Understanding the Role for Novel PK Activation Strategies in Hemoglobinopathies: Developments in Sickle Cell Disease

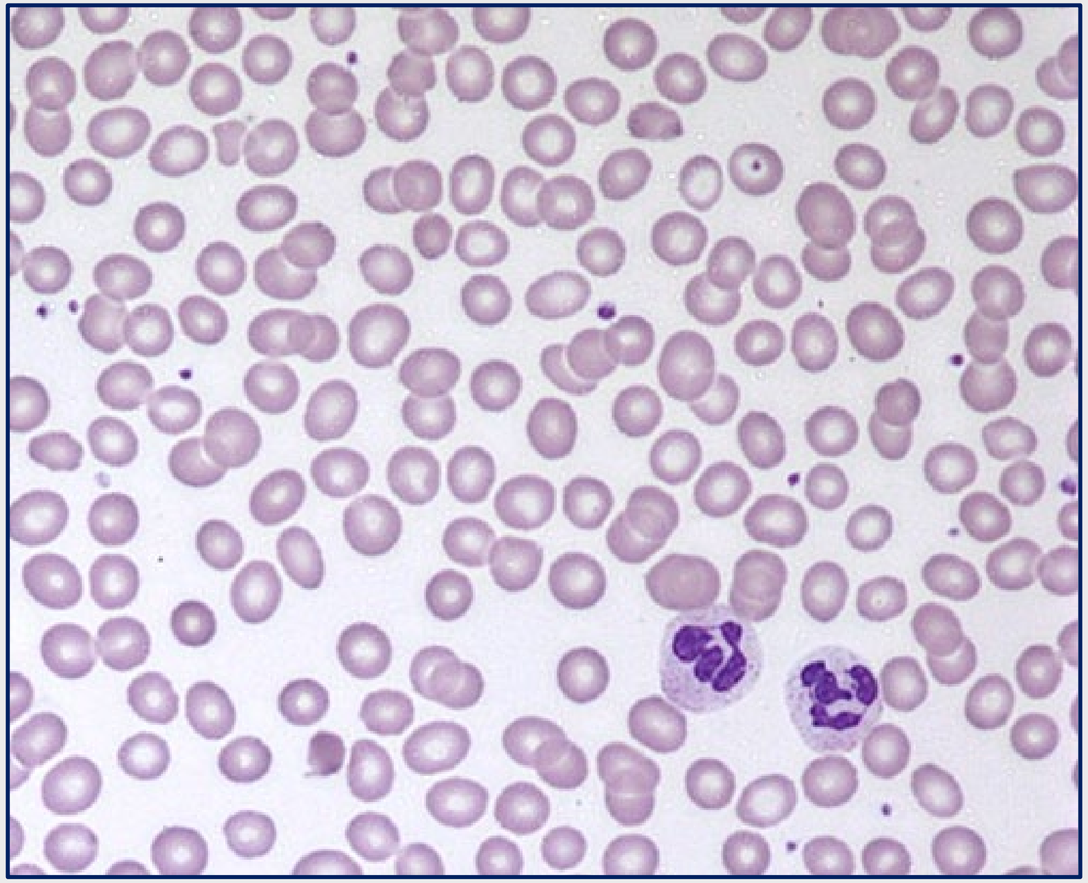
Pre-Read Materials

Approach to Diagnosing Sickle Cell Disease

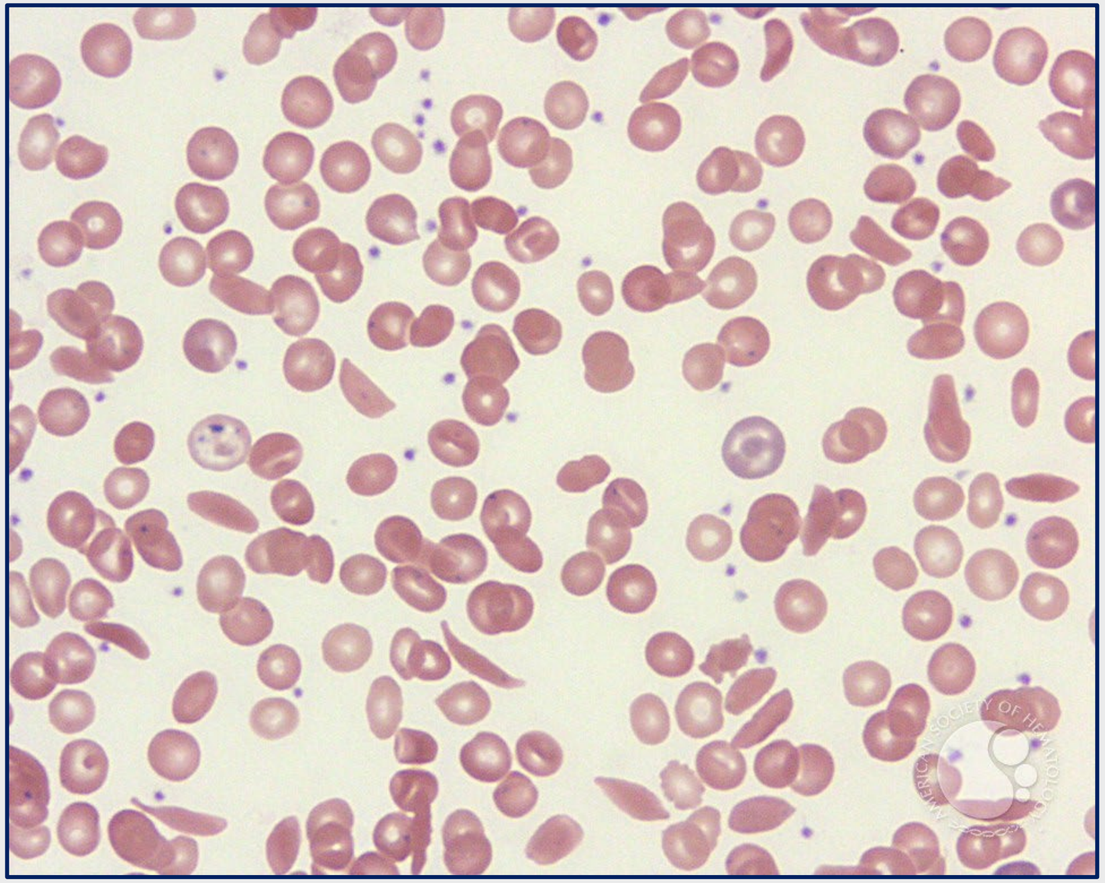
- Take a thorough personal history: Ask about lifelong anemia, history of transfusions, frequent hospitalizations in childhood, acute pain episodes (pain crises), history of pneumonia (acute chest syndrome), or any other SCD-related complications, especially in childhood (eg, dactylitis, splenic or hepatic sequestration, frequent childhood infections)
- Ask about family history and ancestry
- On physical exam, look for conjunctival pallor, jaundice, hepatomegaly, or splenomegaly
- Obtain complete blood count (CBC) with differential and peripheral blood smear
- Obtain confirmatory testing
  - Hemoglobin electrophoresis
  - High-performance liquid chromatography (HPLC)
  - Genetic testing

