobin might stop red blood cells from sickling. Techniques like CRISPR/Cas9 genome editing and creating induced pluripotent stem cells (iPSCs) are being explored to address SCD more effectively. Genome editing has proved beneficial as a treatment choice. CRISPR/Cas9's ongoing revelations have disrupted genetic design and opened the possibility of the idea into an actual clinical reality. We summarize DNA designing software that uses CRISPR/Cas9, causing problems, and the future perspectives of CRISPR/Cas9 being a viable option to treat SCD. A genome-altering technology that is designed to treat SCD alteration of the β-globin's quality in non-hematopoietic hematopoietic cells, red blood cells, which make normal hemoglobin protein, is enhanced by this modification. Suppose a donor template that is homologous to the donor is codified. In that case, we demonstrate that the Transcription Activator-Like Effector Nucleases (TALENs), as well as the CRISPR/Cas9 nuclear cleavage system, could focus DNA sequences in the vicinity of the sickle-cell variant in the gene, allowing for specific site-cutting and facilitating accurate correction. Off-target and on-target cleavage rates for different pairs of TALENs and guide RNAs for CRISPR have been assessed. In vitro, the CRISPR/Cas9 proteins were introduced directly to CD34+ cells, which resulted in more than 18% gene modifications. Furthermore, we demonstrate how sickle cell disease mutation within CD34+ hematopoietic stems derived from bone marrow cells and trails cells from sickle cell disease patients triggers the production of wild-type hemoglobin. Findings suggest promising improvements in gene editing precision and potential treatment outcomes, though challenges still exist regarding off-target effects, delivery systems, and ethical considerations.*

Keywords: CRISPER Cas-9, Sickle Cell Disease, CD34+ Stem Cells, Genome Editing

Introduction

While recognizing the hereditary cause of Sickle Cell Disease (SCD) in 1957, the treatment options available for the condition remain severely limited. However, despite rapid progress in innovation during the latter years, the standard of life for SCD sufferers has not changed significantly (1, 2). Genome editing can be used to develop strategies that include correcting the mutations of Hematopoietic Stem/Progenitor Cells (HSPCs), causing the expression of fetal hemoglobin, and creating corrected Induced Pluripotent Stem Cells (iPSCs) for therapeutic interventions for diseases such as sickle cell anemia (3).

Specific genome editing technologies such as Transcription Activator-Like Effector Nucleases (TALENs) and CRISPR/Cas9 allow exact correction of sickle cell-related mutations in the β-globin gene within the hematopoietic stem cell, which increases the creation of red blood cells containing normal hemoglobin protein. Four medications are approved by the US Food and Drug Administration (FDA) to alleviate the severe side effects associated with SCD. These include hydroxyurea, L-glutamine, voxelotor, as well as crizanlizumab (4).

Hydroxyurea, the invention of CRISPR and Cas9 systems, which are regular, interspersed, clustered small palindromic repeats, has transformed gene therapy, making it possible to pinpoint particular genes (5). It was first discovered in archaea and microorganisms for protection against phages, plasmids, and other bacterial toxins (6). The CRISPR-Cas framework was developed first by E.coli type K12 in 1987 (7).

Genome editing to treat SCD

Genome altering allows the explicit modification of the human genome to counterbalance or address variations in hereditary conditions that are fundamental to us (8). The alteration of the genome typically outcome from DNA 2-fold components breaks DNA double-stranded breaks (DSBs), which are mediated via configurable endonucleases for an architect, such as Zinc Finger Nucleases (ZFNs), recording stimulator-like effector nucleases (TALENs) and the framework CRISPR-Cas9 (9).

In addition to the proteins-driven ZFNs and TALENs, CRISPR Cas-9 nucleases include a specifically created aid guide RNA (gRNA) essential to a perfect objective succession and guide the Cas-9 protein towards the chosen genome to trigger the development of a DSB (10). Non-homologous End Joining (NHEJ) and HDR are two essential solutions triggered by DSBs formation. NHEJ is a straightforward, however blunder-prone pathway leading to cancellations and inclusions at the break location (11).

NHEJ can be used to cause disruptions or for Cis-administrative substances that are high in efficacy. It is found to have rates of greater than 90 percent for High Density Satellite DNA Clusters (HDSCs). In contrast, Homology Directed Repair (HDR) is a sluggish but precise DNA fix trail, which requires co-presented DNA pieces in a form that addresses disease-related changes that may be hidden or obscured among other factors. HDR is a type of

DNA fix trail that HDR pattern is typically transferred to HSCs through vectors based on adeno-associated viruses (AAV) as well as oligo-deoxynucleotides ODNs (oligonucleotides comprising at least two or more one or two strands) (12). Although HDR is restricted to the G2/S/G2 period of cell sequence, the ability to accomplish quality focus on events of greater than 20% the majority of calm long haul the process of repopulating HSCs is still challenging (13).

