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# Science Writing

# Year 11-12


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## Stage 2 Biology SHE Task

**"The Development of CRISPR Gene Therapy to Promote Fetal Haemoglobin Production as a Treatment for Sickle-Cell Disease"**

## Introduction

Being one of the most prevalent inherited disorders worldwide and the first linked to a molecular cause 70 years ago, Sickle-cell disease (SCD) is a fatal blood condition arising from a gene mutation encoding for haemoglobin (Springer Nature Ltd., 2021). Despite the condition's severity, little progress has been made in biomedical research for treatments, consequently leaving patients in severe pain and with a shortened life expectancy (Eisenstein, 2021). However, recent advancements in gene editing have enabled scientists to develop the promising technology of CRISPR-Cas9, which has the potential to improve the efficiency of medical procedures and possibly replace the only curative SCD treatment, a bone marrow transplant which encompasses various complications. Although, CRISPR-Cas9 is still in its early developmental stages, presenting some limitations and possibilities of unexpected consequences, which has in turn prompted public debate.

## Biological Background

SCD is a recessive genetic disease as one faulty copy of the gene from each parent must be obtained to be affected (Springer Nature Ltd., 2021). It is caused by a mutation at the ß-globin gene (HBB) on chromosome 11, where a single base in the DNA sequence is substituted from adenine to thymine (Synthego, n.d.). Consequently, an amino acid is altered from glutamic acid to valine, resulting in an abnormally formed haemoglobin protein (HbS) (Your Genome, 2021). HbS gives rise to sickle-shaped red blood cells, which are inefficient in oxygen transport and have a reduced life span leading to shortages in their supply. Unlike the round and flexible shape of red blood cells that enable easier movement, these rigid cells can get stuck and clog blood flow when travelling through small blood vessels as seen in Figure 1. This is primarily characterised by periodic episodes of extreme pain and possible organ failure (Springer Nature Ltd., 2021).

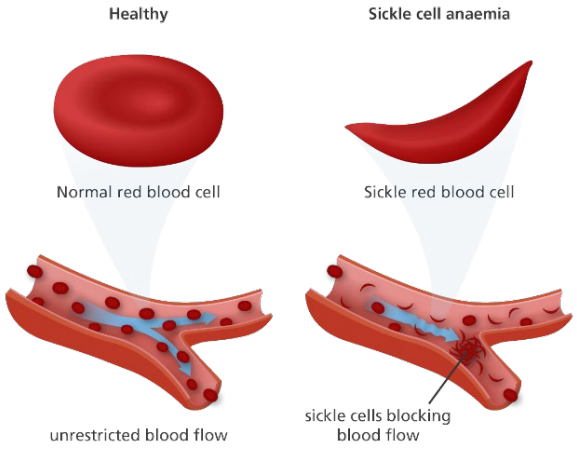


*Figure 1: Diagram of the movement of a healthy and a sickle-shaped red blood cell (Your Genome, 2021)*

However, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), is a recent biotechnology tool that provides opportunity in treating SCD with the capacity to edit genes by adding, removing or altering sections of DNA (Crierie et al., 2018). Short guide RNA (gRNA) molecules are synthesised to match the gene of interest to direct a Cas-9 enzyme to the correct location in the genome (Synthego, n.d.). The Cas-9 is a bacterial endonuclease that cuts DNA across both strands and initiates the cell to recognise its DNA as damaged. At this point, scientists can utilise DNA repair machinery to add or delete specific sections of the gene or replace existing DNA segments with customized sequences to ultimately modify gene function, as highlighted in figure 2 (Sammann, 2020). Although, treatment CTX001 developed by CRISPR Therapeutics and Vertex Pharmaceuticals, does not aim to alter the HBB mutation but rather seeks to boost fetal haemoglobin production (HbF). HbF is highly efficient in transporting oxygen and embryos do not experience any SCD complications, however the BC11A gene encodes a transcription factor that normally represses HbF synthesis at birth where stem cells switch over to HbS production (Springer Nature Ltd., 2021). CTX001 works by using an individual's bone marrow to harvest stem cells where Cas-9 components are directed to BC11A and create a deletion that breaks the gene, enabling cells to continue HbF production and be re-infused into the body (Sammann, 2020). HbF restoration through a somatic edit can compensate for defective haemoglobin and consequently reduce SCD-related symptoms.

