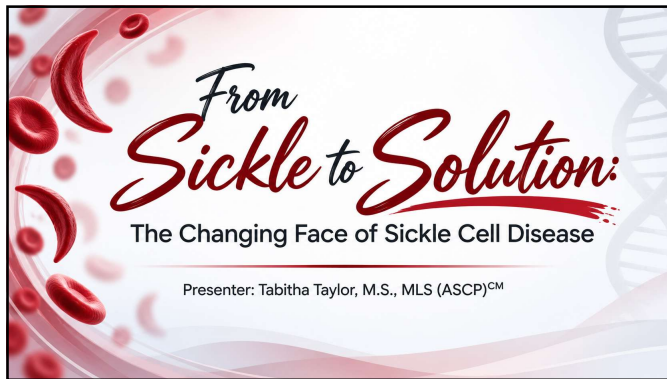
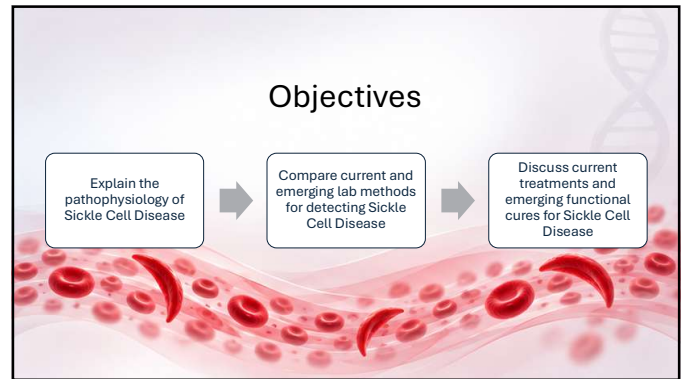
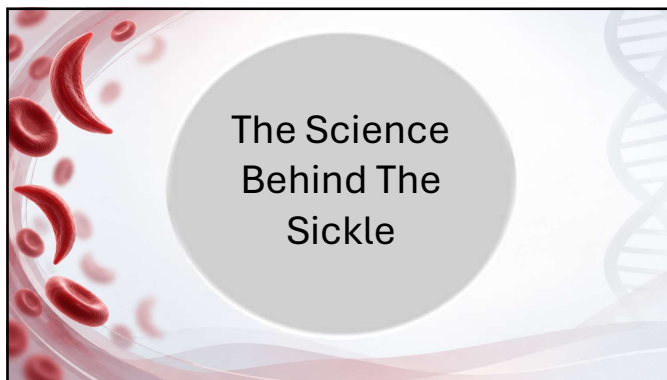




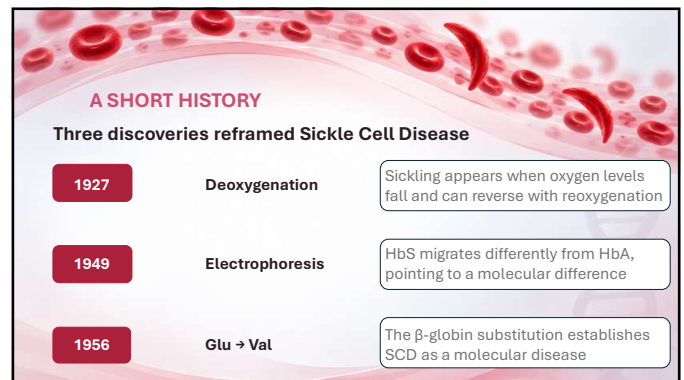
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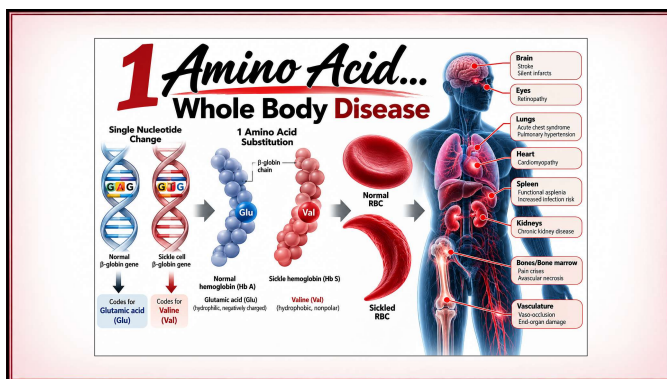
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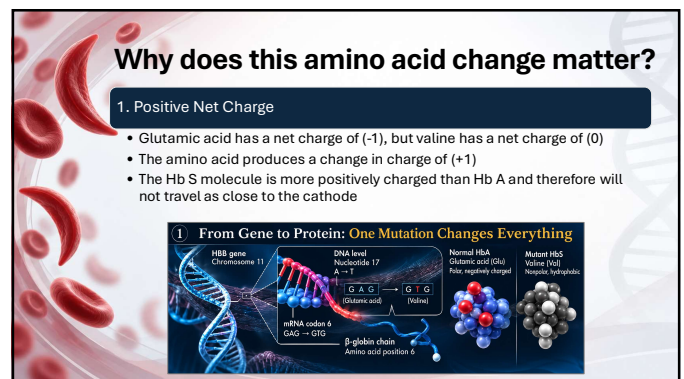
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6