The methods are based on CRISPR Cas-nickases that cut only a single DNA stripe. This group comprises prime editors (PEs) and base editors (BEs). The Cas9 endonuclease can be entirely switched into a Cas9 Nickase, which can display transformations between one of the synergist areas that comprise Cas9. BEs are created when a deaminase-deaminase space is combined into the Cas Nickase. PEs are made up of a Cas9 Nickase that is attached to a reverse transcriptase designed and converts the segment of pegRNA (prime changing gRNA) into DNA in order to display perfect variations like bases that undergo a transformation or include deletions/inclusions of about 80 Bp (16).

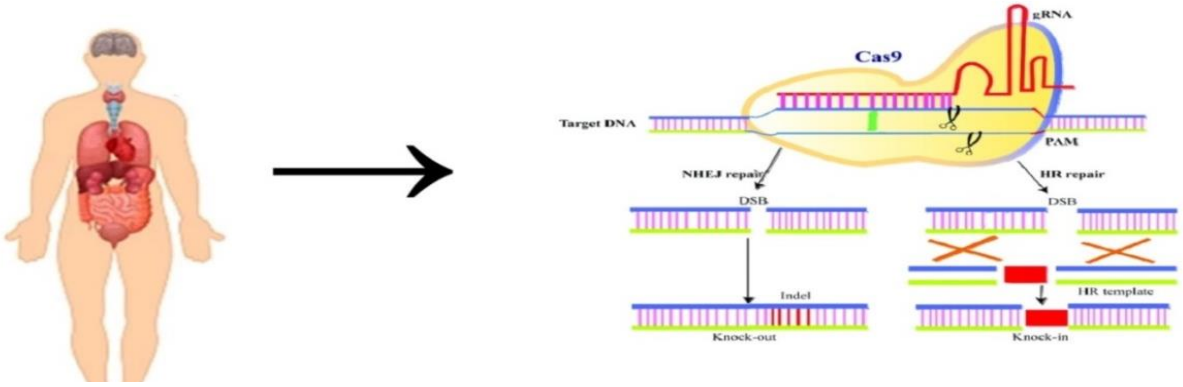

Figure 1: CRISPER-Case 9 Genome editing process for SCD Treatment

Overview of CRISPR-Cas9 Clinical Trials for SCD

The positive effects of fetal hemoglobin (HbF) led to the prior examination of Hydroxyurea (HU), explored by two patients suffering from the Sickle Cell Hemoglobin (HbS) form of anemia, also described as HbS disorder in 1984. The sense that quantifiable and easily manageable increases is that HbF can be achieved with minimal poisoning. However, no change in the direction of the experiment was observed in the study's short time frame. Despite the positive fundamental outcome, the reassuring findings triggered a variety of preliminary clinical trials of Hydroxyurea (HU), first with adults and then with pediatric patients suffering from SCD. The drug was approved by the US Food and Drug Administration (FDA), which approved HU worldwide to treat SCD for adults in 1998 and teens in 2017 because of its overall safety profile and effectiveness. How we might interpret that physiological processes concerned with SCD have likewise been significantly aided by the application of refined sickle with moussing models (14, 15).

Later, it gave us new insights into how sickled red blood cells interact with their surroundings. This includes their connections with blood vessels, monocytes, neutrophils, and platelets and the development of molecules that help cells stick to the blood vessel walls. Atoms, like- and E-selectin, fundamentally used in attachment, as well as the activation of white platelets, specifically neutrophils, to control blood vessel arrangement within an organ or components, have been proven to be able to treat a large portion of the aggravation-related emergency disordered physiological processes and are now treatment goals (16). Since the chain reaction of deoxy-HbS is the major event that aids in triggering the subsequent outcomes of Sickle Cell Anemia, some effective methods have focused on the moderation of this primary driving factor, with both hereditary and chemical opponents of sickle systems. The most effective method is enlisting an HbF combination, accessible not only by the plethora of disease and medical surveillance studies but also by the heat and energy dynamic of the chain reaction. The cause is the irregular flow of HbF enclosed by RBCs; another reason could be the varying medical reactions in SCD patients. In any case, the rise in HbF caused by HU may provide a compelling justification to be mentioned. The main issue is how we look at atomic energy systems that guide children to adults. Changes in Hb have led to a rise in the age of doctors who are not dependent on the trigger of the occurrence of "stress erythropoiesis," and it is a decline to two main collections (17). One alters chromatin's function (for example, gemcitabine when it is acted upon by DNA the methylation process or histone deacetylase inhibitors) in addition to extra elements that impact DNA-restricting information. Concurrent comprehensive association studies identified BCL11A as a commonly used repressor protein for silencing the harmful gamma (g) globin genes. Later, this was linked with zinc finger and BTB domain-containing protein 7A (ZBTB7A), also known as leukemia-related factor (LRF) (18).

