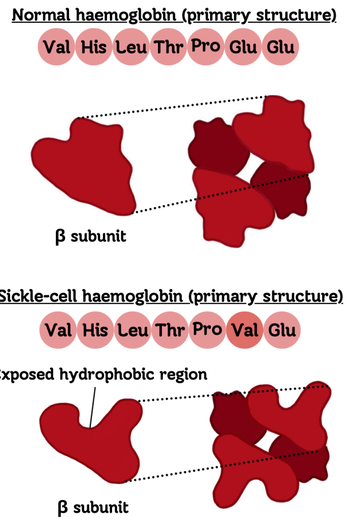
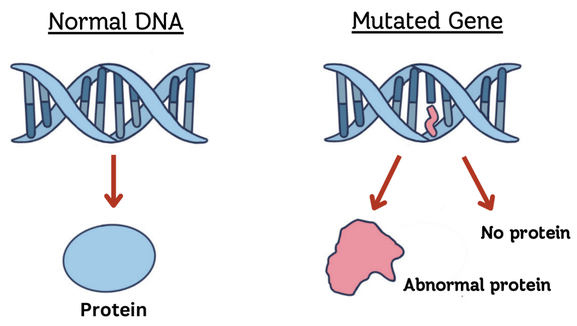
Gene Therapy using CRISPR-Cas9 for SICKLE CELL ANEMIA

What is Sickle-Cell Anemia?

- **inherited disorder** caused by a variation in one of the genes encoding haemoglobin
- **affects the shape of red blood cells**, which carries oxygen to all parts of the body
- caused by **mutation** in a single base in the DNA sequence **of the beta-globin gene (HBB)**, resulting in the formation of **haemoglobin S**
- sickle shape is due to the **substitution of the single amino acid**, causing a change in the 3D shape of the haemoglobin molecule, which makes the molecule **clump together**
- sickle cells become rigid and sticky, slowing or even blocking blood flow, causing severe pain, impairment of normal tissue functions and recurrent infections



What is Gene Mutation?



Gene therapy for Sickle-Cell Anemia

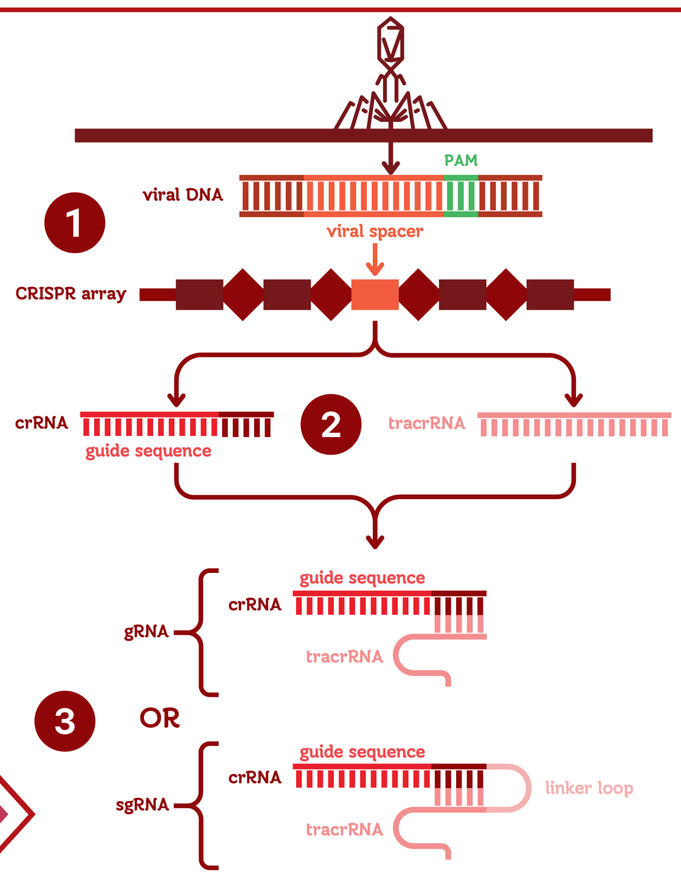
Somatic Cell Therapy

- cure a disease only in the patient, not in the patient's descendants
- prepare **bone marrow extract**, transfect with a retrovirus-based vector and then reimplant the cells
- **construction** of recombinant viruses capable of carrying genes into cells by the process of infection as well as the **use of DNA molecules** that are capable of being used like conventional medicines
- goal: establish site-specific and regulated expression of therapeutic products
- somatic gene transfer is carried out by three methods, in vivo, in situ, and ex vivo