Hemoglobin oxidation: The iron connection

Fe^{2+} Ferrous iron	Fe^{3+} Ferric iron
Functional heme iron in both Hb A and Hb S. Supports reversible oxygen binding.	Oxidized heme iron in methemoglobin. Does not reversibly bind oxygen.

SCD can increase hemoglobin autooxidation and oxidative stress, but Fe^{3+} is not specific to Hb S.

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Sickle Cell Recap

- 1 Glu → Val (Altered Charge)**: Hb A (Glu) vs Hb S (Val)
- 2 Polymerization**: Hb S molecules aggregate into long fibers.
- 3 Phospholipid Redistribution**: The cell membrane becomes rigid and distorted.
- 4 Ferric Iron (Fe^{3+})**: Oxidized iron promotes further polymerization and vaso-occlusion.

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Why does the presence of sickle cells matter?

Deoxygenation and impaired flow reinforce one another

- 01 Hb S Polymerization & Sickling**
Rigid RBCs increase resistance to microvascular flow
- 02 Sluggish Blood Flow**
Longer exposure to low-oxygen conditions
- 03 Conditions Favoring Sickling**
↓ O_2 tension • ↑ pH • ↑ 2,3-DPG

↻ Further Hb S polymerization and sickling

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MICROVASCULAR REALITY

Normal flow vs. vaso-occlusive crisis

From Sickled Cells to Vaso-Occlusion: A Realistic View

VOC is not simply a sickled RBC plugging a vessel: endothelial activation, leukocytes, platelets, adhesion, inflammation, and impaired nitric-oxide biology participate

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Clinical Features

- Vaso-occlusive crisis**
 - Hallmark of SCD and accounts for most hospital and emergency department visits.
 - Acute, painful and occurs with great predictability and severity.
- Splenic sequestration and infarcts**
 - Spleen traps blood and leads to rapid decline in hemoglobin, often < 6 g/dL.
 - Occurs more often in infants and young children whose spleens are chronically enlarged.
- Acute chest syndrome**
 - Acute illness with fever and/or respiratory symptoms.
 - Second most common cause of hospitalization and third most common cause of death in adults with SCD.
- Other complications**
 - Fat embolism syndrome
 - Bone marrow necrosis
 - Bacterial infections
 - Chronic hemolysis
 - Megaloblastic and/or aplastic episodes
 - Cardiac abnormalities
 - Renal abnormalities
 - Skin abnormalities
 - Stroke
 - Retinopathy

17

Lab Techniques for Detection

-Current-

18

From recognition to molecular certainty

Laboratory detection: understanding the mechanism, result, and limitation

RECOGNIZE

Morphology and solubility

What raises suspicion?

CHARACTERIZE

Protein separation

Which Hb fractions are present?

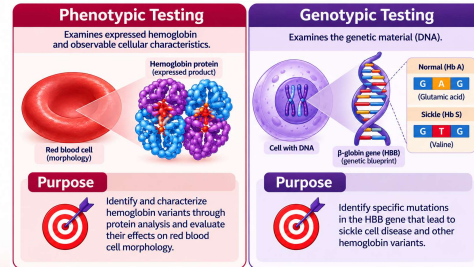
IDENTIFY

Molecular testing

Which variants are encoded?

