Thalassemia

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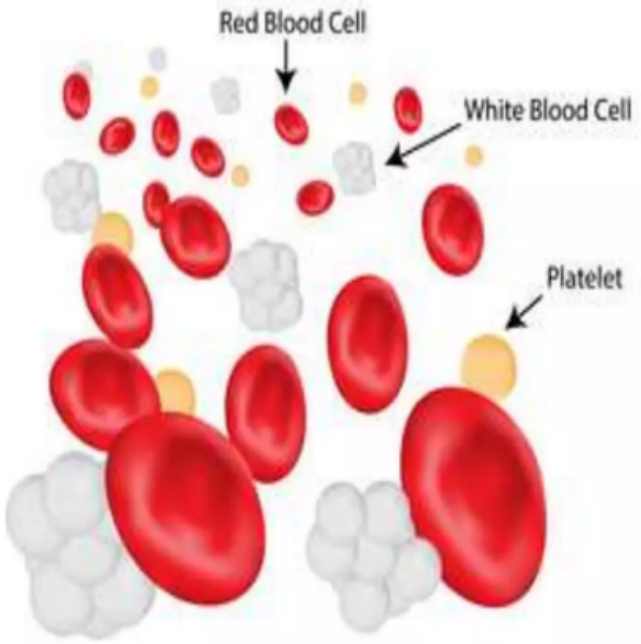
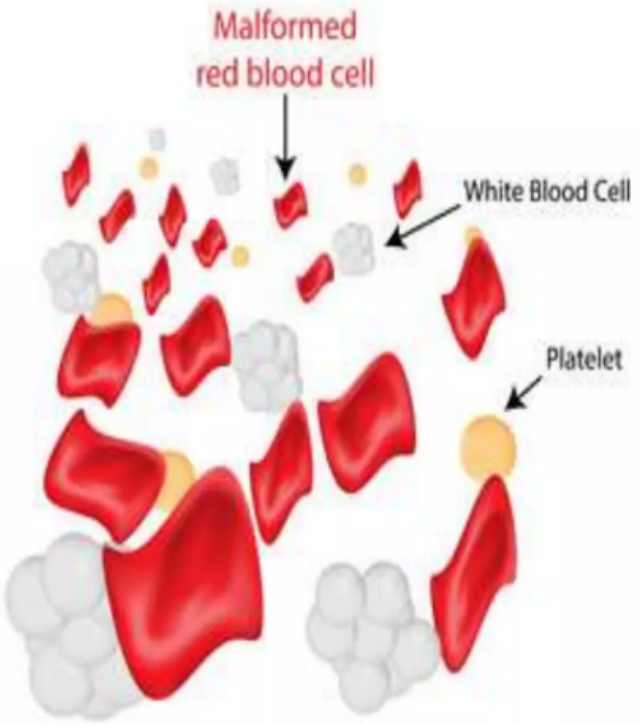
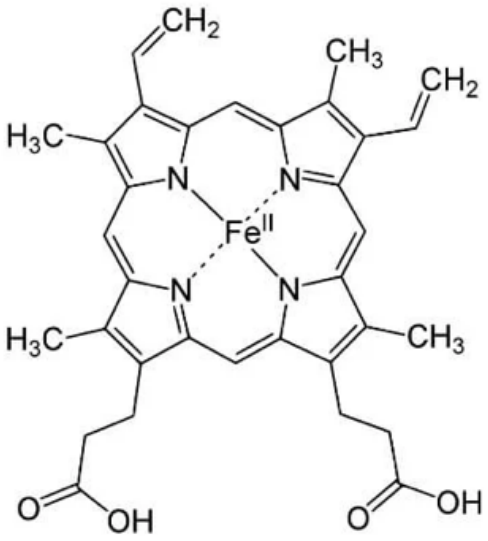
Thalassemia

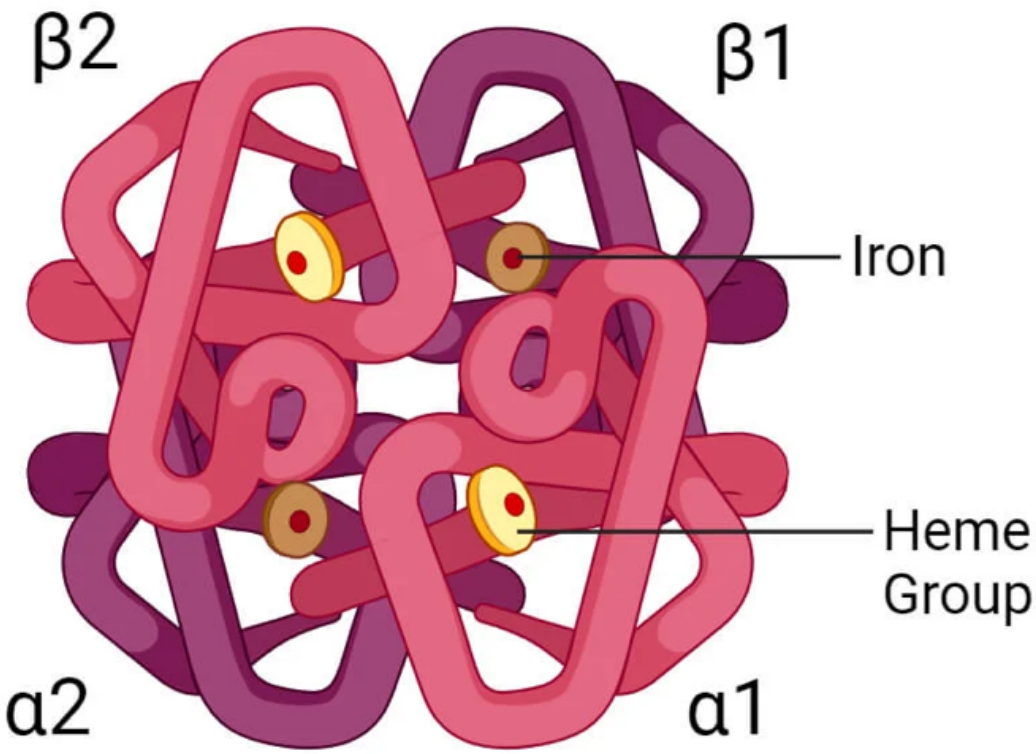




Hemoglobin



heme *b*



Hemoglobin Disorders

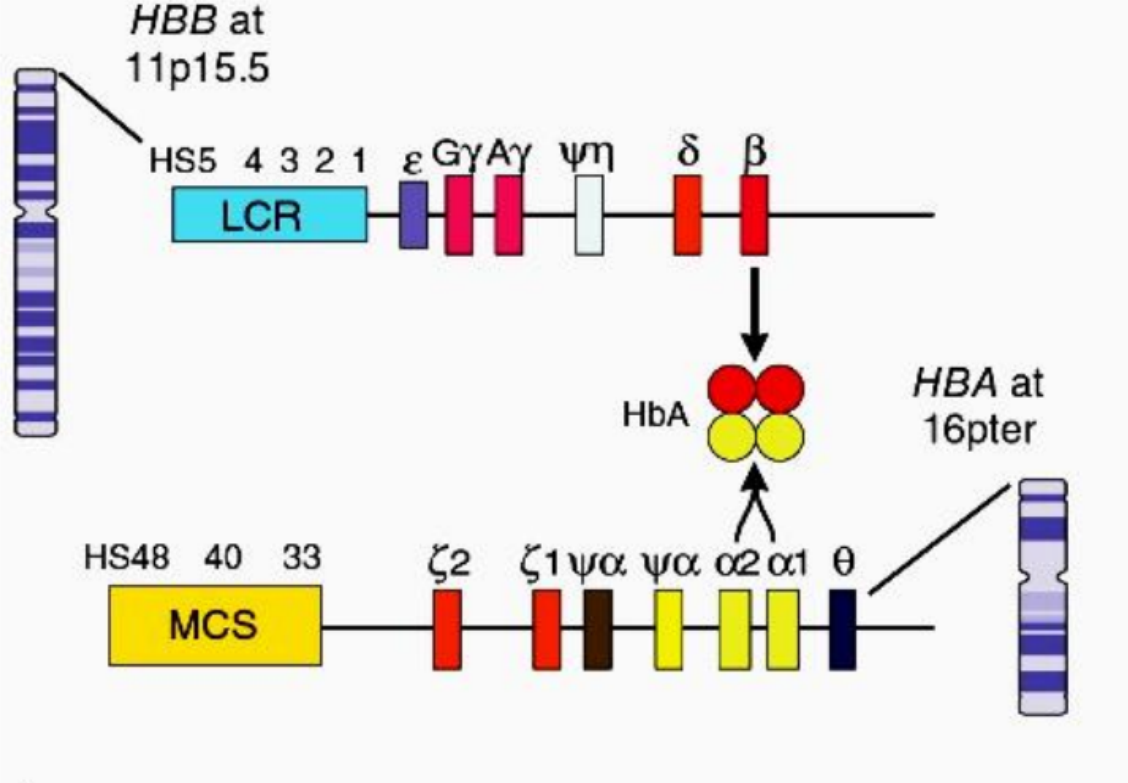
- Most common human mendelian genetic diseases