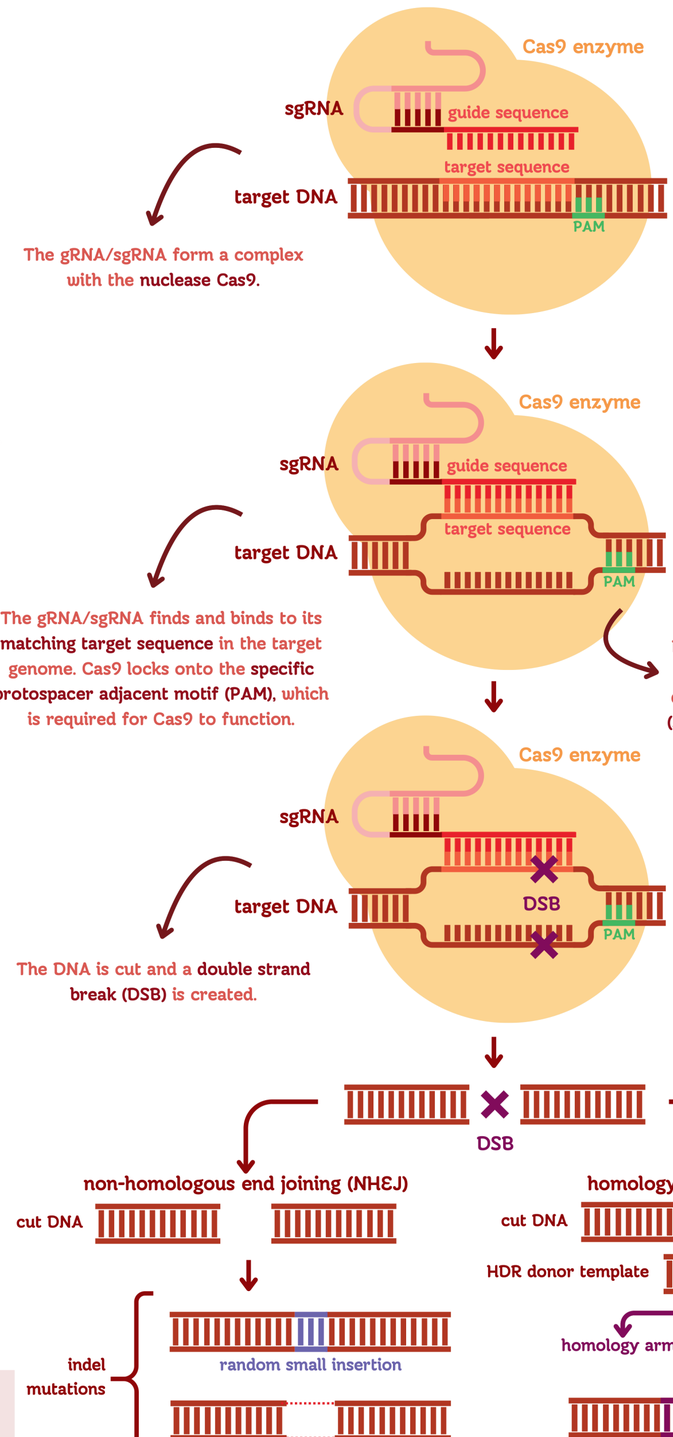
What is CRISPR-Cas9?

The CRISPR-Cas9 mechanism is based on a natural system used by bacteria to protect themselves from invasion by viruses (Bacteriophages).

CRISPR is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria.