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Phenotypic vs. Genotypic testing

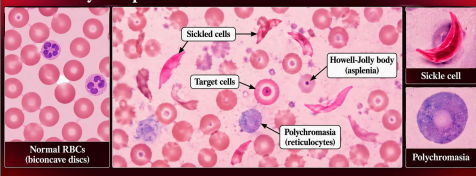


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Spot the sickle

Scientists report sickle cells and other morphology that prompt reflex testing

Laboratory Perspective: What You See in the Smear



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Test Methods

Phenotypic Testing

- Peripheral blood smear
- Solubility testing
- Electrophoresis
- HPLC
- Capillary electrophoresis

Genotypic Testing

- PCR
- DNA variants
- DNA microarrays
- DNA sequencing / NGS

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Solubility test (Sickle Dex)

Based on insolubility of Hb S

- Agent crystallizes HbS and causes it to precipitate the cells, which refracts light and causes turbidity (easy, inexpensive)

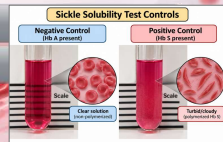
Don't forget there are other hemoglobins that give the positive result.

- Hb C-Harlem (Georgetown), Hb S-Memphis, Hb C-Ziguinchor, etc.
- High serum viscosity, erythrocytosis and marked leukocytosis

False negatives in newborns still expressing Hb F

Can't distinguish between sickle trait and disease

POSITIVE = GENOTYPE



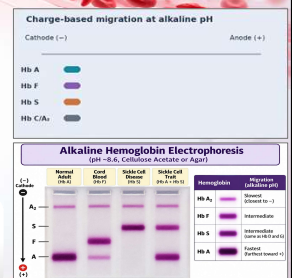
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Hemoglobin Electrophoresis

Electrical field facilitates the migration of electrically charged molecules

Identify hemoglobin variants

Hemoglobin F is relevant in newborn interpretation



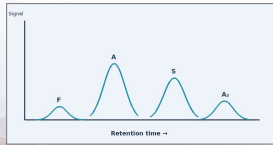
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High Performance Liquid Chromatography (HPLC)

Ion-exchange separation reflects differing interactions with the stationary phase

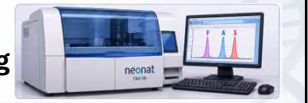
Uses known retention time and shapes of peaks

More sensitive than electrophoresis but can't differentiate between other Hb variants that mimic Hb S



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Newborn Screening



Neonat Fast HB device

- Capillary zone electrophoresis with CAPILLARYS cord blood mode can analyze dried spots on filter paper and liquid cord blood.
- An electric field separates charged hemoglobin fractions within capillaries.
- Automated optical detection produces electropherograms.

Isoelectric focusing

- A pH gradient separates hemoglobins by isoelectric point.
- Hb molecules precipitate and appear as a sharp band
- Can detect Hb S and Hb A in presence of high Hb F concentrations and separated Hb D-Punjab from Hb S (expensive)

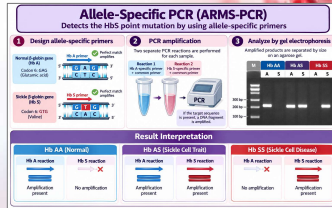
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PCR Based Testing ARMS-PCR

Can detect well known single genes or several genes in a single tube.

Used to detect β chain mutations

Can determine homozygous or heterozygous states based on different band sizes on agarose gel electrophoresis.



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PCR Based Testing Refraction fragment length polymorphism PCR (PCR-RFLP)

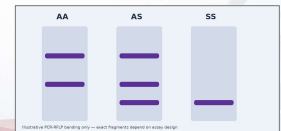
DNA sequences are recognized by an enzyme for cleavage

Sickle cells have a disrupted sequence not recognized by the enzyme for cleavage

HbSS patients have wider bands

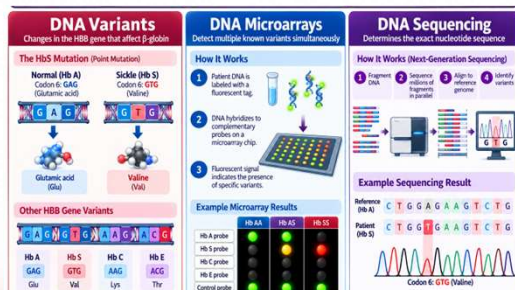
The number of bands indicates the number of mutations

Single band will remain.