Sickled RBCs on Peripheral Blood Smear

Hemoglobin AA



Hemoglobin SS

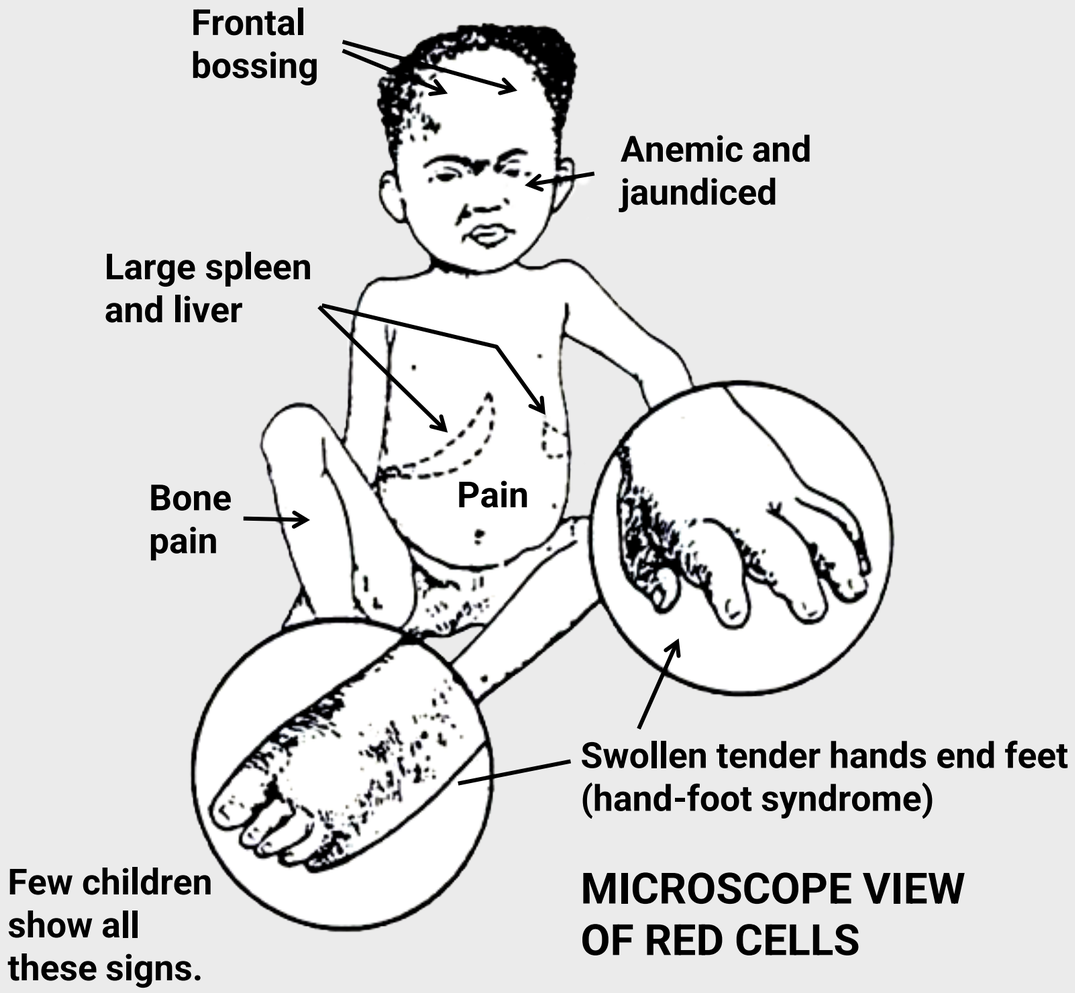


RBCs = red blood cells.

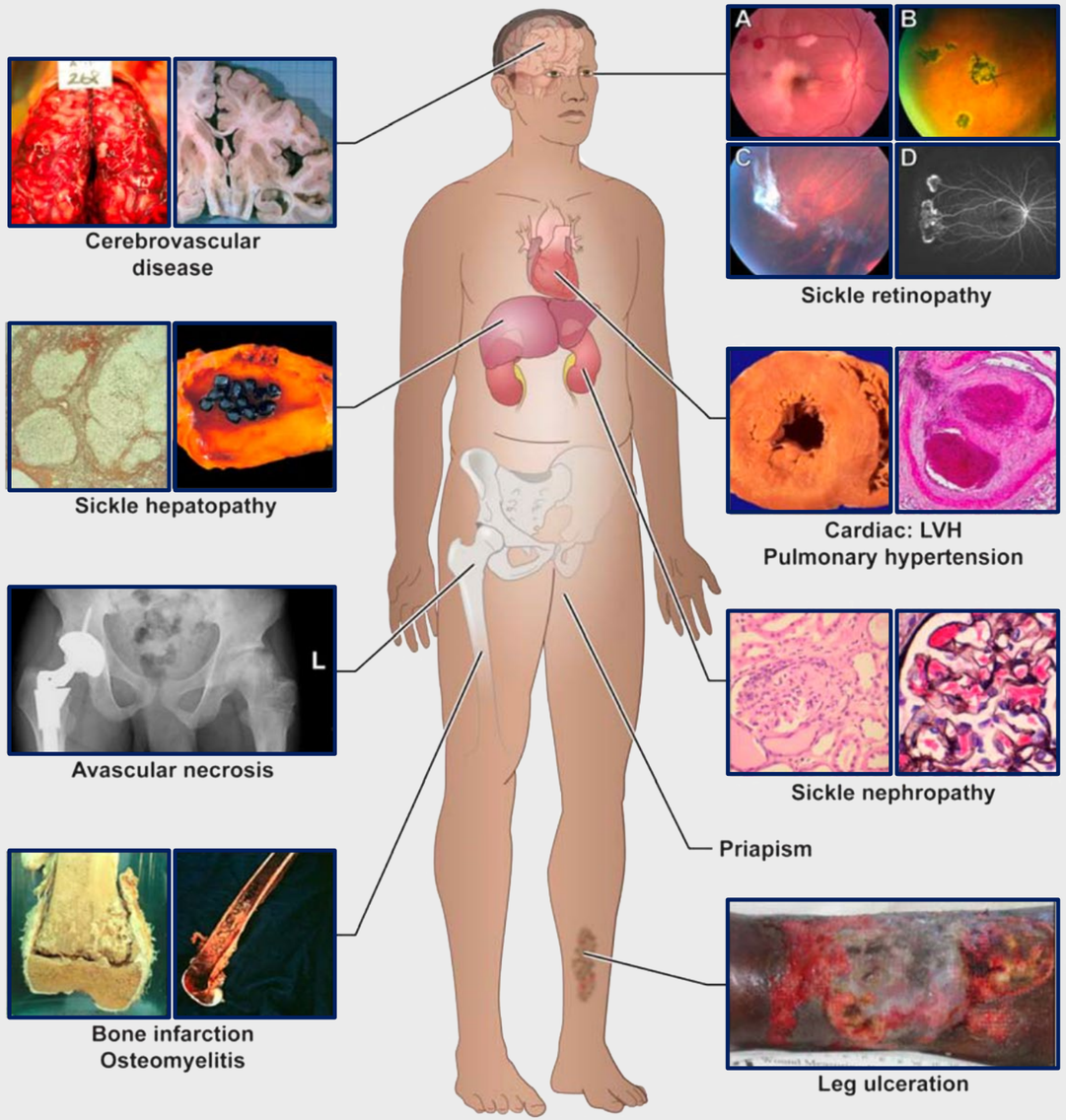
Courtesy of Dr Xu's personal collection.