In 2018, key examinations by two groups displayed that BCL11A and ZBTB7A each tighten toward a related acknowledgment spot inside the g-globin promoter. Besides being a g-globin repressor, BCL11A is likewise fundamental for the expansion of B-lymphoid (19). Identifying the main erythroid-specific companion factors was crucial and significant for the development of medical trials aimed at suppressing BCL11A using two different genetic approaches: lentivirus short guide RNA (sgRNA) and CRISPR/CRISPR-associated nuclease-9 (Cas-9) editing (20).

Another hereditary method for the self-generated globin is to generate vast amounts of HbF to mimic the regular HPFH changes in G-globin advertiser by altering genes to block the restriction to BCL11A and ZBTB7A/LRF repressors. The idea of reversing the transformation of SCD (rs334) can be the fastest method since a similar nucleotide alteration can be found for all bS variants, but the rate constraints homology-coordinated DNA fixes at how rectification can be achieved, and also the age at which it takes for deletions/additions, and the transformation of B-thalassemia quality of the variant (21).

Patient Outcomes and Safety Considerations for SCD

Since 1960, the outlook for people with sickle cell disease has dramatically improved. Sir John Dacia described sickle cell disease as "mainly a childhood disease." He wrote that even with high-quality medical care, many patients did not survive to adulthood. In his 1973 survey given post-mortems, Diggs assessed a middle endurance of 14.3 years, with 20% of the passing happening in the initial two years of lifespan, 33% happening before the fifth year of life, half somewhere in the range of five and thirty years old, and single sixth later the age of 102 (22).

However, according to the newly released Cooperative Study of Sickle Cell Disease (CSSCD), about 90% of children and teenagers with sickle cell anemia (caused by sickle hemoglobin) and 95% of those with sickle cell hemoglobin C disease (a variation involving hemoglobin S and C) are now living into their twenties. Among patients under 20 years old in that study, the highest death rate was between ages 1 and 3, primarily due to infections, especially Streptococcus pneumonia sepsis. Low fetal hemoglobin levels, insufficient hemoglobin, and higher white blood cell counts were all associated with an increased risk of death (23).

When it comes to many studies on deaths among adults, the date of death is determined from the dissection data or the clinical record. These experimental events that lead to passing are not documented similarly. According to the Jamaican studies carried out by Thomas and Co. at the age of 10, chest pain was the leading cause of death. It was then long-term organ damage such as heart or kidney failure as well as a stroke to the brain as well as pregnancy-related issues.

Powers and his colleagues described similar results in a companion of the victim who remained in Los Angeles for quite some duration, observing that endurance tests in the context of this event could remain affected by the variations of exercise in the course of the. For instance, they suggested that the high level of death rate that they found in sickle cell anemia patients could be a result of the high rate of mortality for pregnant women before 1975. It is essential to know the current death rate and the events that occur within a short time prior to dying, as well as the medical situations that can be linked to an increased chance of death (24, 25).

Counseling patients, predicting potentially dangerous clinical scenarios, constructing specific therapies, and executing clinical trials count heavily on this knowledge. The CSSCD has a comprehensive examination of the passing between patients within the CSSCD and emphasizes that the risk of death is a factor for patients who are more than 20 years old (26).

"Off-target Effect and hurdles in cas-9 implementations."

Even though it is true that there have been massive developments here, many specialties should be resolved, such as faulty movements, inadequate Intel, or faulty homology-coordinated fixes (HDR) efficacy and in vivo transmission of Cas frame components and the immune system's response. Chromosome changes triggered by various impacts may accidentally affect gnomically unmatched regions and restrict the use of CRISPR-Cas, switching advancements to beneficial motives. Research has shown that CRISPR-Cas devices could not be as protected from unwanted impacts as some of the other standard techniques for altering quality in light because Cas-9 proteins are monomers, which could inadvertently alter the ID of targets with smaller intervals. Likewise, ZFN and TALEN gatherings are dimeric. TALEN and ZFN collections are dimeric (27).