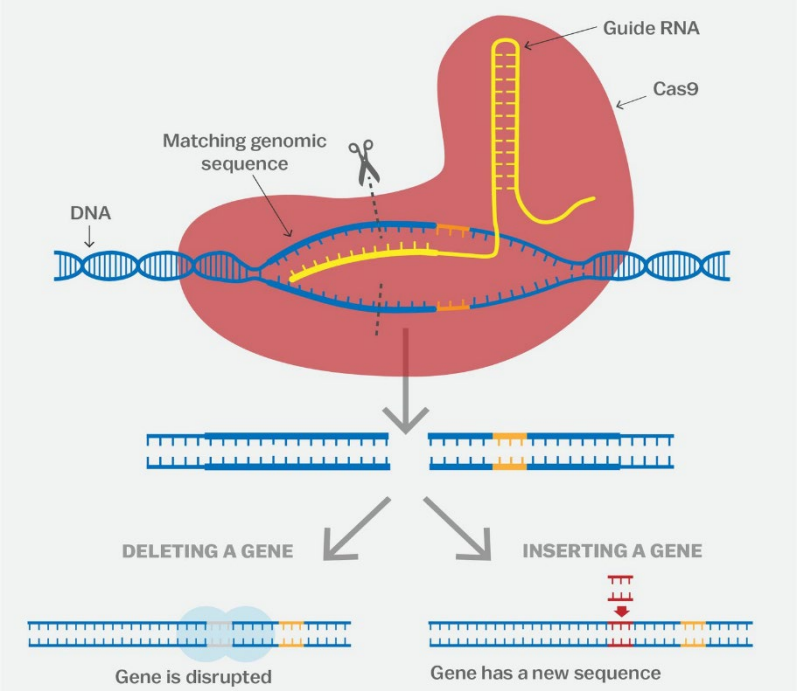


*Figure 2: Diagram of the CRISPR-Cas9 editing process (Zarracina, 2018)*

## Development

Scientific advancements continue to prompt the development of CRISPR to become more accessible, accurate and efficient than previous procedures for treating SCD. In 1997, periodic blood transfusions became the first-recognised SCD treatment, having reduced risks of strokes by 90%, however alloimmunization and hemochromatosis became potential consequences (Howard, 2016). The drug hydroxyurea, in 1995, was reported to reduce pain

crises by promoting HbF production and was FDA approved in 1998 (SCANJ, n.d.). Hydroxyurea was found to be more effective than transfusions due to its fewer complications, however it is greatly limited in accessibility and still has possible long-term effects of neutropenia and leukemia (Springer Nature Ltd., 2021). In the 1990's, bone marrow transplants were applied to SCD patients and found if successful, cured the disease by replacing abnormal stem cells in bone marrow with healthy ones. Currently, this is still the only curative solution, although, under 20% of SCD patients are eligible due to the requirement of a donor of close match to the recipient and the significant risks such as immune rejection and graft-versus-host disease (Synthego, n.d.).

Therefore, CRISPR trials that have taken place have already demonstrated more success than previous SCD treatments. The DNA sequence repeats, defined as CRISPR, were initially discovered in 1987 by microbiologist Francisco Mojica when analysing genes apart of phosphate metabolism in the *E.coli* genome (Ishino et al., 2018). However, it was not until the 2000's that it was discovered that these sequences are involved in the adaptive immune system of prokaryotes, where CRISPR-Cas systems function as a defence mechanism by cleaving complementary viral DNA or RNA sequences. In 2012, CRISPR's first official breakthrough took place, where gRNA was designed to target a specific sequence (Bite Size Bio, 2020).