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DNA Microarrays and Sequencing Techniques



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Lab
Techniques
for Detection
-Emerging-

30

Smartphone Based Screening Tool

Doesn't require blood sampling or specialized training

Uses patient's questionnaire responses and smartphone camera photos of the patient's fingernail beds to supplement existing modalities

Accurately compute hemoglobin level with a sensitivity of 95%

The screening application effectively identified sickle cell patients aged 6 months to 21 years with a sensitivity of 74% and specificity of 76%

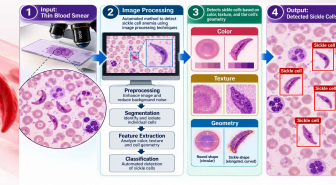


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Imaging Processing Techniques

Automated method to detect Sickle Cell Disease using image processing technique

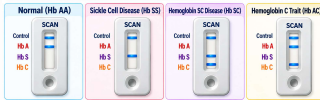
Detects of sickle cells found in thin blood smears based on color, texture, and the cell's geometry



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Lateral Flow Technique

Example Results (SCAN cartridge)



Qualitative detection using polyclonal antibodies on lateral flow chromatographic immunoassay against hemoglobin S, C, and A

- Polyclonal antibodies are attached to the test strip.
- The hemoglobin binds with the corresponding antibody and produces blue lines.

The SCAN cartridge has 4 detection bands: control band, Hb A, Hb S, Hb C

The test isn't affected by high amounts of Hb F

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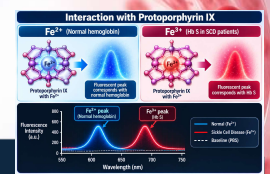
Sensor Based Technique

Fluorescence-Based Optofluidic resonator

- Optofluidic resonator based on the waveguide that identifies the interaction of Fe²⁺ and Fe³⁺ within protoporphyrin IX in phosphate buffer saline

Real time detection using a small sample volume

- Fe²⁺ (ferrous) molecule produces a fluorescent peak that corresponds with normal hemoglobin
- Fe³⁺ (ferric) molecule produces a fluorescent peak that corresponds with Hb S in SCD patients



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Emerging Flow Cytometry

Detects sickle cells based on fluorescent markers or cellular morphology

Based on population analysis and morphological data

- Deoxygenate and classify cells based on morphology
- Impedance

Mechanical Differentiation of Sickle Cells

Mechanically fragile and poorly deformable sickle cells impair blood flow.

Uses RBC deformability, mechanical fatigue, kinetics, and cell membrane fluctuations to detect and monitor treatments of sickle cell disease

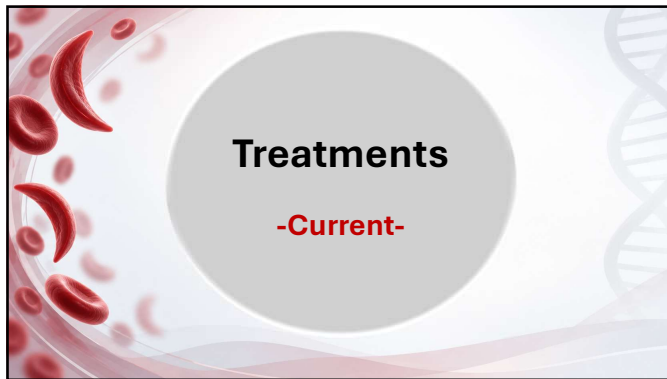
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From sickle characteristics to detection method

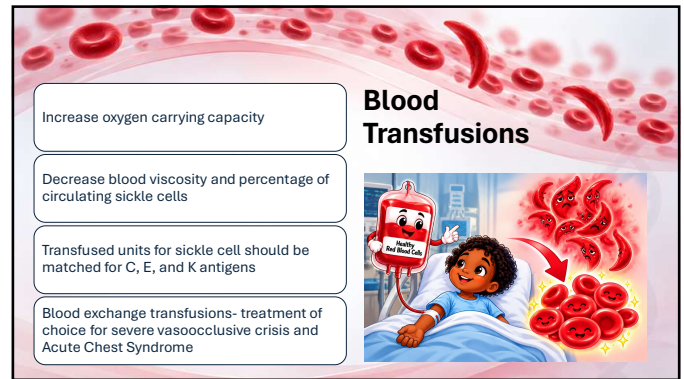
Altered charge	➤	Electrophoresis • Isoelectric focusing
Hb variants / protein properties	➤	HPLC • Capillary electrophoresis • Immunoassays
Deoxy-Hb S polymerization / insolubility	➤	Sickle solubility testing
Altered RBC morphology	➤	Peripheral blood smear • Digital Image processing
β-globin DNA substitution	➤	PCR • Sequencing • Genotyping