➢ Qualitative ---- Hemoglobinopathies like Sickle Cell Disease

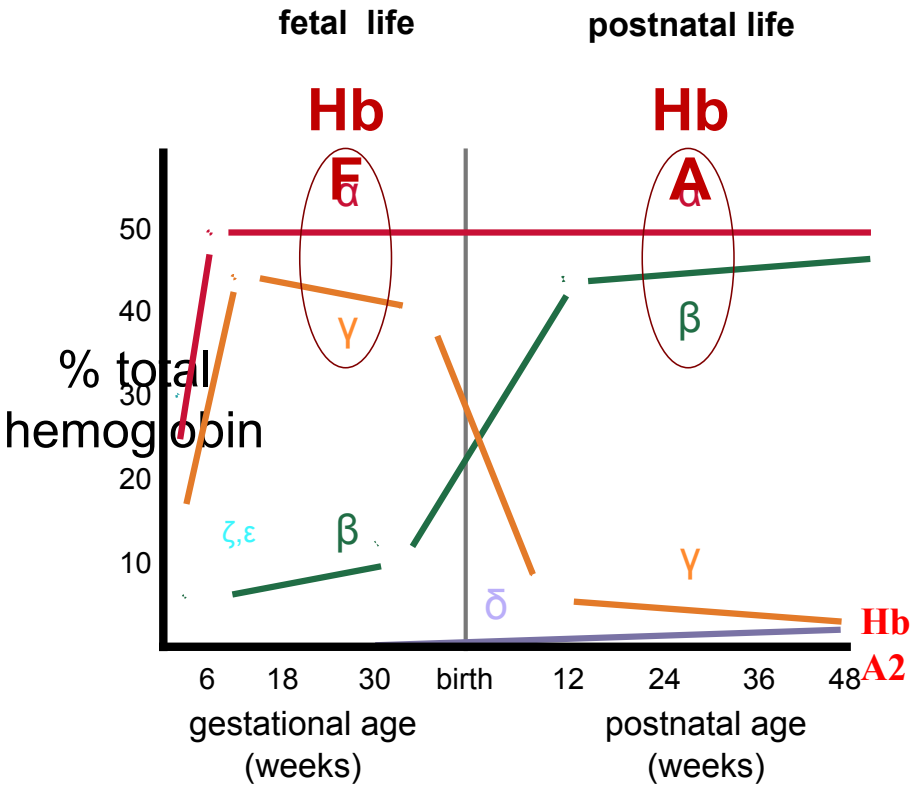
➢ Quantitative ---- Thalassemia

- Almost all are autosomal recessive disorders
- Family history of anemia
- In addition to pallor and jaundice, splenomegaly is often present

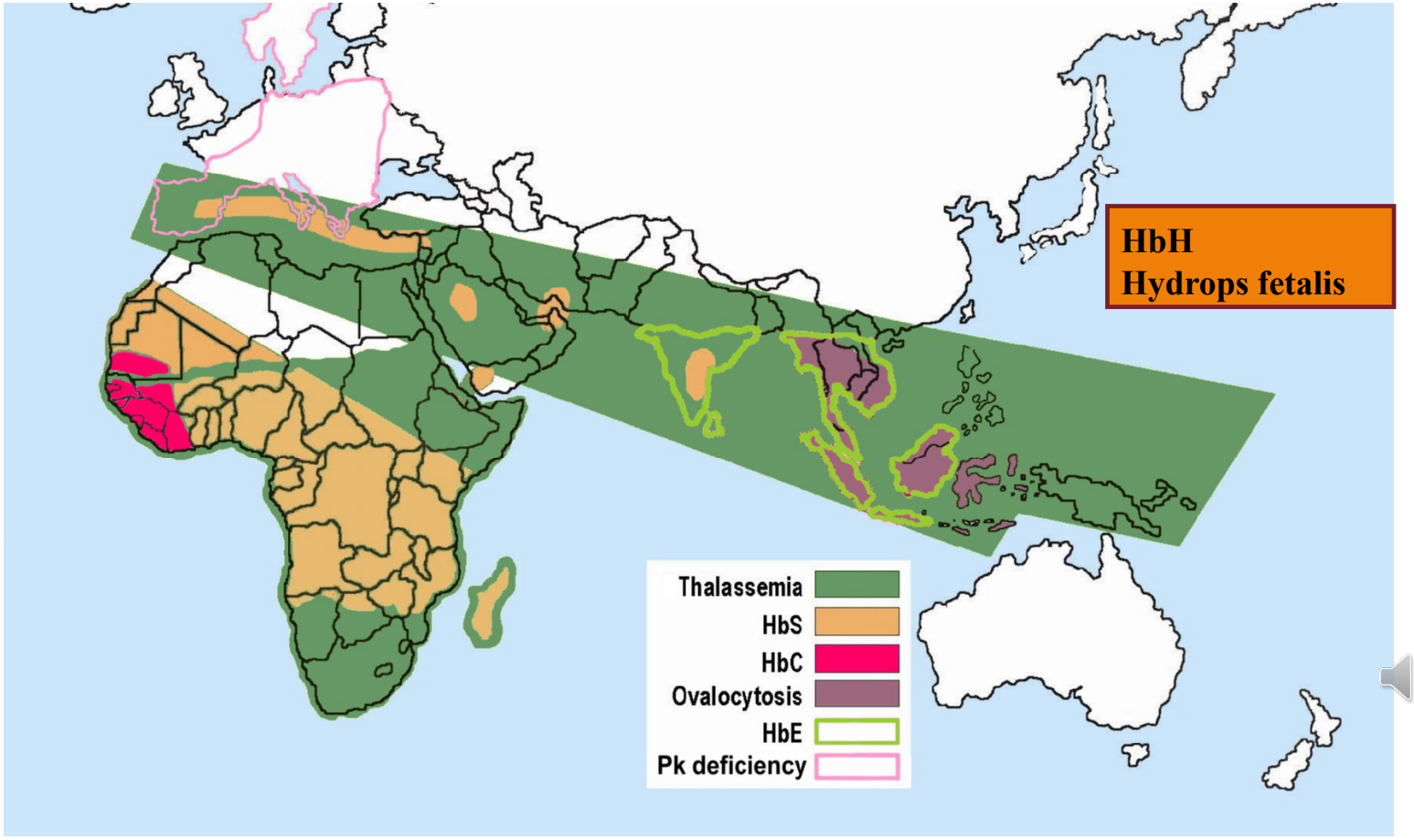
Globin gene clusters



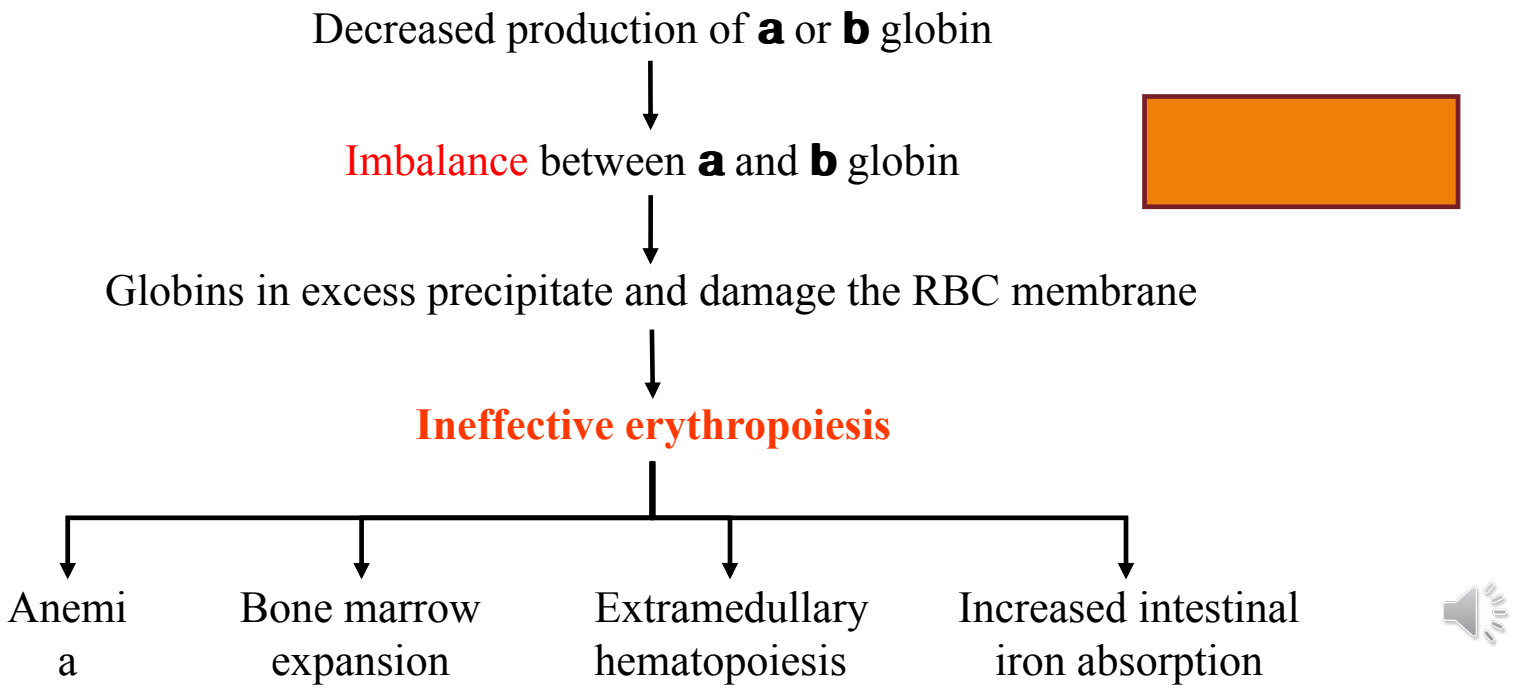
Hemoglobins Throughout Development



Geographical Distribution of Thalassemia



Pathophysiology of Thalassemia



Basics of Electrophoresis

Differentiation of thalassemia trait from mild iron deficiency anemia

Condition	Hgb F	Hgb A2
Beta thalassemia trait	Normal or Increased (1 – 10%)	Increased (3 – 10%)
Alpha thalassemia trait	Normal (0 – 2%)	Normal or Decreased (0 – 2.5%)
Iron deficiency anemia	Normal (0 – 2%)	Normal or Decreased (0 – 2.5%)