Clinical Manifestations of SCD in Children vs Adults

SICKLE CELL ANEMIA



MICROSCOPE VIEW OF RED CELLS

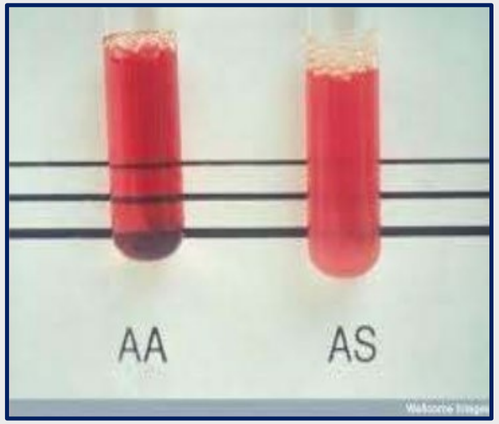


Thein SL, Howard J. *Blood*. 2018;132(17):1750-1760.

Diagnostic Techniques for Hemoglobin Disorders

Methods of screening and diagnosis

- Hematological parameters
- **Sickle solubility assay**
- **Cellulose acetate electrophoresis**
- **Citrate agar electrophoresis**
- **Isoelectric focusing (IEF)**
- Capillary electrophoresis
- **High-performance liquid chromatography (HPLC)**
- Mass spectrometry
- DNA sequencing
- Polymerase chain reaction (PCR) for common deletions

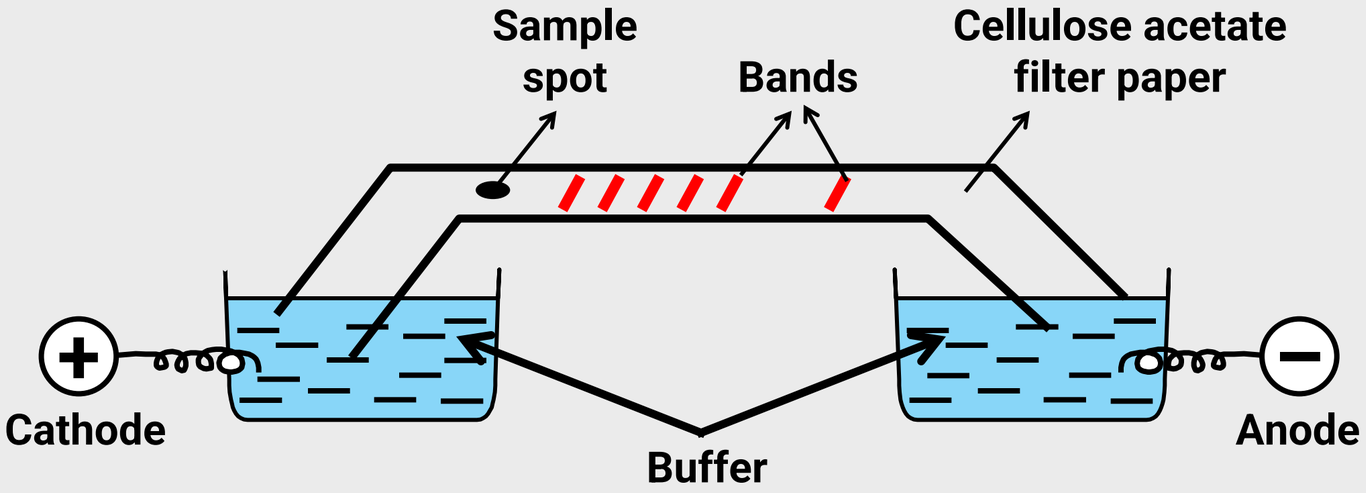


Sickle cell solubility (Sickle screen)