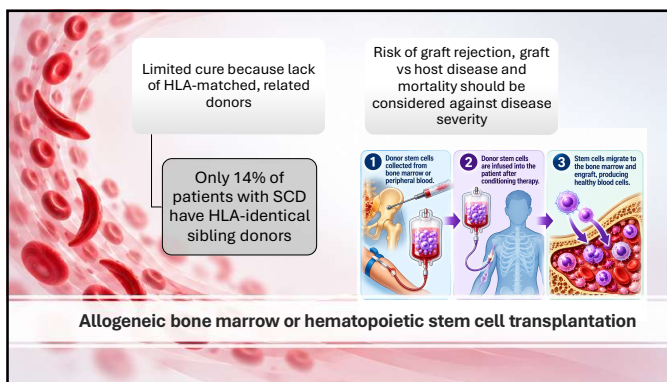
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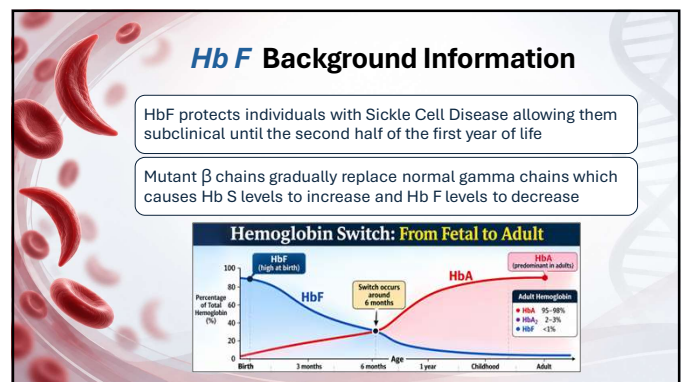
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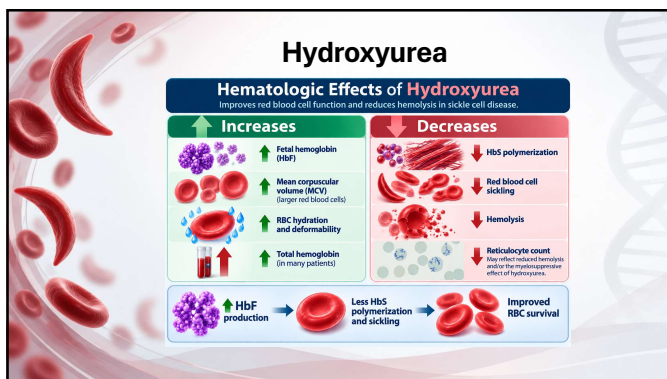
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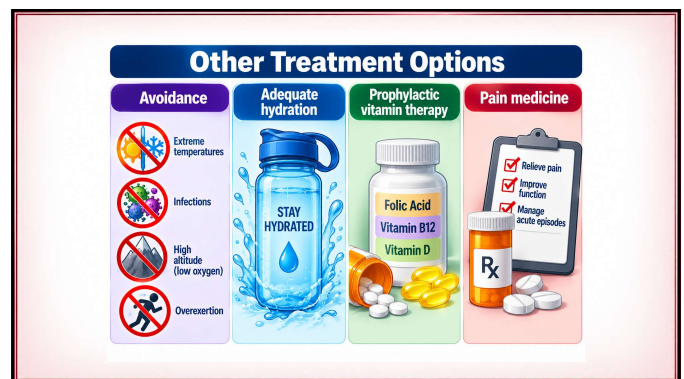
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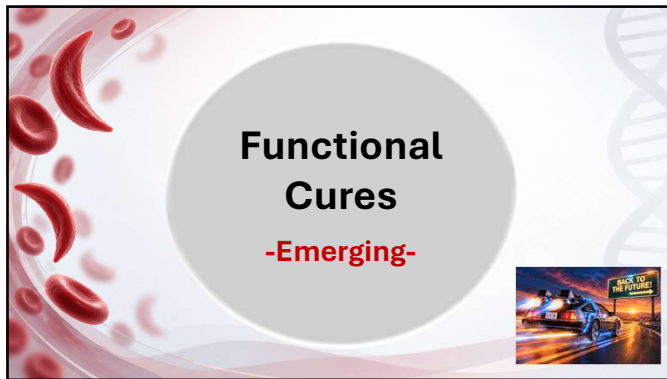