Normal Hgb F: ≈ 0 – 2%* Normal Hgb A2: ≈ 2 – 3%*

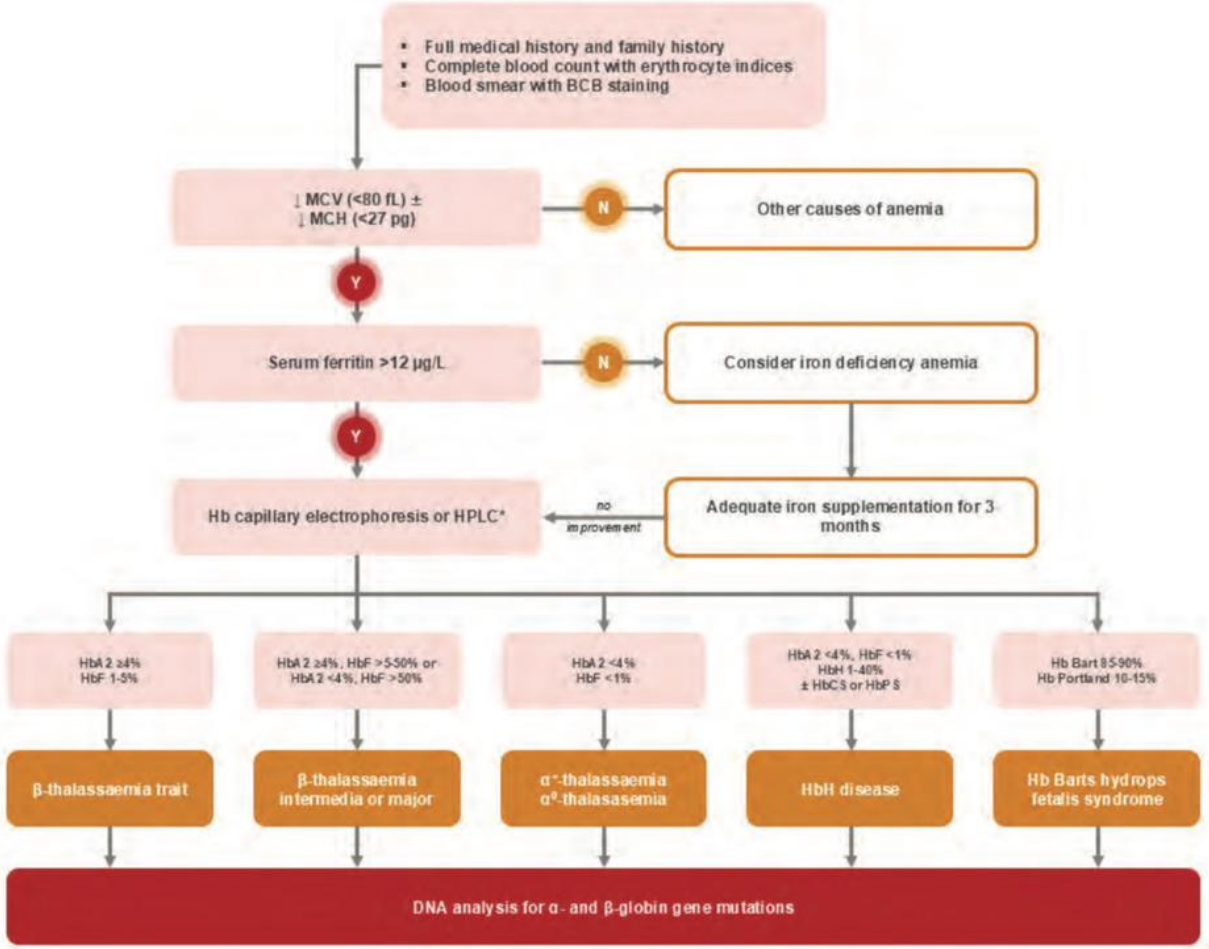
Key Points:

Beta thalassemia trait: diagnosis can be confirmed by electrophoresis.

Alpha thalassemia trait: diagnosis of exclusion (electrophoresis may be normal).

Iron deficiency anemia: mimics a-thalassemia trait, masks b-thalassemia trait.

Treat iron deficiency before performing electrophoresis.


Full medical history and family history
Complete blood count with erythrocyte indices
Blood smear with BCB staining
↓ MCV (<80 fL) ±
↓ MCH (<27 pg)
N
Other causes of anemia
Y
Serum ferritin >12 µg/L
N
Consider iron deficiency anemia
Y
Hb capillary electrophoresis or HPLC*
no improvement
Adequate iron supplementation for 3 months
HbA 2 ≥4%
HbF 1-5%
HbA 2 ≥4%, HbF >5-50% or
HbA 2 <4%, HbF >50%
HbA 2 <4%
HbF <1%
HbA 2 <4%, HbF <1%
HbH 1-40%
± HbCS or HbPS
Hb Bart 85-90%
Hb Portland 10-15%
β-thalassaemia trait
β-thalassaemia intermedia or major
α+-thalassaemia
α0-thalasasemia
HbH disease
Hb Barts hydrops fetalis syndrome
DNA analysis for α- and β-globin gene mutations

α Thalassemia

TABLE 98-6 α Thalassemias

CLASSIFICATION	α-GLOBIN GENE ARRANGEMENT	HEMOGLOBIN LEVEL, g/L (g/dL)/MCV (fL)	CLINICAL FEATURES
α Thalassemia trait	−α/αα −α/−α − −/αα $\alpha^T\alpha/\alpha\alpha$	120–150 (12–15)/65–80	The chromosome with one deleted α gene (−α/) is called α^+ thalassemia (α thalassemia-2); the chromosome with both deleted α genes is α^0 thalassemia (α thalassemia-1); non–gene deletion α thalassemias (α^T) often have a more severe phenotype.
Hemoglobin H disease	− −/−α $\alpha^T\alpha$/− − $\alpha^T\alpha/\alpha^T\alpha$	50–120 (5–12)/60–70	Mild to moderate anemia depending on genotype; non–gene deletion forms of α thalassemia can produce severe HbH disease.
Hb Bart's hydrops fetalis	−−/−−		Fatal in utero or at birth with rare survivors. Hydrops can also result from combinations of gene deletion and non–gene deletion α thalassemia.
α Thalassemia/intellectual disability syndromes (ATR-16) (ATR-X)	− −/αα or − −/−α in ATR-16 αα/αα in ATR-X		ATR-16: Large deletions and rearrangements in chr16p. ATR-X: No α-globin gene deletion or mutation, *ATRX* mutations, X-linked.
α Thalassemia with myelodysplasia (ATMDS)	αα/αα		Mutations in *ATRX*; striking male predominance. Hematologic findings of HbH disease.

Risk of α Thalassemia Major in Offsprings

a-/a-
***trans* deletions**

	a-	a-
a-	□a-□a-	□a-□a-
a-	□a-□a-	□a-□a-

No thalassemia major

--/aa
***cis* deletions**

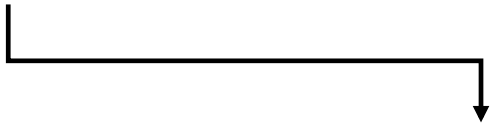
	□-	aa
□-	□□□□□□	□□-□aa
aa	□aa□□-	aa□aa

25% thalassemia major

Pathophysiology

- Gene deletions are the most common α thalassemia events (vs. mutations)
- Unpaired γ-globin chains form γ4 or Hb Bart's; in fetus
- Unpaired β-globin chains, tetramerize as β4 or HbH; in adults

- Both Hb Bart's and HbH have very high O2 affinity and do not unload O2 in tissues



- Severe anemia in Hb Bart's hydrops fetalis result in death
- Unstable HbH leads to oxidative membrane damage with extravascular hemolysis in the spleen and ineffective erythropoiesis

Diagnosis

Trait

- Microcytosis/hypochromia with nearly normal Hb concentrations
- Absence of iron deficiency
- Normal HbA2 level
- Genetic counseling

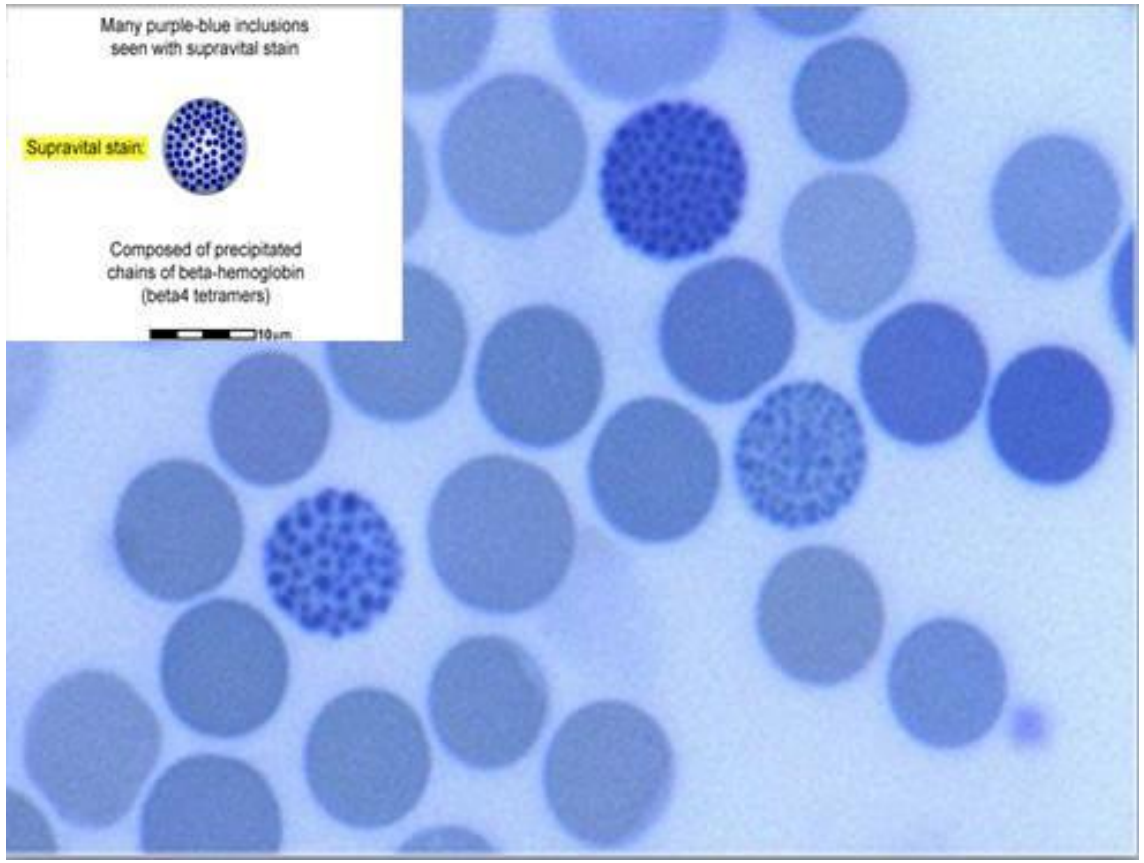
HbH

- HbH disease, varying levels of reticulocytosis, 20–30% Hb Bart's at birth
- In adults, traces to 40% HbH are present
- HbH inclusions staining with brilliant cresyl blue