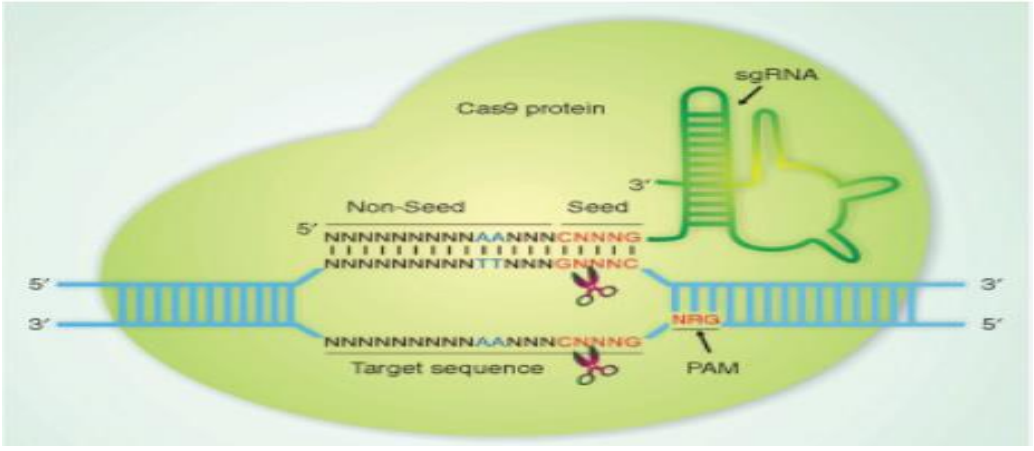

Figure 2: Targeted Sequences and Hurdles in CRISPER Cas-9 Sequencing

which split the spectators (not intended target) and help RNA sense confusion. CRISPR structures that can be induced in life can trigger resistance reactions to unknown substances by expanding people's natural invulnerability and flexibility of vulnerability. Guide RNAs can be used to trigger natural resistance reactions.

laboratory facilities, identifies existing limitations that could restrict the use of CRISPR-Cas frameworks as quality-altering instrument compartments for accuracy medicine, and provides some perspectives regarding the best approach to overcome these obstacles and speed up specialization progression (28).

Ethical Concerns Surrounding Gene Editing for SCD

The issues of equal accessibility and exam guidelines are essential, but there is a risk in focusing solely on accessibility and moderation; global well-being researchers run the risk of leaving experts as well as patients, researchers, and decision-makers without much information regarding other ethical, and legal ethical, or social concerns which could arise during the design and conduct of preliminaries for quality treatment in Africa and beyond, such as the desirable way to ensure that quality physical treatment is conducted in a reliable ethically sound manner (29).

This paper addresses important ethical, legal, and social issues arising from gene therapy trials to treat sickle cell disease (SCD) in Africa (Mununget al., 2023). The ethics of CRISPR have also been evaluated by the US National Academies of Sciences, Engineering, and Medicine (NASEM). In their article, "CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool," authors Carolyn Brokowski and MazharAdli explained that the NASEM report provides a comprehensive analysis of widespread concerns about human genome editing (30).

Significantly, the advisory group leaned toward substantial genome altering but didn't allow genomic alteration for any sort of upgrade" (Brokowski). This entry is incredibly adroit when considering the morals of utilizing CRISPR. As made sense in this statement, the report by the NASEM involved a thorough examination while exploring the morals of human genome alteration. Through this top-to-bottom hunt, NASEM wound up supporting genome altering.

However, they also made a crucial distinction, stating that the edits should not be used to improve a person. This distinction is crucial when considering the ethics of genetic editing in the future. Instead of "designing" genetics to have particular characteristics or appearances, the objective should be to treat and prevent genetic conditions (31).

"Future Directions and Advancement in CRISPR Technology"

The effectiveness of the CRISPR-Cas system is still being debated. Some researchers have raised concerns about its technical limitations, particularly regarding unintended results. Cross-breeding with different kinds of plants is an everyday practice in the commercial development of products and seed multiplication. It allows for the omission of possible off-target consequences in time frames considerably less than the traditional cross-breeding methods for crop development. We believe this is more concerned with academics. There were no tests at all in the context of whole genome sequencing that could discover odd regions of cleavage of the genome by Cas12a as well as Cas9 nucleases found in cotton rice and Arabidopsis (32).