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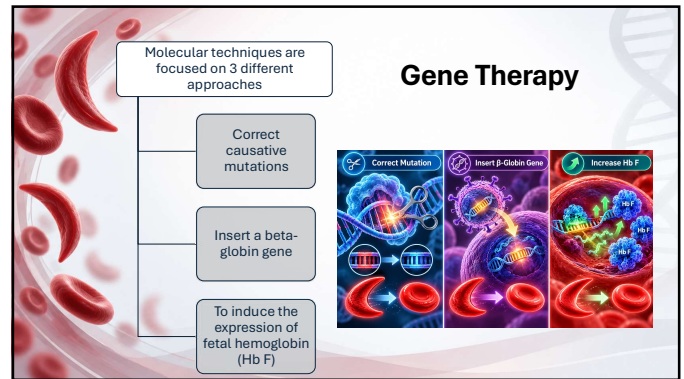
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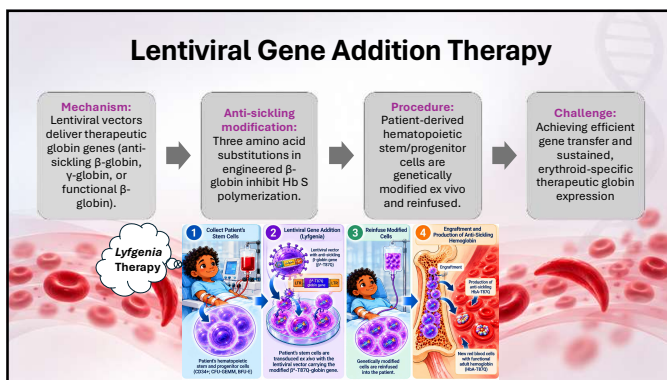
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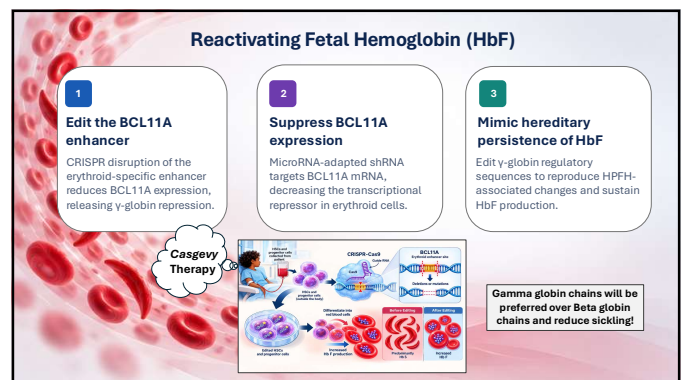
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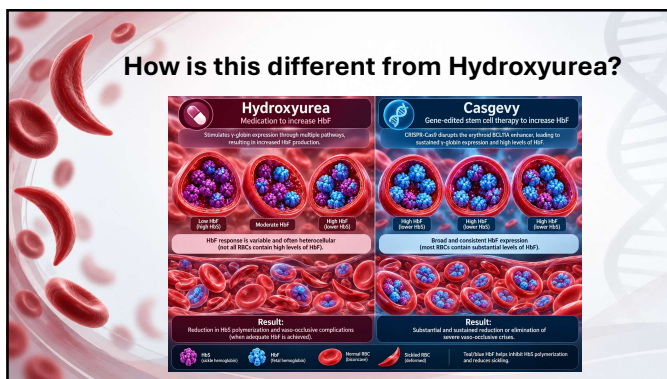
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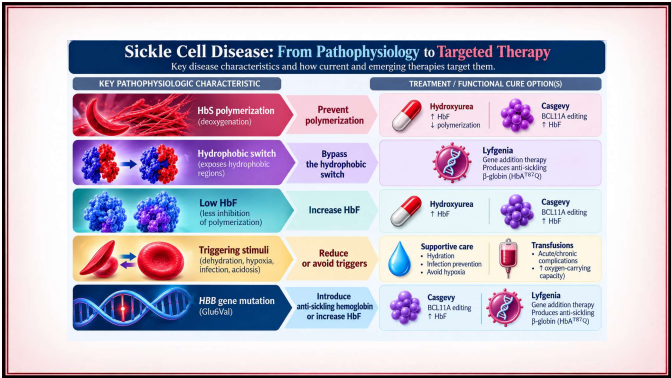