Bart

- Hb Bart's hydrops fetalis is predominantly Hb Bart's with some Hb Portland if the deletion removing α-globin genes preserves the ζ-globin gene

HbH supravital stain



Symptoms severity

Trait

- No symptoms
- Normal hemoglobin

HbH

- Heterogeneous phenotype
- More severe if HbCS is present in genotype
- Hepatosplenomegaly , jaundice
- Thalassemic bone changes in the face
- Growth impairment

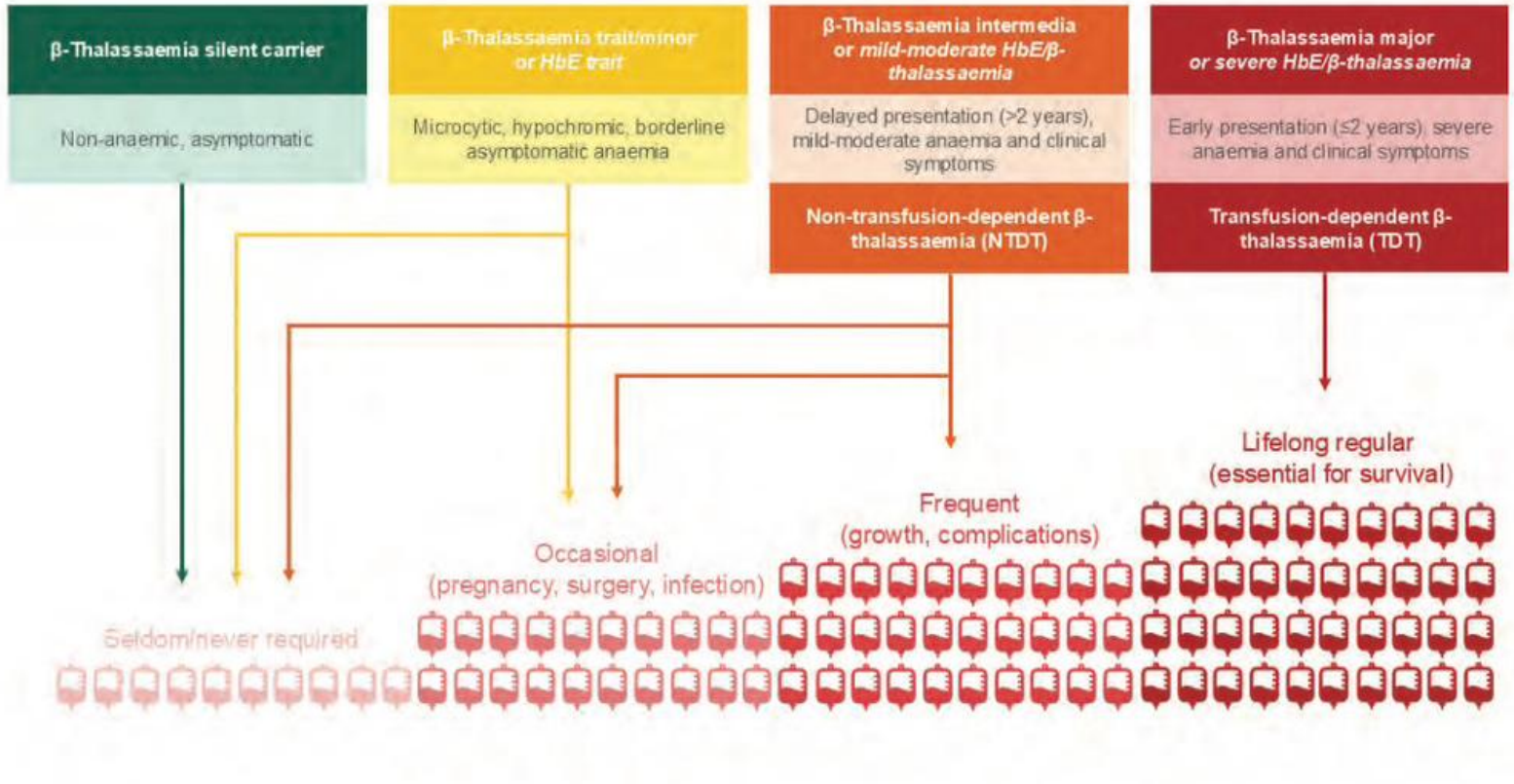
Bart

- Preeclampsia, polyhydramnios, and antepartum hemorrhage

Management

- No transfusion needed unless severe anemia/pregnancy
- No iron supplement unless IDA
- Iron store checks in intervals

- Hb Bart's hydrops fetalis is best prevented by screening couples at risk and antenatal diagnosis
- Intrauterine therapy and perinatal intensive care
- Best to prevent


β-Thalassaemia silent carrier
Non-anaemic, asymptomatic
β-Thalassaemia trait/minor or HbE trait
Microcytic, hypochromic, borderline asymptomatic anaemia
β-Thalassaemia intermedia or mild-moderate HbE/β-thalassaemia
Delayed presentation (>2 years), mild-moderate anaemia and clinical symptoms
Non-transfusion-dependent β-thalassaemia (NTDT)
β-Thalassaemia major or severe HbE/β-thalassaemia
Early presentation (≤2 years), severe anaemia and clinical symptoms
Transfusion-dependent β-thalassaemia (TDT)
Lifelong regular (essential for survival)
Frequent (growth, complications)
Occasional (pregnancy, surgery, infection)
Seldom/never required