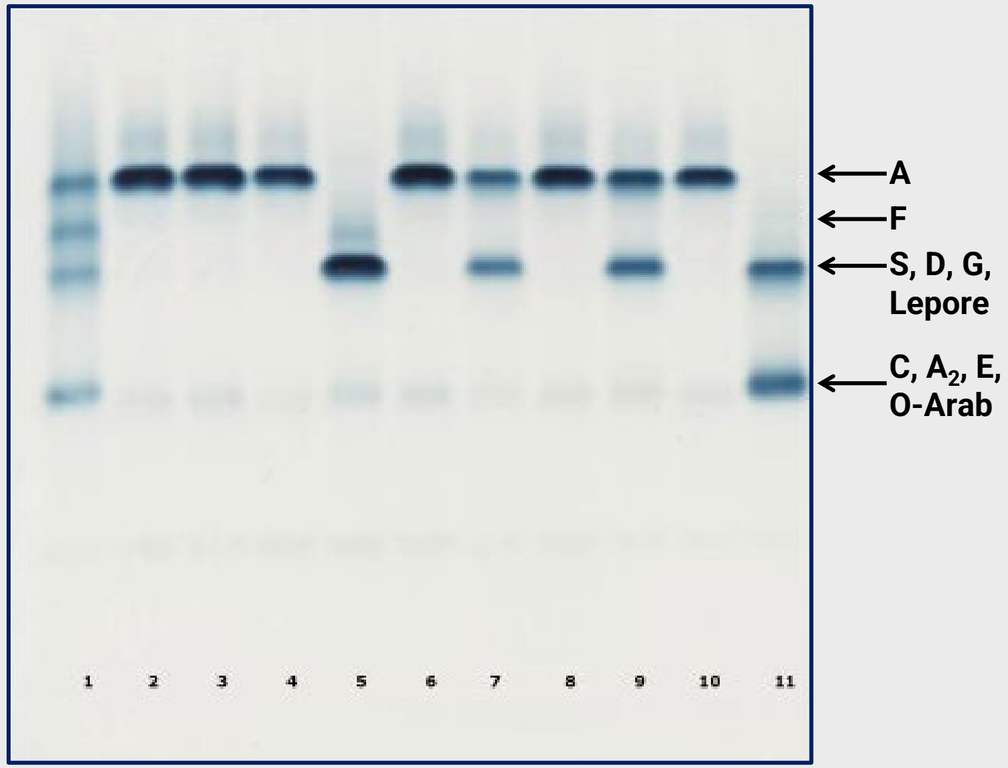
- Lyse RBCs
- Sodium hydrosulfite reduces released hemoglobin
- Reduced hemoglobin S (Hb S) is insoluble and precipitates in buffer
- Cloudy suspension = positive
- Limitations: Anemia (hematocrit <15%), Hb S <10%, high Hb fetal (F)
- Cannot differentiate Hb AS and SS

Twumasi S, et al. *Health Sci Rep*. 2025;8(9):e71223.

Diagnostic Techniques



Cellulose acetate electrophoresis



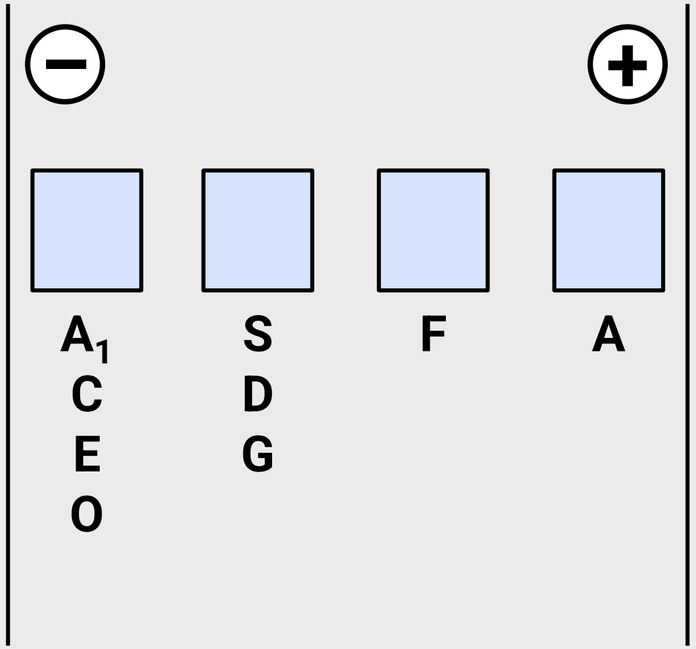
Alkaline pH (8.6)

Separation by electrical charge

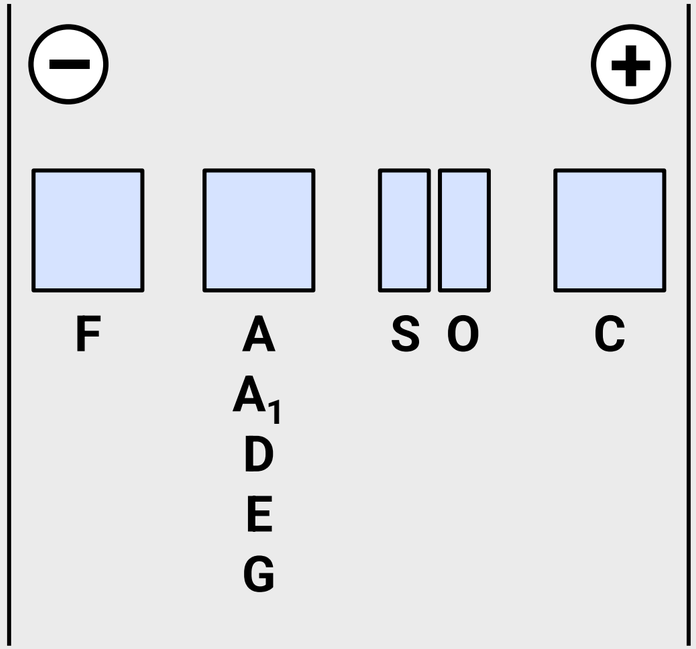
Gummadi S, Kandula VN. Int J Pharm Sci Res. 2020;11(12):6038-6056.

Diagnostic Techniques

Citrate agar gel:
At acidic pH, separation by electrical charge AND interaction with agar