1. When a bacteriophage invades the bacterium, a new spacer (fragment of non-coding DNA) is derived from the virus and integrated into the bacterium's **CRISPR sequences**
2. **CRISPR RNA (crRNA)** and **Trans-activating CRISPR RNA (tracrRNA)** is produced
3. **crRNA & tracrRNA** combine to form a dual-RNA **guide RNA (gRNA)**. Alternatively, the **crRNA and tracrRNA** can also be combined into a single RNA transcript called the **single guide RNA (sgRNA)**



CRISPR-Cas9 can be manipulated to cut **any DNA sequence** precisely by **synthesising a gRNA/sgRNA** that **matches the target sequence**, and by harnessing **DSB repair mechanisms**, DNA can be **modified precisely**. Thus, specific gene targeting and correction **beyond bacterial cells is possible.**

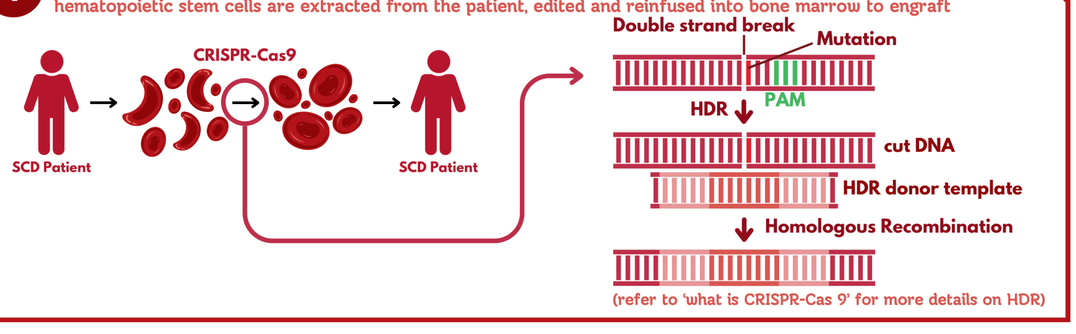
In NHEJ, DSB is repaired without HDR template, but is **error prone.** Introduces scars in the form of **random insertions or deletions (indels)** at the break point.
Indels can cause a **frameshift mutation** and create a **premature stop codon**, disrupting a gene's expression and function. This is known as **gene knock-out.**

In HDR, a **homologous piece of DNA** acquired from a donor, such as plasmids or single-stranded DNA oligonucleotides (ssODNs), is used as a **HDR template** that guides DSB repair, and allows **high fidelity and precise editing.** This insertion of an exogenous gene is known as **gene knock-in.**

Application of CRISPR-Cas9 Technology → Treating Sickle Cell Anemia

1 Restoring Adult Hemoglobin (ex vivo)

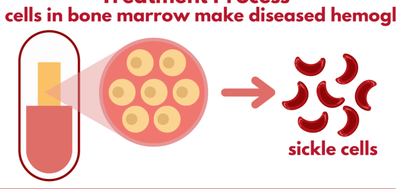
hematopoietic stem cells are extracted from the patient, edited and reinfused into bone marrow to engraft



(refer to 'what is CRISPR-Cas 9' for more details on HDR)

2 Promoting Fetal Hemoglobin Production

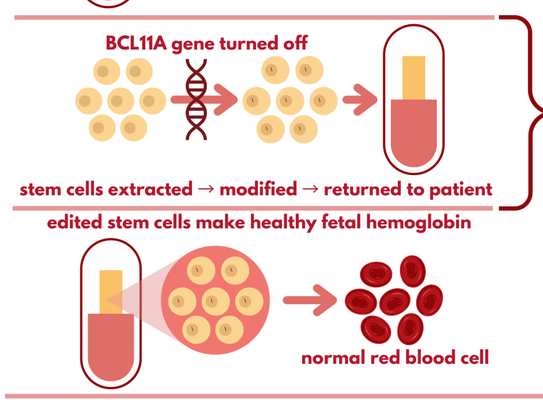
Treatment Process
stem cells in bone marrow make diseased hemoglobin