β Thalassemia

- Mediterranean / Cooley anemia

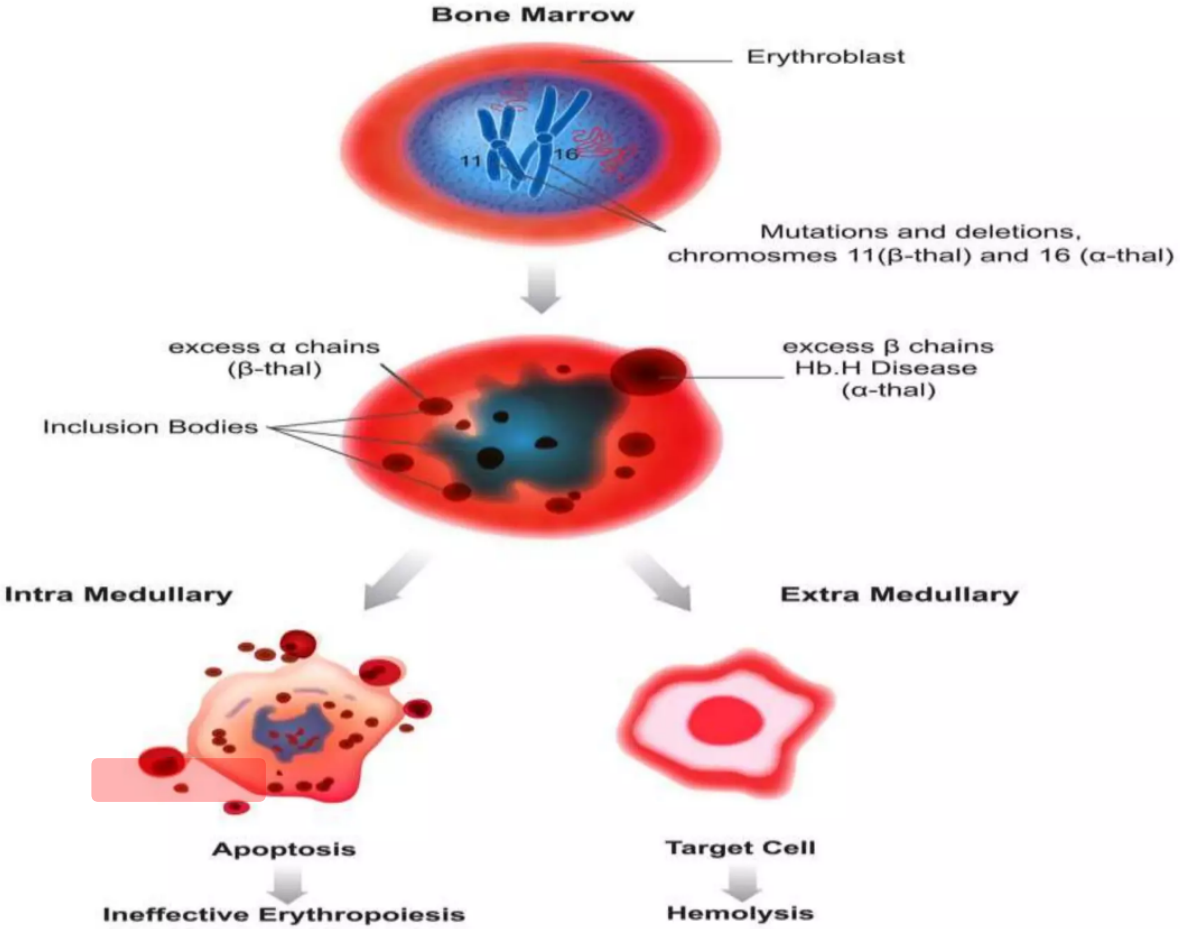
TABLE 98-4 β Thalassemias

CLASSIFICATION	HEMOGLOBIN (g/dL)/ MCV (fL)	HEMOGLOBIN FRACTIONS (%)	CLINICAL FEATURES
β Thalassemia trait	100–140 (10–14)/60–80	HbA: 94 HbF: 1–2 HbA_2: 4–6	Heterozygosity for β^+ or β^0 thalassemia mutations; "silent" carriers can have normal HbA_2 and red cell indices.
Non-transfusion-dependent β thalassemia (thalassemia intermedia)	70–120 (7–12)/65–80	HbA: 60–90 HbF: 10–40 HbA_2: 4–6	Defined by infrequent or no transfusion requirement; caused by many different genotypes including homozygosity for "mild" β^+ mutations, combinations of β and α thalassemia, homozygous β thalassemia with high HbF producing capacity, and many others. Iron loading, thromboembolic disease, and pulmonary hypertension are major clinical events.
Transfusion-dependent β thalassemia (thalassemia major)	20–40 (2–4)/50–80	HbA: 0–5 HbF: 90–100 HbA_2: 2–5	Caused by many different genotypes including homozygosity and compound heterozygosity for β^0 and β^+ mutations, combinations of β and α thalassemia; transplantation curative; iron chelation required.

Pathophysiology

- Single nucleotide changes are the most common β thalassemia mutations (vs. gene deletions)
  - Promoter elements causing mild and sometimes silent β+ thalassemia
  - Translation mutations causing β0 thalassemia
  - Nonsense mutations causing β0 thalassemia
- Unstable α-globin chains accumulate in excess, causing membrane lipid oxidation and damage
- Intramedullary destruction of erythroid precursors known as ineffective erythropoiesis
- Extra- and intravascular hemolysis
- Severe anemia leads to bone marrow expansion;

Pathophysiology



Diagnosis

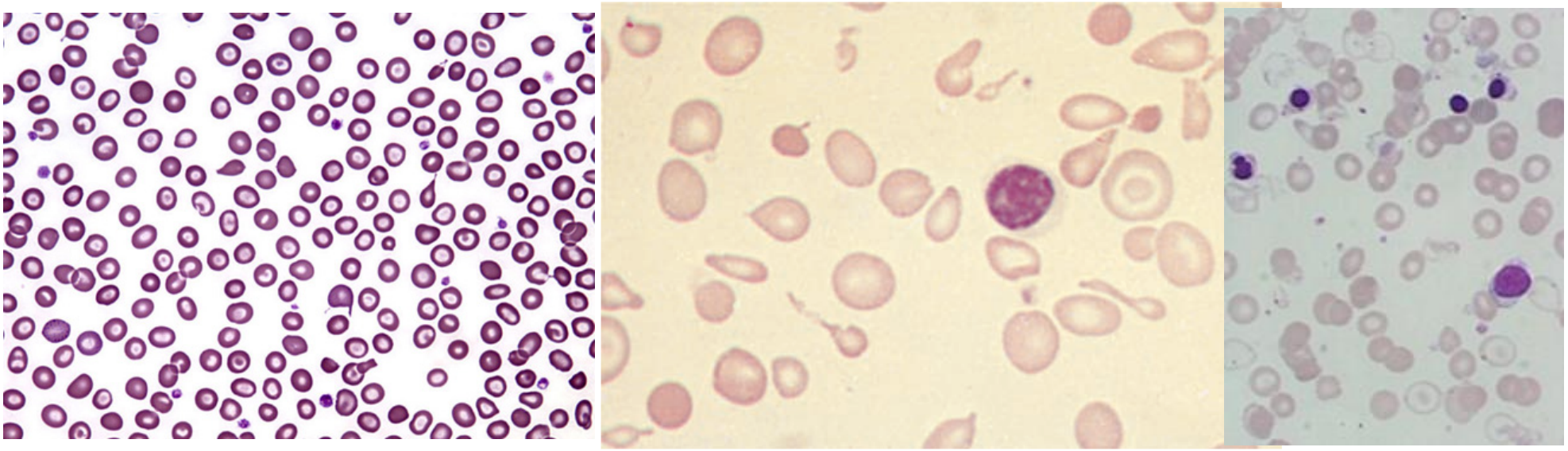
Carrier/Trait

- Mild or no anemia
- Microcytic/hypochromic erythrocytes with no increase in reticulocyte count
- Elevated level of HbA2 and perhaps HbF
- Genetic counseling

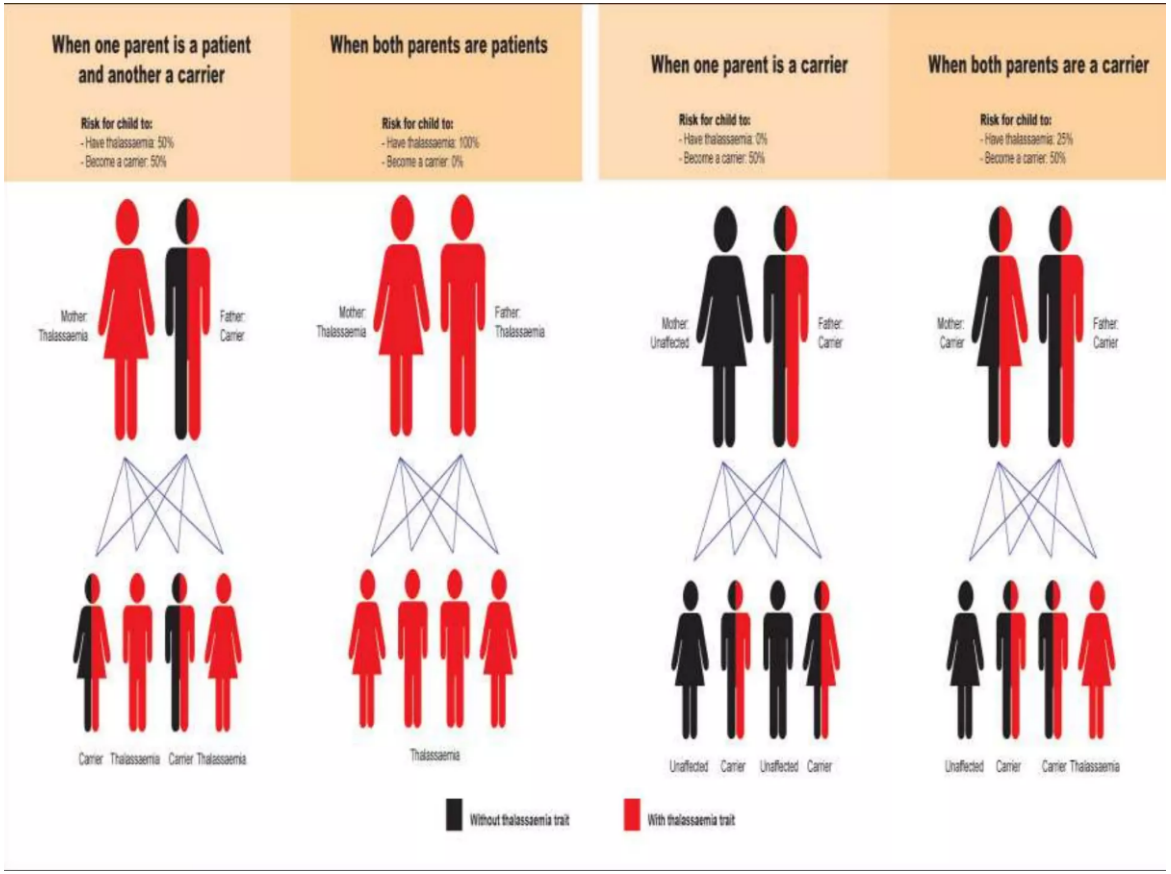
Major

- Hemolytic anemia
- Hypochromia, microcytosis
- Reticulocytosis, marked anisocytosis, and poikilocytosis with nucleated red cells

β Thalassemia smear



Inheritance



Complications

TABLE 98-5 Complications of β Thalassemia

COMPLICATION	INCIDENCE, DIAGNOSIS, AND FEATURES
Growth retardation	Most often a feature of delayed or inadequate transfusions but can occur in well-transfused children.
Delayed puberty; secondary amenorrhea	50% and 25%, respectively.
Splenomegaly	Can trap 1–40% of red blood cell volume; increases plasma volume, worsening heart failure. Splenectomy indicated when transfusion requirement to maintain ideal hemoglobin increases. Prophylactic penicillin after splenectomy.
Heart	Due to chronic anemia, heightened sensitivity to iron toxicity, thromboembolic pulmonary hypertension, other causes. Progresses through stages to congestive failure and arrhythmias. Assessed by T2* on MRI. The available chelating agents might have differential effects on different measures of cardiac function and can be used in combination.
Leg ulcers	Common in thalassemia intermedia.
Hepatic disease	Fibrosis progressing to cirrhosis is related to hepatic iron concentration that can be monitored by MRI. Hepatitis also plays a role.
Lung disease/ pulmonary hypertension	Fibrosis, chronic thromboembolic disease, restrictive pathophysiology, intravascular hemolysis, and reduced nitric oxide bioavailability
Thromboembolism	Multifactorial etiology including platelet activation, red cell–endothelial interactions, thrombocytosis; endothelial activation; splenectomy.
Endocrinopathies	Diabetes, hypothyroidism, hypoparathyroidism, adrenal insufficiency; hypogonadism; hypothalamic-pituitary axis might be especially sensitive to iron.
Bone disease	Caused by bone marrow expansion, severe iron loading, hypogonadism; osteoporosis in ~50% of patients, even those well treated. Extramedullary hematopoietic masses are a feature of thalassemia intermedia.
Infections	Transfusion associated; linked to iron overload (*Yersinia*); malaria.