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TREATMENT vs. FUNCTIONAL CURE vs. GENETIC CURE		
What does "cure" mean in Sickle Cell Disease?		
TREATMENT	Manages or modifies disease and reduces complications.	Hydroxyurea increases HbF and reduces sickling complications.
FUNCTIONAL CURE	Sustained absence or major suppression of clinically significant disease manifestations; the HbS mutation may remain.	Casgevy increases HbF; Lyfgenia produces anti-sickling HbA ¹⁰⁰ Q.
GENETIC / MOLECULAR CURE	Corrects the underlying disease-causing genetic alteration in relevant cells.	Direct correction of the HbS mutation; neither Casgevy nor Lyfgenia does this.
KEY DISTINCTION: Functional cure ≠ removal of the HbS mutation		

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References

Arishi, W. A., Alhadrami, H. A., & Zurob, M. (2021). Techniques for the detection of sickle cell disease: A review. *Micromachines*, 12(5), Article 519. <https://doi.org/10.3390/mi12050519>

Borba, R., Lima, C. S. P., & Grotto, H. Z. W. (2003). Reticulocyte parameters and hemoglobin F production in sickle cell disease patients undergoing hydroxyurea therapy. *Journal of Clinical Laboratory Analysis*, 17(2), 66-72. <https://doi.org/10.1002/jcla.10070>

Keohane, E. M., Otts, C. N., & Walenga, J. M. (Eds.). (2020). *Rodak's hematology: Clinical principles and applications* (6th ed.). Elsevier.

Ramadier, S., Chalumeau, A., Felix, T., Othman, N., Aknoun, S., Casini, A., Maule, G., Masson, C., De Cian, A., Frai, G., Brasseur, M., Concordet, J.-P., Cavazzana, M., Cereseto, A., El Nemer, W., Amendola, M., Wattel, B., Meneghini, V., & Miccio, A. (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.mth.2021.08.018>

Scaramellino, N., Panzeri, D. L., & Cappellini, M. D. (2025). Gene therapy for sickle cell disease and thalassemia. *Current Opinion in Hematology*, 32(3), 120-129. <https://doi.org/10.1097/HCT.0000000000000862>

Vital, E. F., J. Cabell, M. H., Manning, R. G., McGinn, P. T., & Lam, W. A. (2025). Non-invasive, smartphone image-based screening for sickle cell disease at the point-of-need. *Heliyon*, 11(2), Article e41830. <https://doi.org/10.1016/j.heliyon.2025.e41830>

Chou, S. T., et al. (2020). American Society of Hematology 2020 guidelines for sickle cell disease: Transfusion support. *Blood Advances*, 4(2), 327-355. <https://doi.org/10.1182/bloodadvances.201901143>

Frömmel, C. (2018). Newborn screening for sickle cell disease and other hemoglobinopathies: A short review on classical laboratory methods—isoelectric focusing, HPLC, and capillary electrophoresis. *International Journal of Neonatal Screening*, 4(4), 39. <https://doi.org/10.3390/ijns404039>

Centers for Disease Control and Prevention. (2026). Prevention and treatment of sickle cell disease complications. <https://www.cdc.gov/sickle-cell/about/prevention-and-treatment.html>

American Society of Hematology. (n.d.). Clinical practice guidelines on sickle cell disease. <https://www.hematology.org/about/clinical-practice-guidelines-and-quality-care/clinical-practice-guidelines/sickle-cell-disease-evidence>

U.S. Food and Drug Administration. (2026). CASGEVY (exagamglogene autotemcel). <https://www.fda.gov/vaccines-blood-biologics/casgevy>

U.S. Food and Drug Administration. (2023). LYFGENIA (lontotibeglogene autotemcel). <https://www.fda.gov/vaccines-blood-biologics/lyfgenia>

Manning Family Children's. (2026, June 22). Manning Family Children's makes history as Louisiana's first sickle cell disease gene therapy patient rings the bell, marking his functional cure and restoring the dream of becoming a pilot. <https://www.manningfamilychildrens.com/news/2026/06/22/manning-family-childrens-makes-history-as-louisiana-s-first-sickle-cell-disease-gene-therapy-as-pilot>

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