Paragon (alkaline) Hb

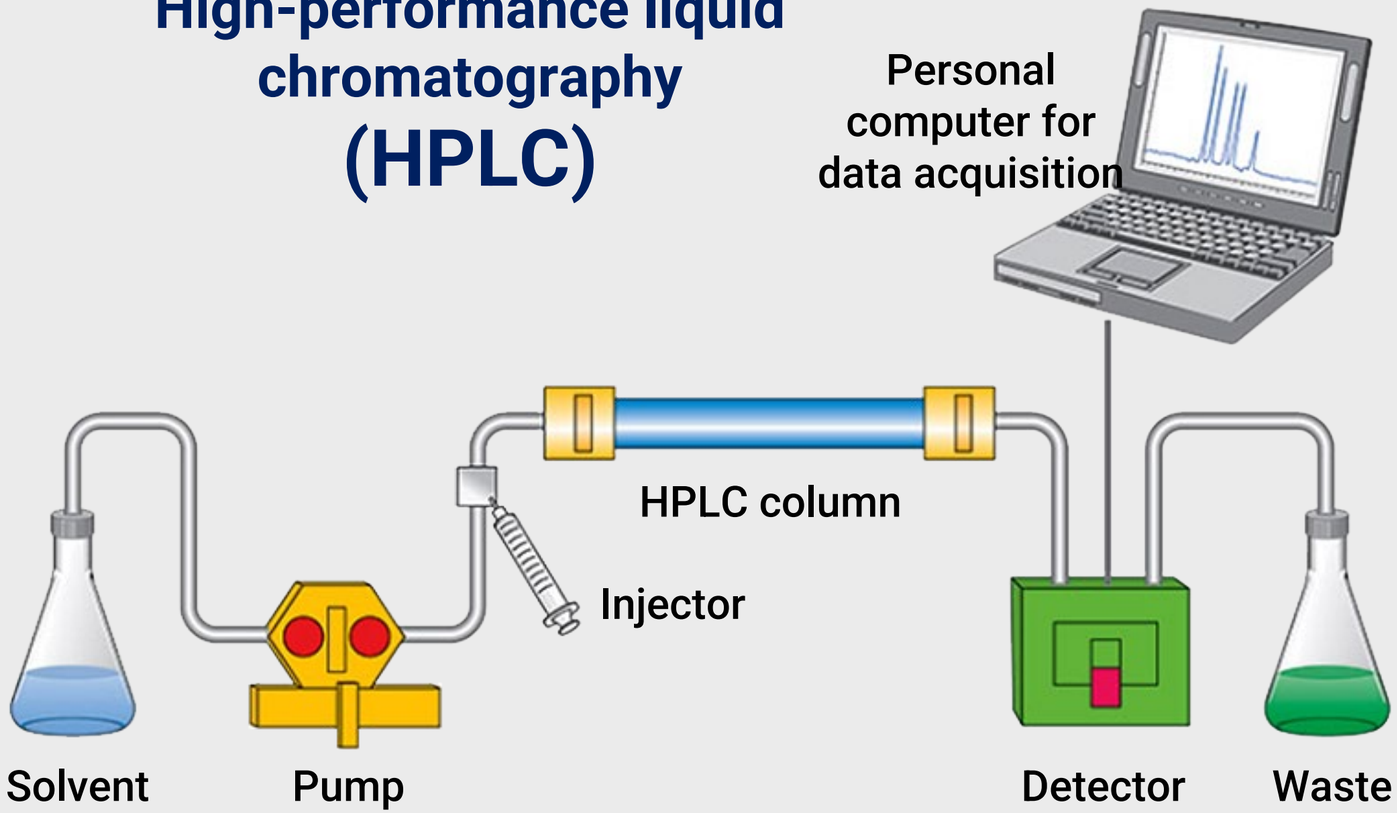


Paragon acid Hb

HemePathReview website. Hemoglobin electrophoresis (https://hemepathreview.com/Heme-Review/HemoglobinElectrophoresis.pdf). Accessed 9.17.2025.

Diagnostic Techniques

High-performance liquid chromatography (HPLC)