There is evidence that Cas12a and Cas9 nucleases are extremely clear, and off-target effects could be significantly reduced by establishing clear one-guide sequences of RNAs (sgRNAs). Furthermore, various platforms are being developed to clarify the Cas9-connected base editors. For instance, using Ribonucleoproteins (RNP) to transport bases and Cas9-HF1 for linking, as well as broadening the grouping directed by sgRNA, in the plant kingdom, quality thump has been largely ignored compared to mammals (33). In this regard, the development of a reliable arrangement, the ideal strategy, the transmission of the process, and the direction for the DNA fixing pathway are most important. Improving the process is crucial since it is essential to eliminate the possibility of an unintentional change that may occur in ideal hereditary material (34).

Contrasting CRISPR-Cas9 with Traditional Therapies

With the progression of CRISPR/Cas9 innovatiogbggbn, autologous transfer of quality-altered hematopoietic immature microorganisms might fix most patients with SCD. The upside of this methodology over the regular foundational microorganism transplantation is that it diminishes the requirement for immuno-suppressive medications and the gamble of joining versus having sickness (35).

Moreover, ongoing mechanical advances can lessen the off-target impacts; however, long-term observation is expected to guarantee the unwavering quality of these techniques in the clinical setting. This audit investigates the viability and security of blend treatments and stands out this option from the difficulties that exist with sickle cell quality treatment utilizing CRISPR.

Over the past decade, CRISPR/Cas systems advancements have made genome editing a transformative and potentially curative treatment for human diseases. Expanding the clinical use of CRISPR treatments has involved revisiting the traditional drug development model used for previous generations of medications. Essentially, this has required a reassessment of verifiable clinical pharmacology and interpretation methodologies that have generally been taught in these past classes of constantly managed specialists. This audit must exclude foundationally controlled in vivo and ex vivo CRISPR treatments (36).

Even though significant advances have been made in locally administered CRISPR treatments, such as Edit 101 for blindness caused by Leber congenital amaurosis 10 (delivered through a subretinal injection), the focus on systemically administered CRISPR treatments is necessary to address the full range of potential considerations rather than just those specific to locally administered treatments (37).

Potential Advantages and Limitations of CRISPER Cas-9

We agree that CRISPR/Cas9 is a widely used development for changing quality, but it has off-target effects, efficacy, and safety issues. The absence of an effective delivery system is another significant obstacle when applying CRISPR-Cas technology to living things. Including CRISPR components in a delivery vehicle is challenging because Cas proteins are relatively large. Scientists can use artificial intelligence and computational tools to design smaller, more efficient Cas proteins to address this. An ideal CRISPR/Cas9 delivery method should have high transfection efficiency, high concentration capacity, and be easy to produce on a large scale (38).

Nonetheless, current systems are still far from accomplishing these objectives. The thought of the PAM grouping is essential for the sgRNA plan and is restricting,

albeit fundamental, for CRISPR/Cas9. Making designable PAMs with the assistance of artificial brainpower is vital to extending the utilization of CRISPR/Cas9. The CRISPR/Cas9 method may not be able to enter therapy if antibodies against Cas9 are found in many people. Likewise, focusing on the insusceptibility made via transporters, particularly infections, is another test because the patient may have proactively been associated with that infection. A significant constraint and hazard in quality treatment utilizing the CRISPR/Cas9 procedure has an off-target impact. The plan and determination of significant gRNA highlights that foreseeing the productivity of an interaction requires decent information on the system of CRISPR quality altering, which exists and is not entirely settled. Besides, include recognizable proof that can be stretched to speculation age, CRISPR plan, and profound learning tests (39).

Although a few PC programs have enhanced the plan of sgRNA, its particularity can't be completely ensured, and maybe artificial reasoning can help specialists better foresee impacts. The Deep CRISPR evaluation, for instance, demonstrated that even when data augmentation methods are utilized, insufficient data renders predictive models ineffective. Nonetheless, various techniques for joining informational indexes can prompt various outcomes (40).

Conclusion

Analysts prefer CRISPR/Cas9 for its work efficiency, simple construction, and low trial cost. It is widely used in cancer research due to its applications and reduced off-target effects. The technology offers a new approach to genetic treatment for hepatocellular carcinoma. CRISPR-Cas9 is the most popular type used in genome editing and has therapeutic potential. It is more relevant and cost-effective compared to other gene-editing tools. The RNP system is highlighted for its fast action and minimal antigenicity reactions. The CRISPR-Cas9 system has proven to be a valuable tool for cancer treatment research in vivo and in vitro.

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All data generated or analyzed during the study are included in the manuscript.
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The authors declared the absence of a conflict of interest.

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