Therapy for Thalassemia Intermedia/Major

- **The goals of treatment for the thalassemia syndromes are to:**
- maintain optimal levels of Hb for growth and development in children, appropriate activity at all ages.
- minimize the complications related to ineffective erythropoiesis, by adequately suppressing patient's own (endogenous) marrow activity.
- prevent complications related to the therapy of the disease.
- improve longevity while maintaining a good quality of life.

It is important to approach management with the idea that there is spectrum of severity between thalassemia major and thalassemia intermedia, and that patients may move on that continuum with increasing severity of clinical disease

Therapy for Thalassemia Intermedia/Major

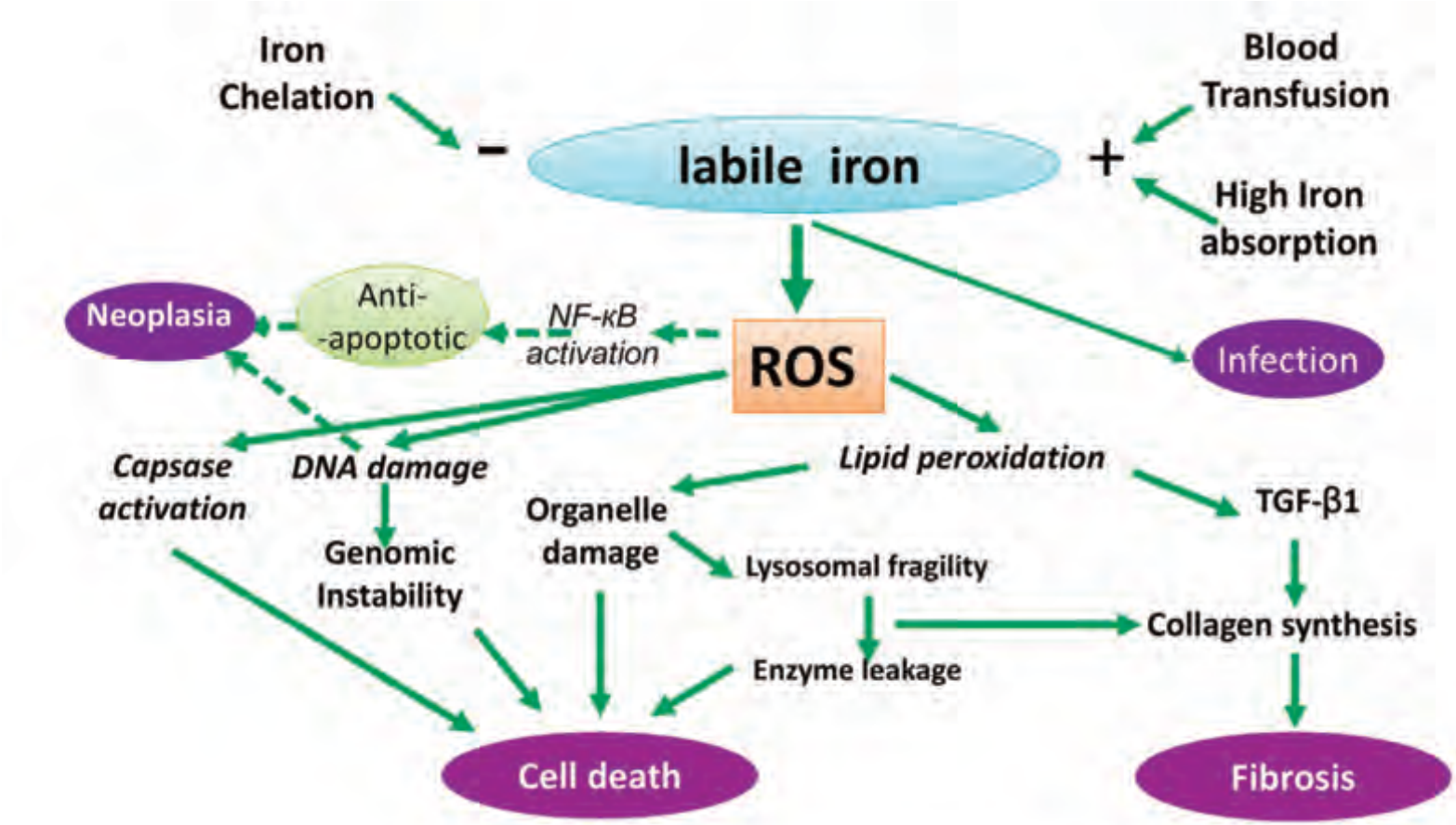
- Chronic transfusions ("hyper transfusion")
  - Every 2-4 weeks (goal Hb ≈ 9-10.5 g/dL)

    in patients with clinical thalassemia intermedia, (complex and involve patient and family preference)

    degree of anemia and bone changes related to ineffective erythropoiesis, degree of extramedullary hematopoiesis, and complications such as spinal cord compression, nonhealing leg ulcers, or progressive symptoms related to anemia.
  - Stress times: severe infections, surgery , or pregnancy
- limited extended phenotype of the recipient red cells, including

- Chelation therapy for iron overload or secondary hemochromatosis:
- serum ferritin levels , liver biopsy and quantitative iron measurement(magnetic resonance techniques) …….recommended annually.
- liver iron concentration,less than 1.5 mg/g dry weight,
- myocardial iron level measures as a T2* value, normally longer than 20 milliseconds.(not performed until the child is older than 10 years)
- Chelation will start when: serum ferritin of 1000 mcg/dL or received 15 transfusions
- Oral chelating agents; deferasirox and deferiprone
- Intravenous chelator; deferoxamine

- Started early, be uninterrupted, and continue lifelong


Iron
Chelation
–
labile iron
+
Blood
Transfusion
High Iron
absorption
Neoplasia
Anti-
-apoptotic
NF-κB
activation
ROS
Infection
Capsase
activation
DNA damage
Lipid peroxidation
TGF-β1
Organelle
damage
Genomic
Instability
Lysosomal fragility
Collagen synthesis
Enzyme leakage
Cell death
Fibrosis

Organ	Consequences
Pituitary	Hypogonadotropic Hypogonadism
Thyroid	Hypothyroidism
Parathyroid	Hypoparathyroidism
Heart	Cardiomyopathy
Liver	Cirrhosis, carcinoma
Pancreas	Diabetes

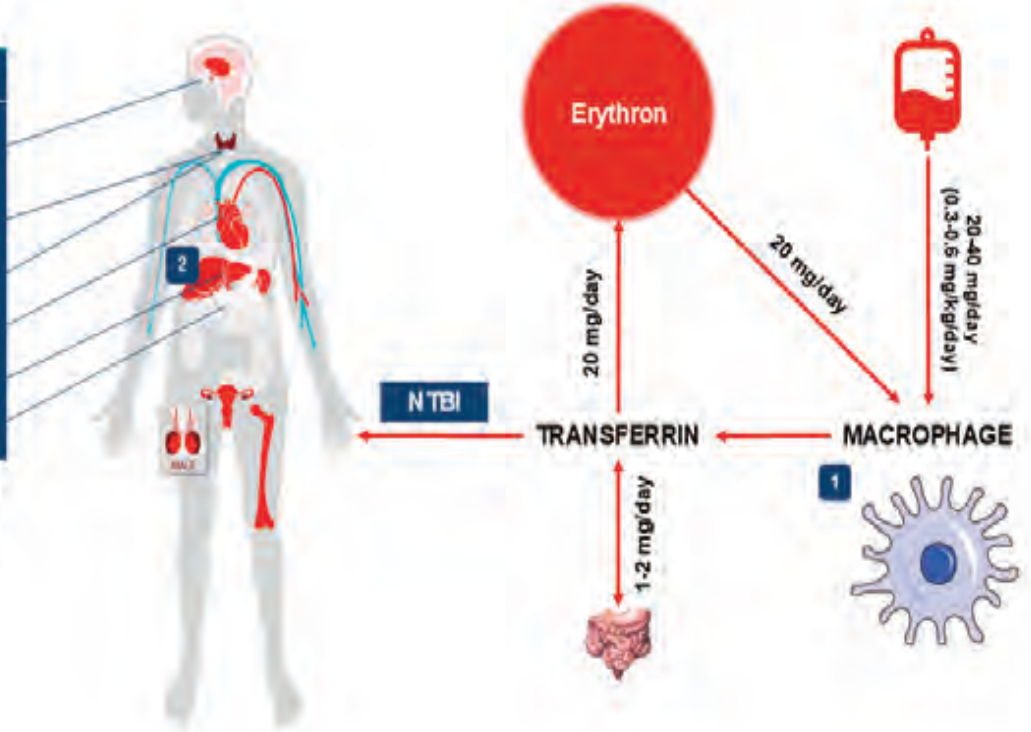


Table 5. *Licensed indications, dosing, contraindications, and interactions for iron chelators in transfusion-dependent β-thalassaemia [88-93].*

Parameter	DFO	DFP	DFX
Indication in TDT by age	First line in ≥2 years	**USA:** first line in ≥3 years (oral solution) or ≥8 years (tablets) **Europe:** second line when current chelation therapy is contraindicated or inadequate in ≥6 years	**USA:** first line in ≥2 years **Europe:** second line when DFO is contraindicated in 2-5 years, first line in ≥6 years
Administration	Parenteral (sc, IM, or IV)	Oral, tablets or oral solution	Oral, FCT or sprinkle granules (previously DT)
Dosage and frequency	20-60 mg/kg, 5-7 day/week (50 mg/kg in Europe), children's dose up to 40 mg/kg	75 -99 (USA) / 75-100 (Europe) mg/kg/day in 3 divided doses daily	14-28 mg/kg/day once daily for FCT and granules (20-40 mg/kg/day for DT)
Contraindications	• Pregnancy/breast feeding (no data, but has been used in 3rd trimester) • Hypersensitivity • Severe renal disease or anuria	• Pregnancy/breast feeding • History of agranulocytosis or recurrent neutropaenia • Hypersensitivity (Henoch-Schönlein purpura. Urticaria, and periorbital oedema with skin rash)	• Pregnancy/breast feeding • Hypersensitivity • Estimated GFR <40 mL/min/1.73 m^2 (USA) or creatinine clearance <60 mL/min (Europe) • Platelet count <50 x10^9/L (USA) • Severe hepatic impairment (Child-Pugh Class C)