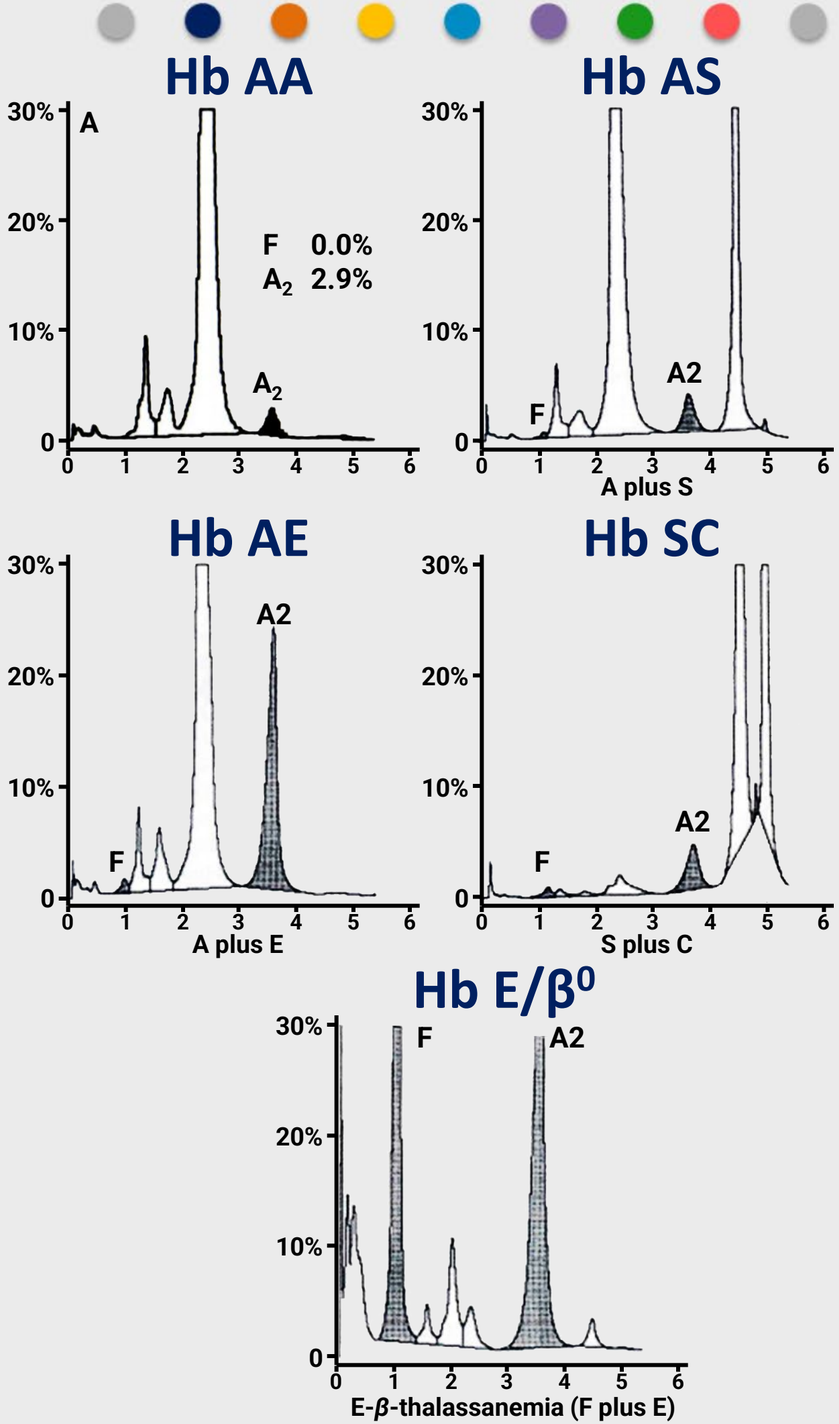
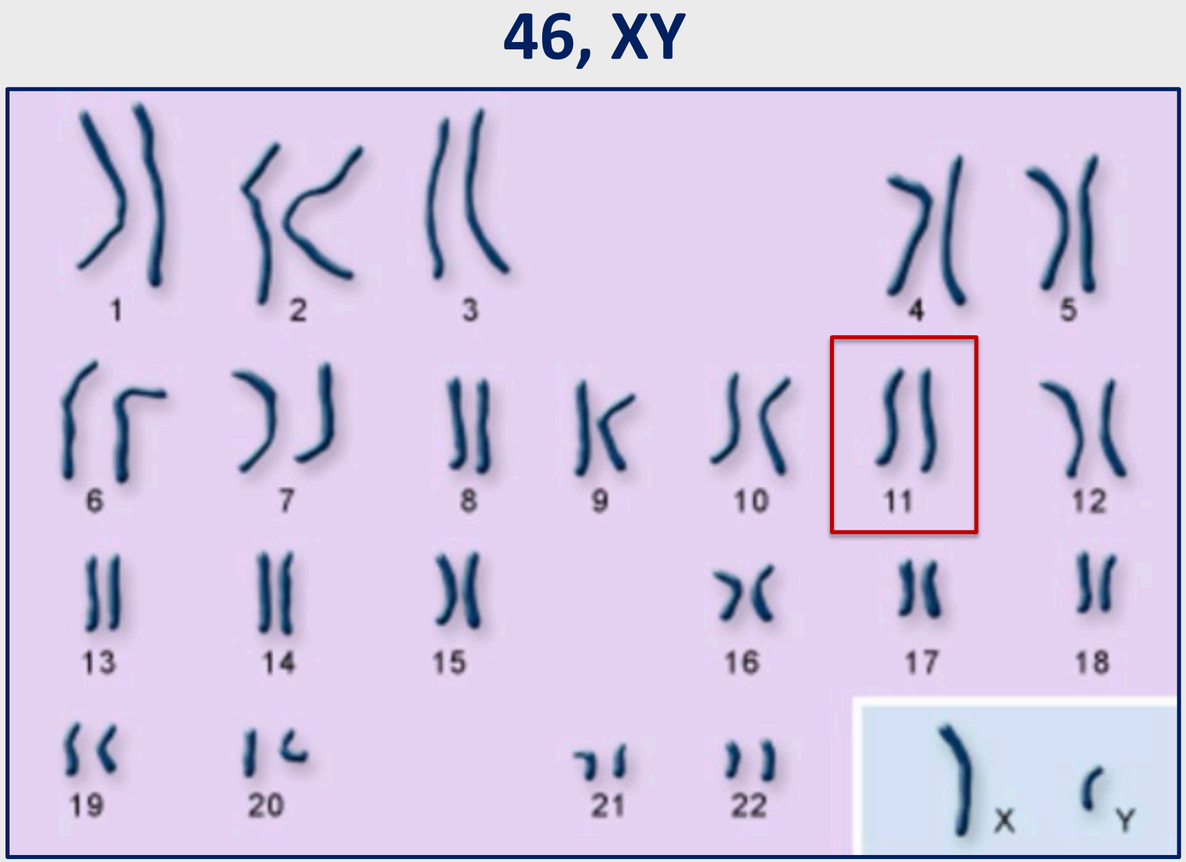
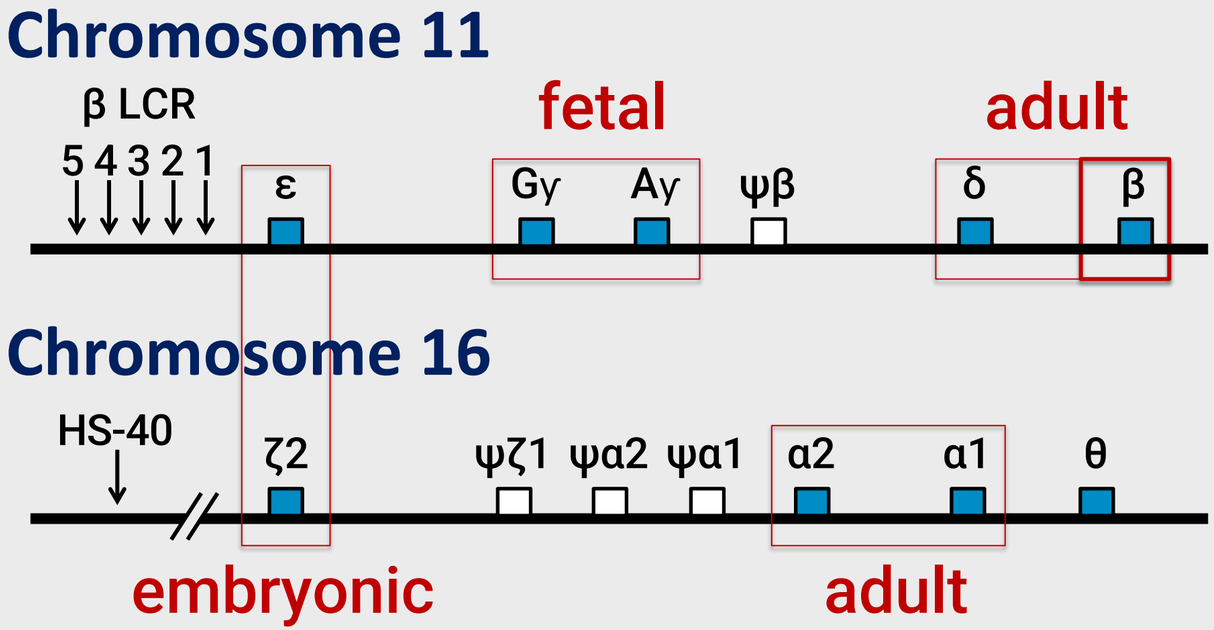


Image source: Sartorius AG.

