Exploring Solutions to Genetic Problems

Chef Complaint

James, 6 years old, was brought in to the clinic experiencing fatigue and joint pain.

Patient Background

James is a quiet 6-year-old boy who leads a relatively sedentary lifestyle. When he is home, he spends most of his time in his room reading books or watching cartoons. Three months ago, he began attending first grade and seems to enjoy it. Recently, his mother (Sheryl) noticed that James has been appearing sluggish and she has had to work even harder to get him up for school in the morning. While she suspected something was not quite right, she reasoned that he's a growing boy and his body must need the rest.

On Monday, Sheryl received a phone call informing her that James wasn't feeling well and that he needed to be picked up from school. When his mother asked him what was wrong, James indicated that he felt really tired and his arms and legs hurt. While looking him over, Sheryl noticed that his extremities seemed a little swollen. She immediately scheduled a doctor's appointment and brought her son in for further examination.

Upon physical examination his physician confirms that his extremities are swollen and finds that his blood oxygen is also low. A urine and blood sample were taken and sent to the laboratory for analysis.

Diagnosis

James' blood sample came back positive for hemoglobin S, a defective version of the hemoglobin A protein found in red blood cells. His physician diagnosed James with Sickle Cell Anemia.

TASK

Use the following models to better understand James' Sickle Cell condition and explore current methods for treating it. Then, construct a model to illustrate how gene editing tools like CRISPR Cas9 can be used to correct genetic conditions like Sickle Cell Anemia.

Model 1: **Origin of Sickle Cell Anemia**

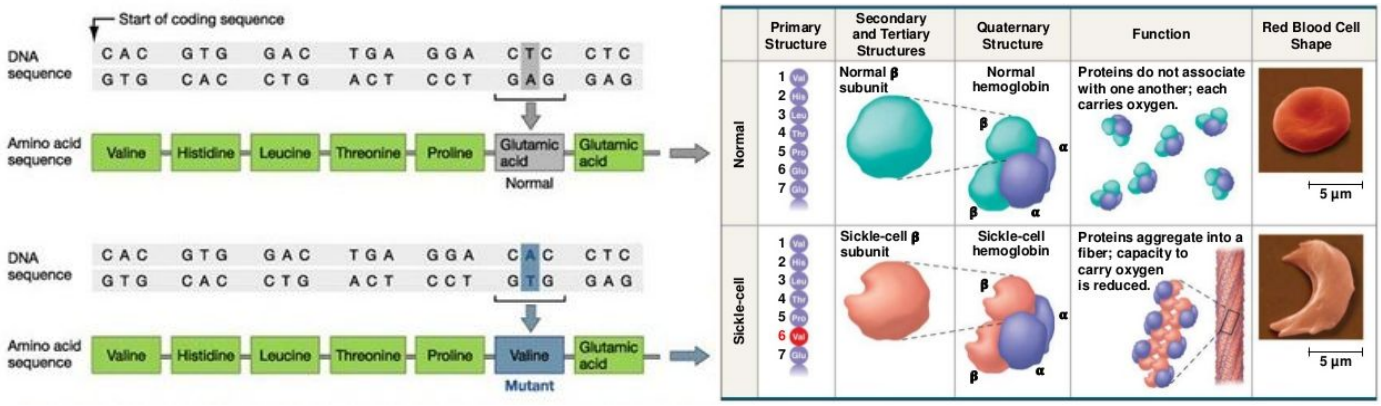


Image sources: https://ct-stem.northwestern.edu/curriculum/preview/28/2/; https://www.studyblue.com/notes/note/n/03-biomolecules-watts/deck/1859402

Questions:

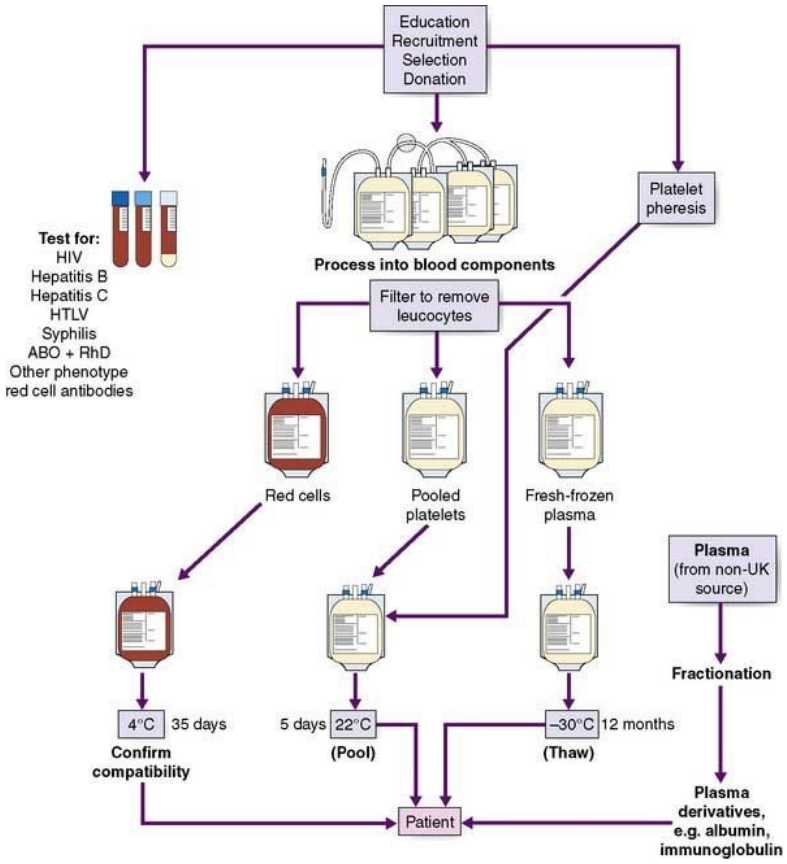
1. What is the primary function of red blood cells in your body and why is this function essential to one's survival?