System*	DFO	DFP	DFX
Vision	• Retinopathy, typically only at high absolute doses or with continuous IV infusions • Symptoms may include: night blindness, reduced colour vision, impaired visual fields, or reduced acuity • Eye exam (visual acuity tests, slit-lamp examinations, funduscopy) annually • Electroretinography may detect early changes before symptoms are obvious, recommended in continuous or high dose treatment	• Retinopathy not generally an issue • Isolated reports of loss of vision (central scotoma)	• Retinopathy not clearly related to DFX • Diffuse retinal pigmentation in one case report • Electroretinographic effects previously seen with DFO have not been described • Eye exam before initiating treatment and annually thereafter
Hearing	• Bilateral sensorineural hearing loss at high doses relative to iron overload • Tinnitus bilaterally in severe cases • Audiometry annually, especially if SF <1000 ng/mL	• One study reported continued audiometric deterioration after switching from DFO to DFP, significance uncertain	• Very rare, significance uncertain, not clearly related to DFX • Audiometry before initiating treatment and annually thereafter
Hepatic	• Generally, well tolerated by liver • Improvement of deranged liver enzymes in severe iron overload with DFO use • ALT before initiating treatment and monthly thereafter	• Raised ALT reports variable: rates 7.5% (of 642 cases) to 65% • Liver histology fibrosis reports variable: stability or fibrosis progression • ALT before initiating treatment and monthly thereafter • Interrupt therapy if persistent increase in ALT	• Low incidence of abnormal LFT in adults, in EPIC 0.6% of 1115 TDT patients showed AST >10x ULN • Improvement in liver pathology in 219 TDT patients on DFX for ≥3 years • Contraindicated in severe (Child–Pugh C) hepatic impairment • Reduce starting dose by 50% in patients with moderate (Child–Pugh B) hepatic impairment • LFT and bilirubin before initiating treatment, every 2 weeks during the first month and monthly thereafter • If there is a persistent and progressive increase in LFT that cannot be attributed to other causes, treatment should be interrupted

Parameter	DFO	DFP	DFX
Key drug-drug interactions	• Co-administration with prochlorperazine may lead to temporary impairment of consciousness • Imaging results may be distorted by rapid urinary excretion of deferoxamine-bound Gallium-67 • Discontinuation 48 hours prior to scintigraphy is advisable	• Theoretical interactions with UGT1A6 inhibitors (e.g., diclofenac, probenecid, or silymarin [milk thistle]) • Avoid concomitant use with drugs associated with neutropaenia • Gallium-67 as with DFO • With oral preparations containing polyvalent cations (e.g., aluminium containing antacids and zinc), allow at least a 4-hour interval	• Theoretical interactions with drugs metabolised by CYP3A4 (e.g., midazolam) • Theoretical interactions with drugs metabolised by CYP1A2 (e.g., theophylline) • Gallium-67 as with DFO • Oral preparations containing polyvalent cations as with DFP

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; TDT, transfusion-dependent β-thalassaemia; sc, subcutaneous; IM, intramuscular; IV, intravenous; FCT, film-coated tablets; DT, dispersible tablets; GFR, glomerular filtration rate.

SPLENECTOMY

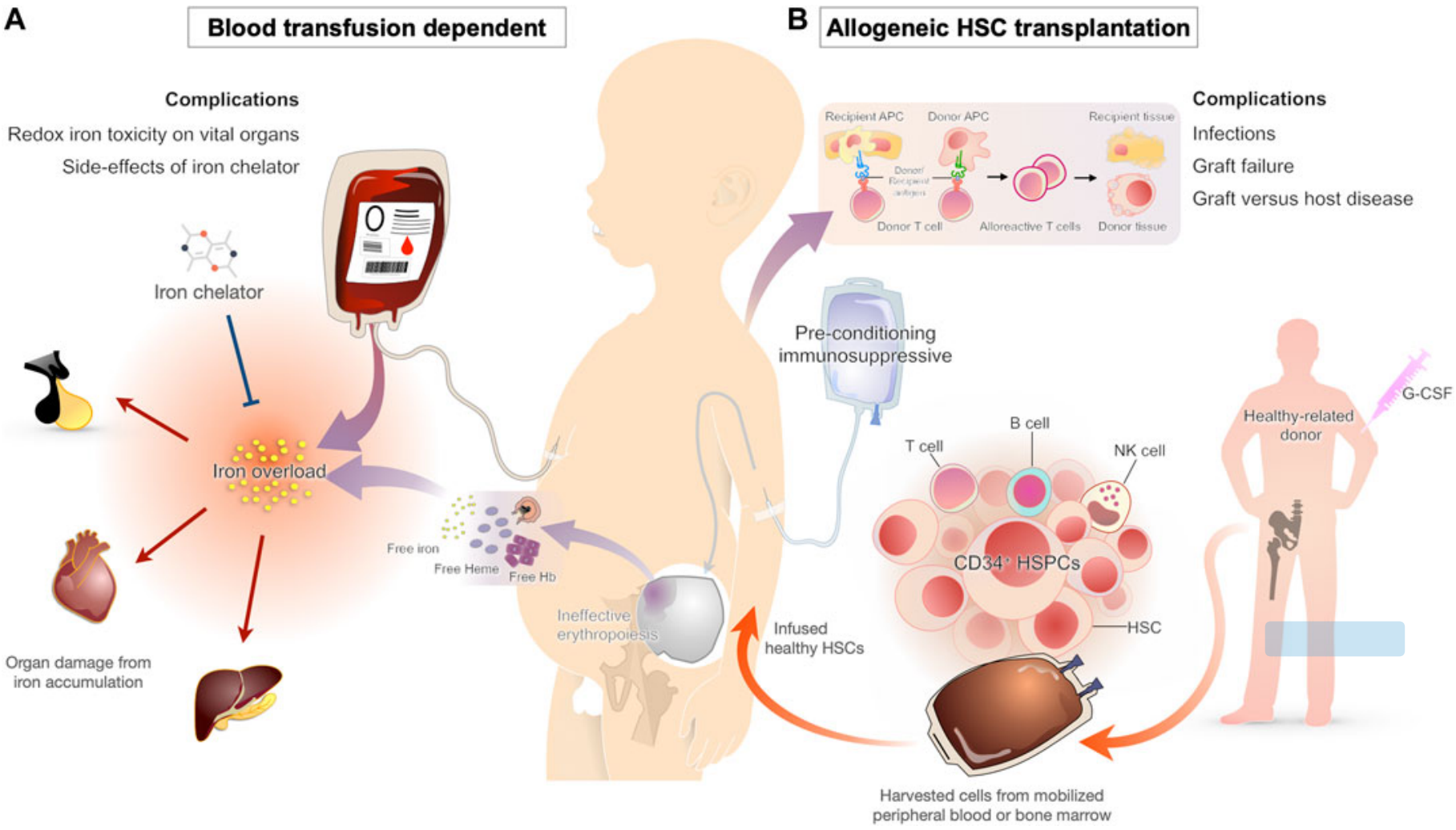
- Splenectomy : (TDT and NTDT)
- **To** minimize the risk of spontaneous or traumatic rupture of a large spleen ,the spleen was surgically removed.
- **Avoid** dropping Hb level,
- Compliation:
- Thrombocytosis/ increases risk of developing vascular disease/pulmonary hypertension/silent cerebral infarcts
- **Contraindication:**
- children younger than5 years ….. risk of pneumococcal infection
- Prophylaxis of vaccines /Empirical antibiotic

SCT for Thalassemia Major

- For patients with available donors
- Quality of life in successfully transplanted patients is better
- Transplantation from matched sibling donors is curative in >80%

- The best results are in the youngest patients who have been effectively chelated and received fewer transfusions