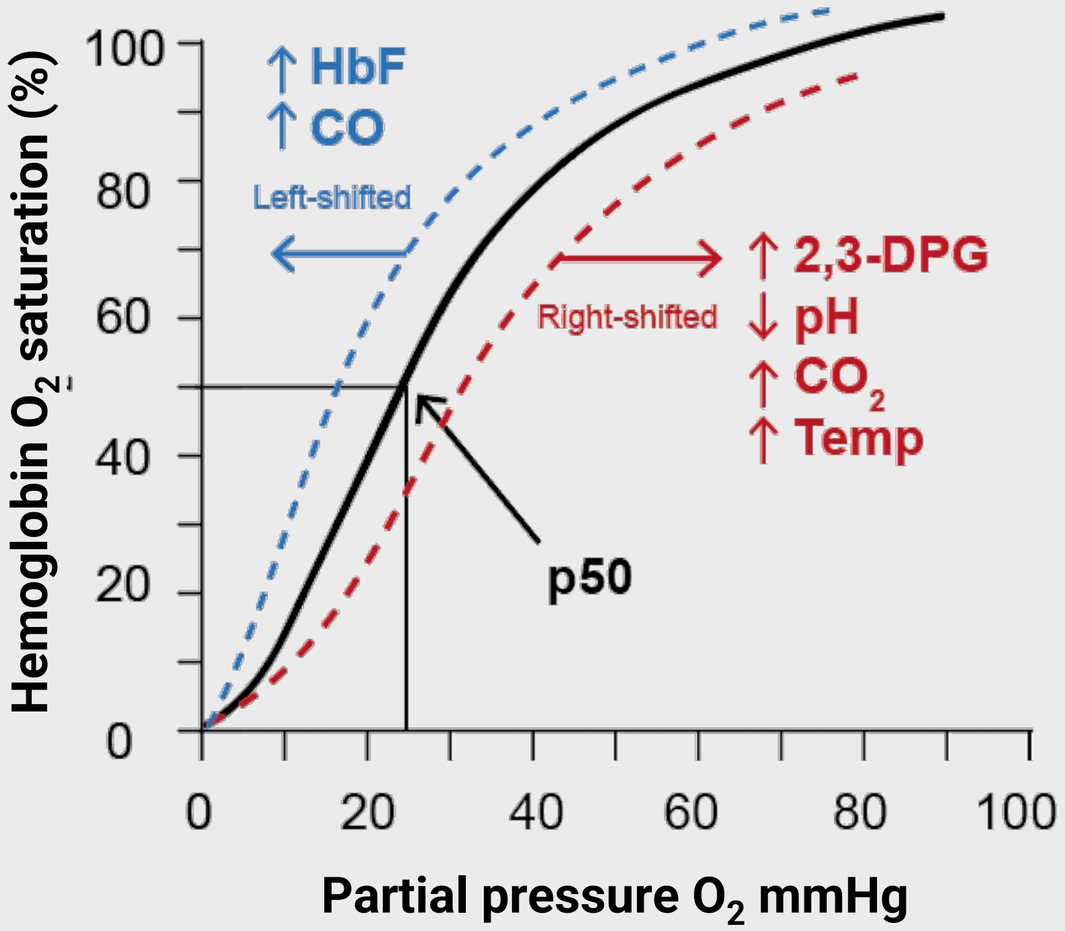
Genetics of Sickle Cell Disease



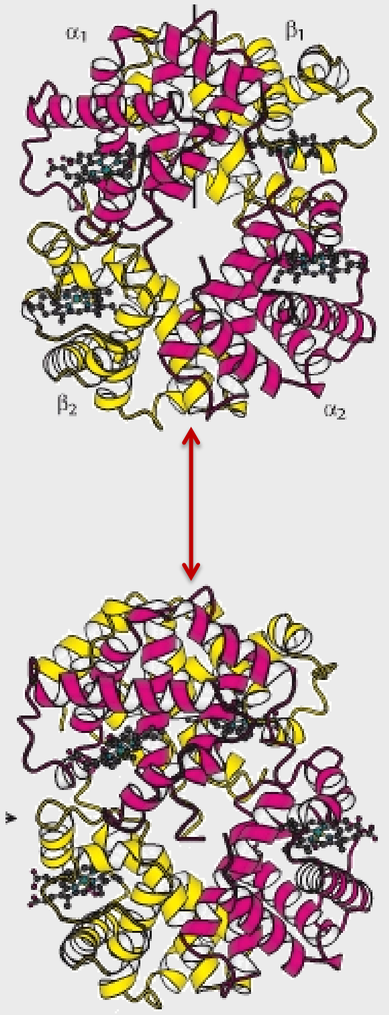


Sayani F, et al. Thalassemia, sickle cell disease, and other hemoglobinopathies. In: Cuker A, et al (eds). *American Society of Hematology Self-Assessment Program (ASH-SAP)*, 7th ed. ASH; 2019.

Pharmacological Inhibition of Hb S Polymerization



2,3-Diphosphoglycerate (2,3-DPG): Glycolytic intermediate that stabilizes deoxy-Hb



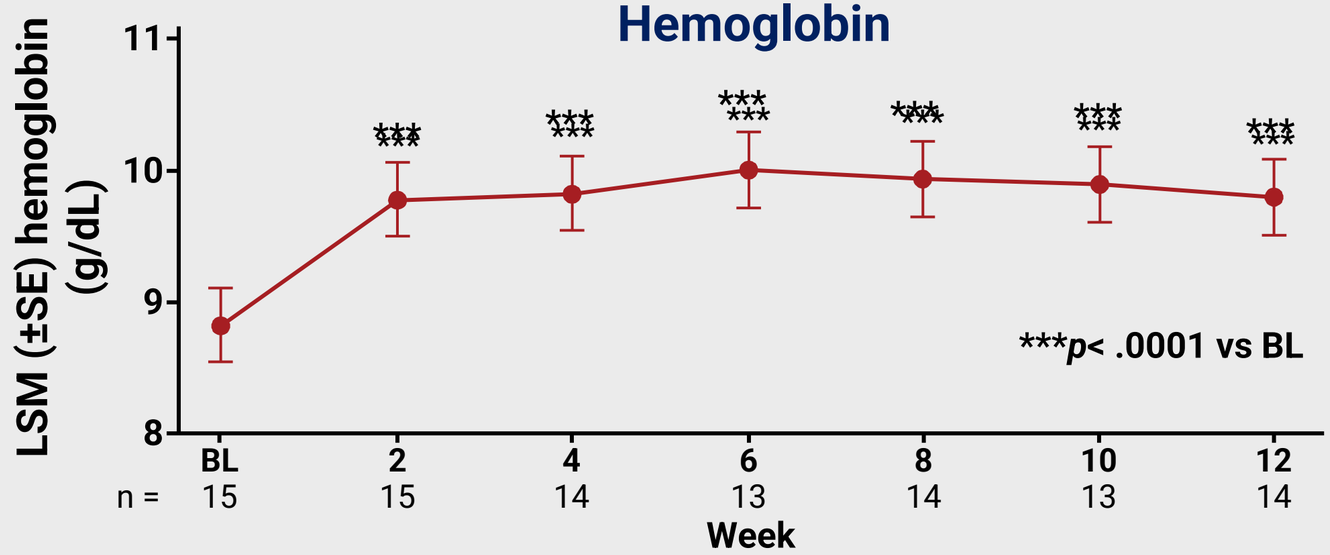
T state (deoxy-Hb)