CTX001 CRISPR trials have reported that one-year post-treatment, patient HbF levels exceeded the expectation of 20% of haemoglobin being HbF, instead achieving 46%, indicating the modified cells have continued to survive in the body for extended periods (Sammann, 2020). As a result, patients experienced instant pain relief within a week and have not since suffered with any major SCD-related complications. CTX001 has presented as a more efficient treatment with its reduced hospitalisations, greater eligibility opposed to transplants and increased accuracy as the technique makes highly precise cuts compared to restriction enzymes previously used that do not have the flexibility to recognise a vast range of specific target sites (Crawley, 2020). CRISPR's programmability has enabled rapid development of therapeutic approaches and hence is more time efficient as it eliminates the need to engineer a specific nuclease to the target sequence, instead only requiring an RNA strand (Lino et al., 2018). Consequently, CRISPR cannot cure SCD as the HBB mutation is still present but the technology can facilitate an improved quality of life through alleviating symptoms and extending life expectancy.

## Application and Limitation

The application of knowledge surrounding genetics has enabled scientists to develop CRISPR as a therapeutic solution to SCD, however its use may be subject to unexpected consequences and limitations. CRISPR is still progressive and accordingly it is too early to draw conclusions about its long-term effectiveness and safety, enforcing scientists to adopt a cautious approach, monitoring and evaluating risks through engaging in further trials. At present, CTX001 is just one of the six major SCD CRISPR trials as there is still much to cover before widespread implementation (Synthego, n.d.). In regards to progress, the other five trials are

still in early phases but have nonetheless reiterated promising outcomes in restoring HbF and also correcting the HBB mutation.

However, accessibility and the economic impact arise as difficulties as transporting individualised therapy from a trial setting to Africa, where SCD is most prevalent, will be an expense (Springer Nature Ltd., 2021). It is estimated that the treatment currently costs $2 million per patient and hence this price point will need to be significantly reduced for the greater community (Thomas, 2021). However, CRISPR has great potential as a treatment as it can keep SCD patients out of hospital which leads to lower associated costs with no ongoing blood transfusions and medication required.

With regards to the social impacts, the CTX001 treatment induces harrowing side effects as it requires recipients to undergo chemotherapy to reduce bone marrow to allow space for modified stem cells (Sammann, 2020). Unintended edits of the Cas-9 enzyme also act as a potential consequence as short gRNA sequences can bind to another sequence that resembles what it is aiming for, causing mutations (Delft University, 2018). Although, if successful, CTX001 can increase work productivity, so life and career ambitions are no longer put on hold for patients with SCD.

Science is greatly influenced by public debate as CRISPR arises with much controversy from religious and ethical standpoints. Genome manipulation of organisms raises the concerns of 'God's image' being violated, particularly germline editing, where changes can be passed through future generations (Joseph, 2016). This can also possibly lead to superficial usage where parents are able to choose their child's traits, therefore it is of public opinion that there is no safeguard against the exploitation and commercial gain of using CRISPR. In order to understand society's outlook, there are surveys in progress, encompassing the knowledge and beliefs surrounding CRISPR gene therapy for SCD patients, care givers and health staff (Synthego, n.d.). Therefore, it may take some considerable time before CRISPR technology becomes convincing to the public in its safety and effectiveness. With a multitude of genetic disorders possibly being treated with CRISPR, funding and research in the medical field can be directed to other areas of health and improve quality of life for people with various other conditions. Future directions for CRISPR technology can also reach beyond the medical field and be applied to areas of agriculture for improving crop disease resistance or sustainability to reduce dependence on plastic from petroleum-based resources.

## Conclusion

It is evident that progress in genetics has continually facilitated breakthroughs in CRISPR technology as a SCD treatment which has reduced complications, increased time efficiency and accuracy. Although, possible consequences, public opinion and economic and social impacts challenge scientists in implementing CRISPR into real-life settings. Therefore, further monitoring and evaluation of risks through trials are required before CRISPR-based therapies can be applied in modern society to treat genetic disorders and be used beyond.

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