2. How do red blood cells affected by the Sickle Cell condition phenotypically differ from "normal" red blood cells?

3. How might these phenotypic changes account for James' symptoms?

4. What is the source of this phenotypic difference between normal and sickled cells? Elaborate on how this difference can produced a new phenotype using your understanding of the central dogma.

Model 2: **Transfusion**



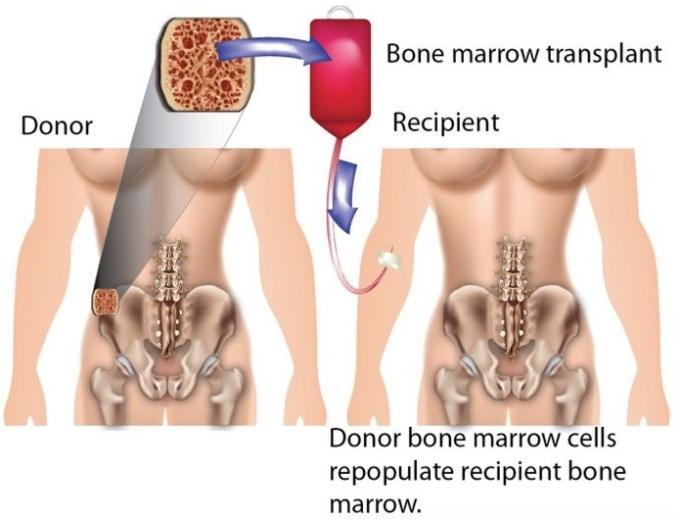
Questions:

5. After blood has been donated, its components can be separated and transfused according to the recipient's needs. Which portion of the donor sample would James require and how will it help treat his condition?

6. Will this treatment address the symptoms or cause of James' condition? (Is this a temporary or long-term solution?)

7. What limitations exist with this treatment?

Model 3: **Transplantation**



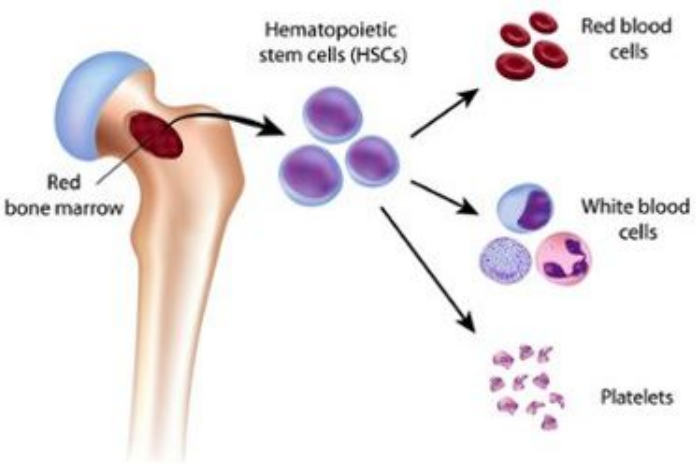


Image sources: Health Library for People (http://www.healthlibrary.com/); lyceum.algonquincollege.com

Questions:

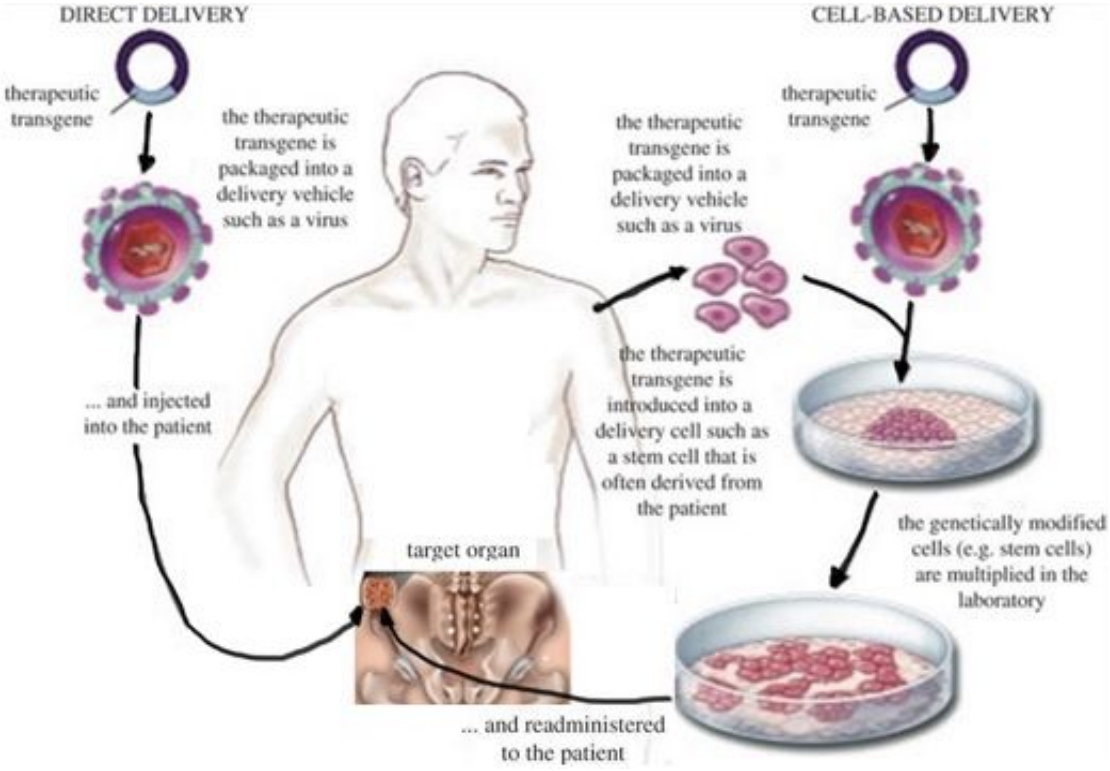
8. How will a bone marrow transplant treat James' condition similarly to a blood transfusion?