R state (oxy-Hb)

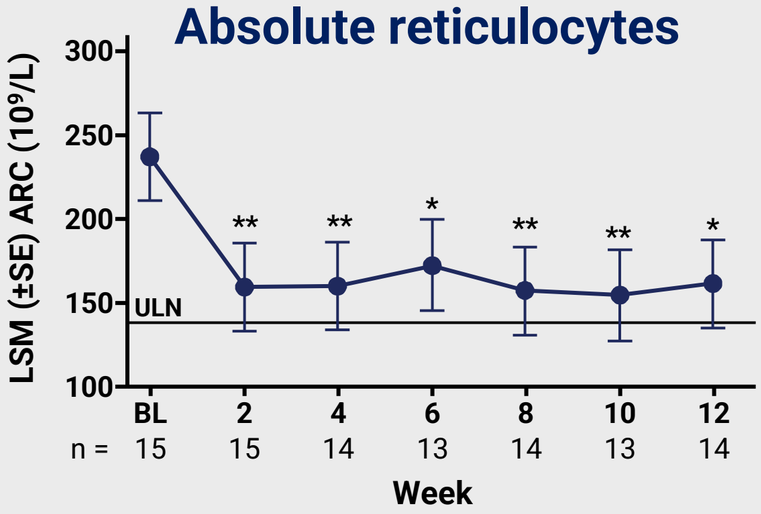
Xu JZ, Vercellotti GM. *Hematology Am Soc Hematol Educ Program*. 2023;2023(1):107-113.

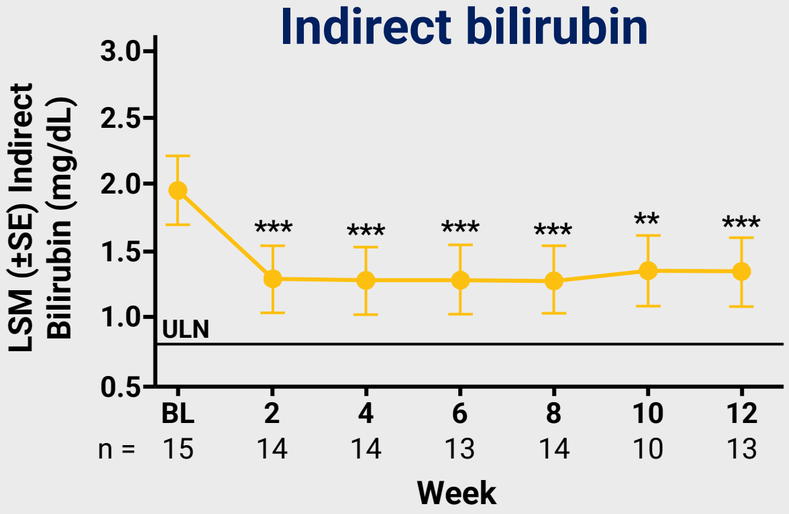
Eaton WA, Bunn HF. *Blood*. 2017;129:2719-2726.

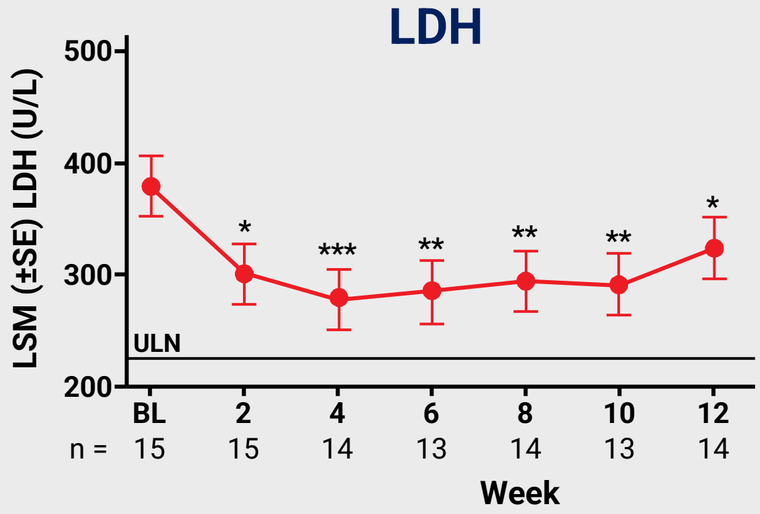
Clinical Laboratory Response to Etavopivat



Responder parameter	12-week cohort N = 15
Maximal Hb increase, mean (range), g/dL	1.5 (0.5–2.3)
Hb increase >1 g/dL on treatment[a] n (%)	11 (73.3)
Maximal Hb increase in patients with >1 g/dL response, mean (range), g/dL	1.8 (1.2–2.3)







Least squares mean (LSM) values presented are estimated from a mixed model repeated measures analysis. *P< .05, **P< .001, or ***P< .0001 vs BL.

[a]In patients who received etavopivat 400 mg once daily for at least 2 weeks (median 12 weeks, mean 11.3 weeks of etavopivat treatment).

BL = baseline; SE = standard error.

Brown JR, et al. *Blood*. 2020;136(1):19-20. Forma Therapeutics ASH Investors Briefing 12/13/2021 (https://www.sec.gov/Archives/edgar/data/1538927/000119312521354602/d231942dex992.htm). Accessed 9/17/2025.