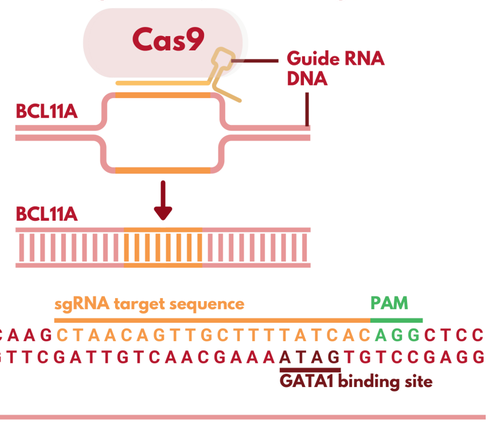


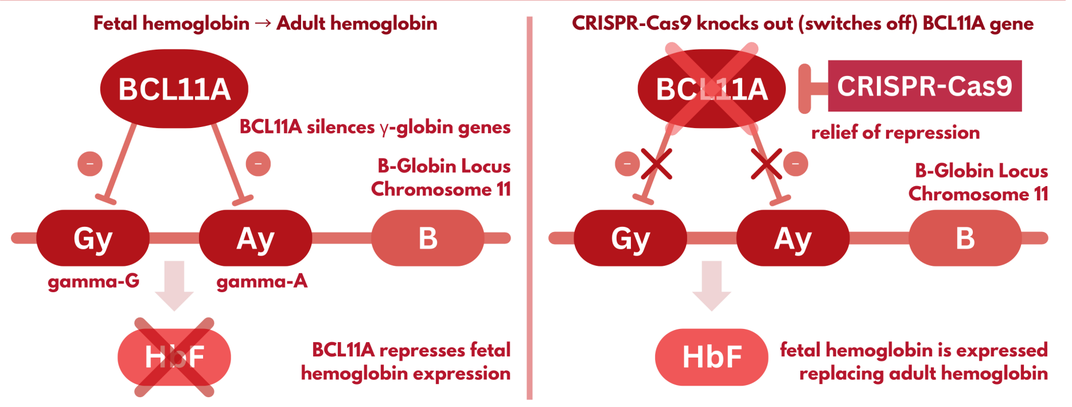
CRISPR-Cas9 gene editing targets *BCL11A* in erythroid lineage, increasing HbF expression in place of sickle hemoglobin

- Exagamglogene Autotemcel (sold under brand name Casgevy)

CRISPR-Cas9 modifies gene that encodes BCL11A reducing its activity and lowering production

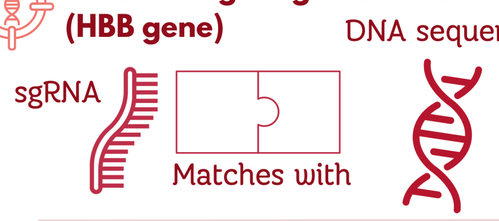






Advantages:

Precise targeting of the mutated gene responsible for sickle cell anemia (HBB gene)



Acts as a guide for Cas9 to target the specific location in the HBB gene

Cas9 cuts both DNA strands at the targeted site. Cell's repair mechanisms (NHEJ/HDR) can correct the mutation causing sickle cell anemia

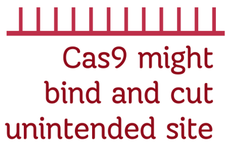
PCR and sequencing used to confirm the desired genetic modification

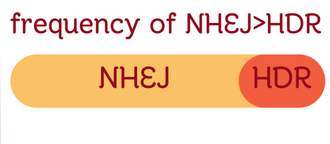
Offers the possibility of making permanent genetic corrections, potentially curing the disease

Successful treatment could reduce or eliminate the need for blood transfusions, improving the quality of life for patients

Disadvantages:

Possibility of off-target effects, where unintended changes occur in the genome, potentially leading to new health issues





Risk of unexpected complications that unintentionally prevent the function of another important gene.

Long-term safety of CRISPR-Cas9 treatments is still not fully understood; e.g. the potential for unintended genetic mutations or immune responses

Treatments can be costly and may not be widely accessible to patients

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[All graphics and diagrams were hand drawn or constructed using Canva.]

This infographic poster, entitled <Gene Therapy using CRISPR-Cas9 for Sickle Cell Anemia>, was created by **Natasha Ang Sze Teng, Ng Jing En, Yang Zhixuan and Ho Eu Leong Zavier of Dunman High School,** as an entry to the GMAC Students Challenge 2024. The contents of this infographic may not necessarily reflect the views of GMAC.