9. How will a bone marrow transplant treat James' condition differently from a blood transfusion?

10. Will this treatment address the symptoms or cause of James' condition? (Is this a temporary or long-term solution?)

11. What obstacles might exist in receiving a successful bone marrow transplant?

Model 4: **Gene Therapy**



12. What therapeutic transgene would you expect to find in a plasmid designed to treat Sickle Cell Anemia?

13. How will this therapeutic gene be introduced into the host/recipient of the treatment?

14. Compare and contrast treatment via gene therapy with that of a bone marrow transplant.

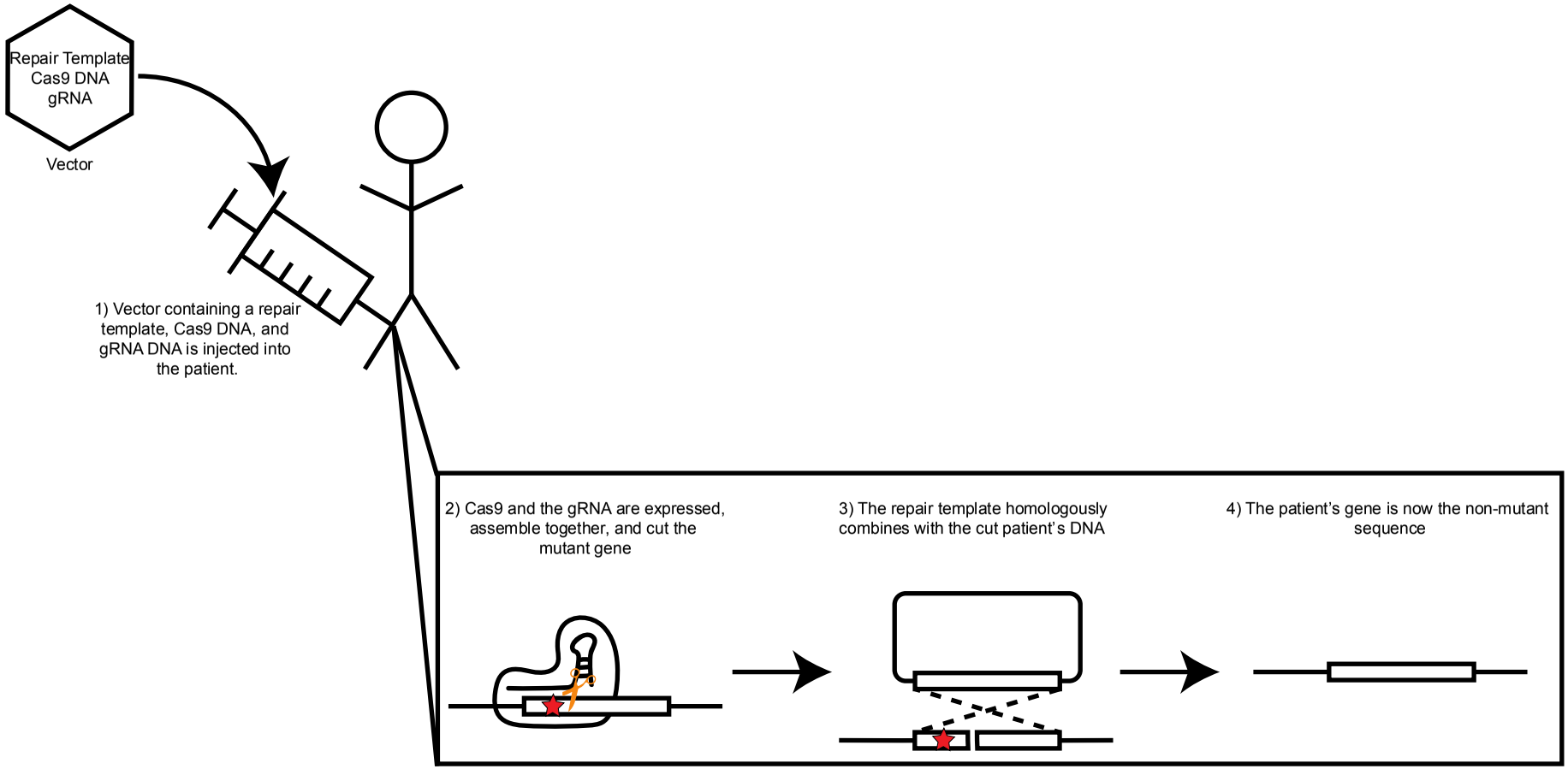
Model 5: **Gene Editing**

15. Create a model demonstrating how the CRISPR Cas9 complex could treat James' Sickle Cell condition. *Hint: You may find Model 1 helpful in completing this task.

16. How would using CRISPR technology provide an advantage in treating individuals with Sickle Cell Anemia over each of the previous treatments modeled?

KEY:

1) Red blood cells carry oxygen inhaled by the lungs to tissues throughout the body. They also carry carbon dioxide produced by tissues to the lung to be exhaled. This is critical to survival as all cells need oxygen for cellular respiration and would be poisoned by carbon dioxide otherwise.
2) Sickle cell affected cells have a different cell shape and a reduced ability to carry oxygen.
3) James experienced fatigue, which may be due to his body's inability to make enough energy (ATP). Low amounts of oxygen in the tissue would prevent respiration and allow for little ATP to be made. Sickle cell affected cells have a lower capacity for carrying oxygen.
4) The Sickle cell phenotype is caused by a mutation in the hemoglobin gene. A point mutation in the DNA encoding hemoglobin causes a different RNA transcript to be made, ultimately resulting in a valine amino acid in position 6 of hemoglobin instead of a glutamic acid amino acid.
5) The red cells component.
6) Transfusing red blood cells will help alleviate James' symptoms, but is a short-term solution since his body will continue to make Sickle cell affected red blood cells.
7) Limitations with this treatment are that it does not fix the source of the problem (the Sickle cell mutation) and the treatment is dependent on the availability of donated blood that passes through quality control.
8) A bone marrow transplant will also give James functional red blood cells.
9) A bone marrow transplant will continually give James functional red blood cells and not require transfusions.
10) A bone marrow transplant is more permanent solution than red blood cell transfusion.
11) A bone marrow transplant also requires a donor, but the donor must be compatible with the recipient. It also requires invasive surgery rather than a simple transfusion.
12) A corrected copy of the hemoglobin gene, one that does not contain any mutations.
13) Viral delivery directly to patient OR implantation of genetically modified cells (similar to bone marrow transplant)
14) Gene therapy is independent of tissue compatibility since the tissue used is the patient's own tissue.
15) See Next Page
16) Using CRISPR to edit a patient with Sickle Cell Anemia allows the patient a more permanent solution as their own cells' DNA is edited in the process. This also avoids problems with finding donors and donors being compatible with the patient (bone marrow transplant and red blood cell transfusion).


Repair Template
Cas9 DNA
gRNA
Vector
1) Vector containing a repair template, Cas9 DNA, and gRNA DNA is injected into the patient.
2) Cas9 and the gRNA are expressed, assemble together, and cut the mutant gene
3) The repair template homologously combines with the cut patient's DNA
4) The patient's gene is now the non-mutant sequence

Teacher Notes:
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