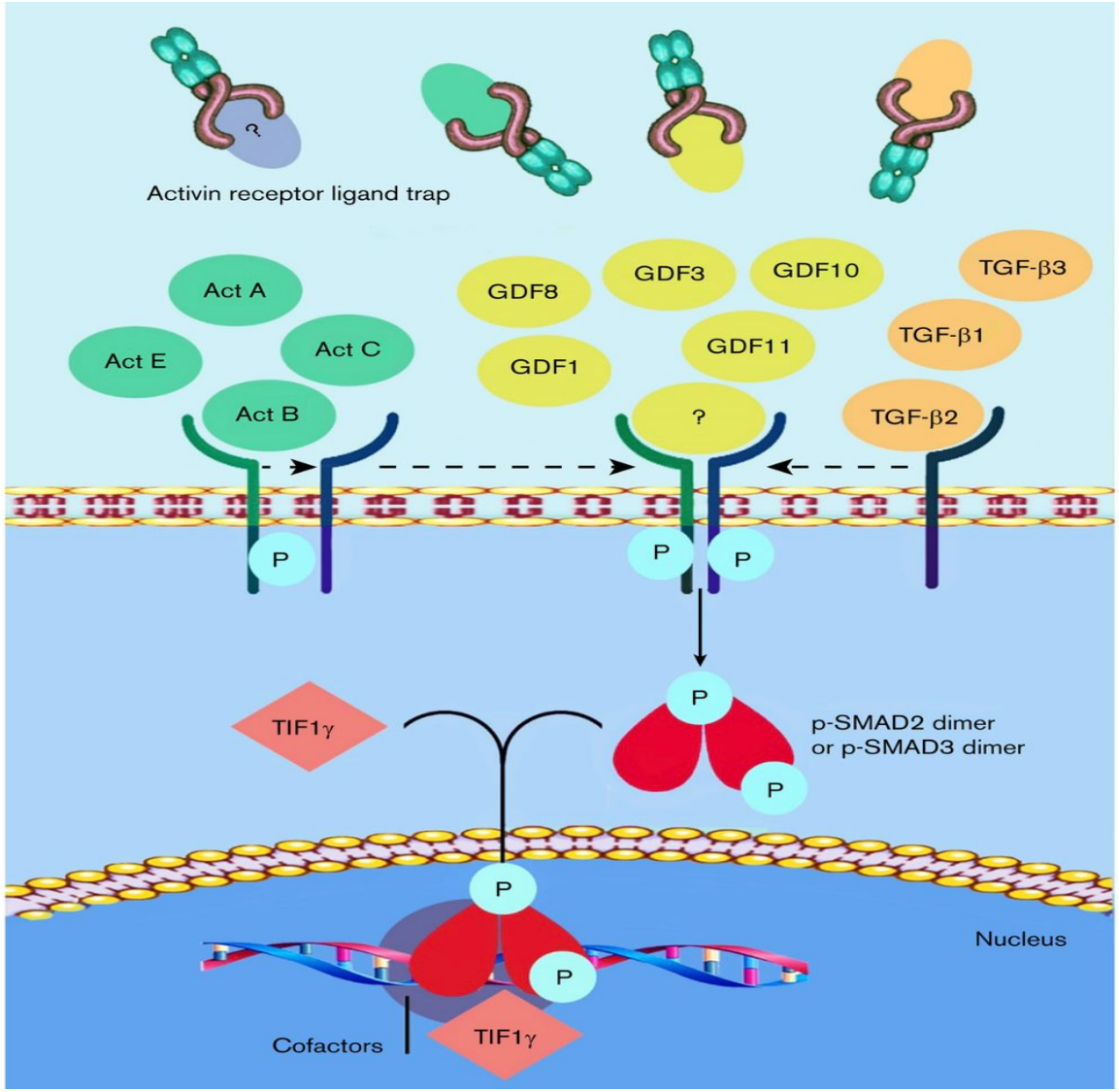
- Results of haploidentical and unrelated donor transplants are improving but lag those of matched sibling donors


A
Blood transfusion dependent
Complications
Redox iron toxicity on vital organs
Side-effects of iron chelator
Iron chelator
Iron overload
Organ damage from iron accumulation
Free iron
Free Heme
Free Hb
Ineffective erythropoiesis
B
Allogeneic HSC transplantation
Recipient APC
Donor APC
Recipient tissue
Donor T cell
Alloreactive T cells
Donor tissue
Complications
Infections
Graft failure
Graft versus host disease
Pre-conditioning immunosuppressive
T cell
B cell
NK cell
CD34+ HSPCs
HSC
Infused healthy HSCs
Healthy-related donor
G-CSF
Harvested cells from mobilized peripheral blood or bone marrow

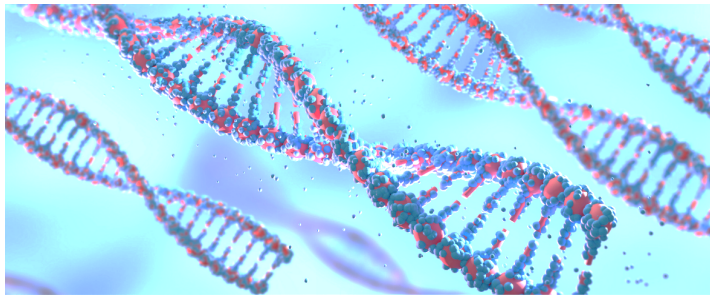
Luspatercept

- A fusion protein containing the extracellular domain of human activin type IIB receptor and the Fc domain of human IgG,
- Approved for treatment of transfusion-dependent thalassemia

- Enhances late-stage erythropoiesis
- Subcutaneously, 1 mg/kg every 3 weeks
- 33% reduction in transfusion requirements


Activin receptor ligand trap
Act A
Act C
Act E
Act B
GDF8
GDF3
GDF10
TGF-β3
GDF1
GDF11
TGF-β1
?
TGF-β2
P
P
P
P
TIF1γ
p-SMAD2 dimer
or p-SMAD3 dimer
P
P
P
Nucleus
Cofactors
TIF1γ

Gene therapy



- Lentiviral mediated gene therapy using autologous CD34+ hematopoietic stem cells for patients who lack a matched donor

- The initial results of CRISPR/Cas editing to downregulate BCL11A in β thalassemia have eliminated the need for transfusion and normalized hemoglobin levels

- (autologous CD34+ cells encoding the β A-T87Q-globin gene) **(Zynteglo)** to treat transfusion-dependent β+ thalassemia in patients 12 years and older, who are eligible for stem cell transplantation but do not have a matched related donor.(June

Other thalassemia syndromes

HbE-β thalassemia	50–80 (5–8)/60–70	HbE: 50–70 HbF: 30–50	Common in SE Asian populations; in some parts of the word, the most prevalent severe thalassemia; in HbE-β^0 thalassemia, only HbE and HbF are found; in HbE-β^+ thalassemia, HbA is present. Transfusion dependence depends in part on the thalassemia mutation.
δβ Thalassemia and hemoglobin Lepore	110–120 (11–12)/65–75	HbA: 70 HbF: 7–13 HbA_2: 2	Rare; deletions removing the δ- and β-globin genes cause δβ thalassemia; Lepore hemoglobins are fusion globin chains; values are for heterozygotes; homozygotes have 100% HbF with hemoglobin 10–11 g/dL.
Gene deletion HPFH	120–140 (12–14)/75–85	HbA: 70 HbF: 15–30 HbA_2: 2	Rare; large deletions removing the δ- and β-globin genes; values are for heterozygotes; homozygotes, who are asymptomatic, have 100% HbF without anemia.

Other hemoglobinopathies

TABLE 98-7 HbC, HbE, and Rare Hemoglobinopathies

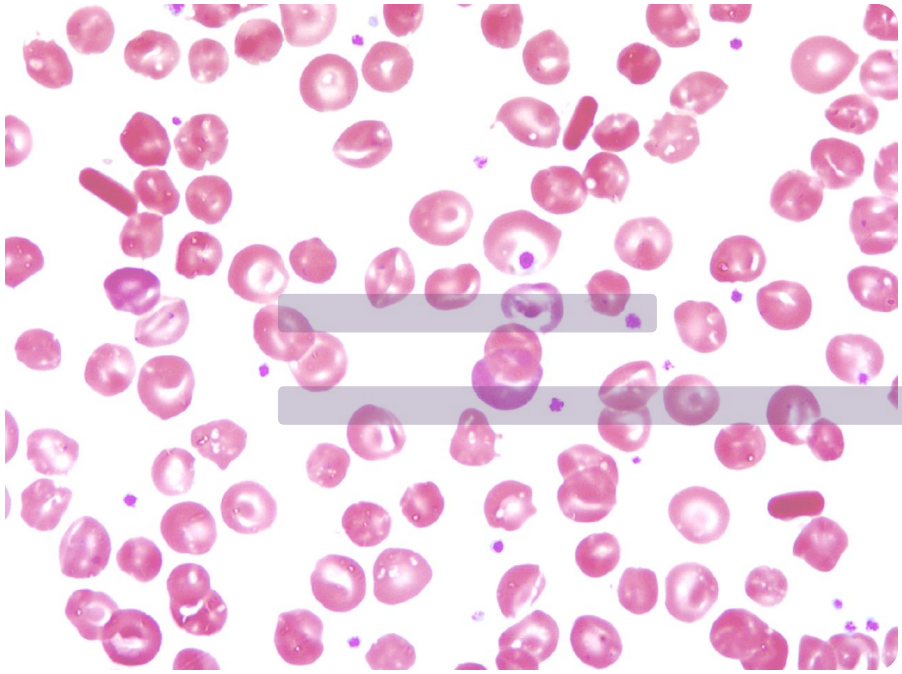
CLASSIFICATION	CLINICAL ABNORMALITIES	HEMOGLOBIN LEVEL, g/L (g/dL)/MCV, fL	HEMOGLOBIN FRACTIONS (%)
HbC trait	2% of African Americans; target cells; no disease	Normal	HbC: 30–40 HbA_2: 2–3
HbC disease	Target cells; HbC crystals; mild reticulocytosis; splenomegaly	100–130 (10–13)/60–70	HbC: >95 HbF: 2–4 HbA_2: 2–3
HbE trait	50% incidence in some Asian populations; a few target cells; clinically normal	120–140 (12–14)/80–90	HbE: 27–31[b] HbF: 1 HbA_2: 3
HbE disease	No hemolysis; 20–80% target cells; no splenomegaly	100–120 (10–12)/65–75	HbE: 85–95 HbF: 3–7 HbA_2: 3
High O_2 affinity hemoglobins	Isolated erythrocytosis; often familial; no splenomegaly; no *JAK2*^{V617F} mutation	150–200 (15–20)	Variants in α- and β-globin genes; patients are heterozygotes; ~25–50% variant
Low O_2 affinity hemoglobins	Asymptomatic mild anemia; cyanosis	100–140 (10–14)	~50% variant
Unstable hemoglobins	Pigmenturia; hemolysis; reticulocytosis; splenomegaly	90–140 (9–14)/70–90	20–35% variant; rare hyperunstable variants can be undetectable and have the phenotype of thalassemia
M hemoglobins	Some have mild hemolysis; few symptoms	100–140 (10–14)/80–90	20–50% variant depending on gene affected

mild

mild

Hb C Disease

Hb C crystals



Questions