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Hematology

Fast Facts

Thalassemia Syndromes

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HEALTHCARE

Fast Facts for Healthcare Professionals

Hematology

Thalassemia Syndromes

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Glossary and list of abbreviations

ASO: antisense oligonucleotides

ATP: adenosine triphosphate

CRISPR-Cas: clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases; enzymes used in gene editing

FDA: Food and Drug Administration

GDF11: growth and differentiation factor 11; a regulator of the maturation of reticulocytes and the formation of red blood cells

Hb: hemoglobin

HbA: a hemoglobin heterodimer comprising two α-globin chains and two β-globin chains

HbA_2: a hemoglobin heterodimer comprising two α-globin chains and two δ-globin chains

Hb Barts: a hemoglobin tetramer comprising four γ-globin chains; detection of Hb Barts during newborn screening is suggestive of α-thalassemia

HbCS: hemoglobin Constant Spring; a form of hemoglobin resulting from a mutated stop codon in an α-globin gene, leading to an elongated α chain

HbE: a structural variant of β hemoglobin

HbF: a hemoglobin heterodimer comprising two α-globin chains and two γ-globin chains; this form of hemoglobin predominates during fetal life and shortly after birth, but then declines

Hb Gower 1: a form of hemoglobin produced during embryonic development comprising two ζ chains and two ε chains

Hb Gower 2: a form of hemoglobin produced during embryonic development comprising two α chains and two ε chains

HbH: a form of hemoglobin formed by tetramers of β globins

HbH disease: a form of α-thalassemia resulting from inactivation (following mutation or deletion) of three α-globin genes leading to an excess of β-globin chains and the formation of HbH. There are two forms of HbH disease: deletional HbH disease, in which three α-globin genes are deleted; and non-deletional, in which two α-globin genes are deleted and one is affected by a mutation, such as Constant Spring

Hb Portland: a form of hemoglobin produced during embryonic development comprising two ζ chains and two γ chains

HPFH: hereditary persistence of fetal hemoglobin; a benign condition in which significant production of HbF continues postnatally rather than declining after birth

HSCT: hematopoietic stem cell transplantation

IE: ineffective erythropoiesis

KLF1: erythroid Kruppel-like factor; a transcription factor that controls the switch from fetal to postnatal ('adult') hemoglobin production by activating human β-globin gene expression and the *BCL11A* gene. KLF1 is a key repressor of the γ-globin gene

LIC: liver iron concentration

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acid

NTDT: non-transfusion-dependent thalassemia, also called thalassemia intermedia

PK: pyruvate kinase

RBC: red blood cell

siRNA: small interfering RNA

TALENS: transcription activator-like effector nucleases

TDT: transfusion-dependent thalassemia, also called thalassemia major

TGF-β: transforming growth factor-β

TMPRSS6: transmembrane serine protease 6

ZFN: zinc finger nucleases; enzymes used in gene editing

Introduction

The thalassemia syndromes are a heterogeneous group of inherited disorders with the common underlying theme of disordered production of hemoglobin (Hb). The spectrum of disease is broad, with the most severe transfusion-dependent forms imposing a significant burden on affected individuals and healthcare systems. The impact on patients with non-transfusion-dependent thalassemia can also be substantial.

The management of thalassemia is complex and requires expertise from a multidisciplinary team of healthcare professionals. Blood transfusions, either lifelong or occasional, remain the central therapeutic approach. Survival is improving, thanks to better monitoring and management of the iron overload associated with both transfusion-dependent and non-transfusion-dependent forms of the disease.

Better understanding of the pathophysiology underlying the thalassemia syndromes has led to the identification of potential targets for new therapies and the development of novel treatment strategies that aim to reduce or abolish the requirement for transfusion, improve Hb levels and prevent or reduce iron overload.

Fast Facts: Thalassemia Syndromes provides a concise, comprehensive introduction to the thalassemia syndromes and current approaches to treating them and their associated morbidities. It also offers an insight into some of the novel therapies that are currently in clinical trials or that have recently been approved (see Chapter 5), which may have the potential to transform the lives of patients with thalassemia. We hope that readers will find this resource both interesting and useful.

Hematology

1 What are the thalassemia syndromes?

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The thalassemia syndromes are inherited quantitative disorders of hemoglobin (Hb) that result in a wide spectrum of disease, ranging from a mild asymptomatic carrier state to a severe transfusion-dependent form. At their most severe, the syndromes impose a high burden of disease, with myriad complications, resulting in significant morbidity and a potential reduction in life expectancy.

Thalassemia was first described in 1925 by Cooley and Lee in patients with severe anemia, splenomegaly and bone disease, and was initially named 'Cooley's anemia', likely describing beta-thalassemia.[1] 'Thalassemia' is a Greek term that roughly translates as 'the sea in the blood'. The sea referred to is the Mediterranean and the term was coined because this anemia was originally most frequently seen in people from the Greek and Italian coasts and nearby islands. The term now refers broadly to a heterogeneous group of disorders with the common theme of disordered globin chain biosynthesis.

Over the past century our understanding of the inheritance and pathophysiology of the thalassemia syndromes has grown considerably, and we now realize that there is much heterogeneity in their clinical manifestations, though the underlying basis of the disorders is very similar. The type of syndrome depends on which globin chain is affected, with alpha- (α-) and beta- (β-) thalassemias being the most common; gamma- (γ-), delta- (δ-) and other thalassemias are much less common.

Epidemiology

The thalassemias are some of the most common genetic disorders worldwide.[2] They occur across the globe, in almost all ethnic groups, but are most common around the Mediterranean and in tropical and subtropical areas of Asia and Africa. The so-called 'thalassemia belt' extends from the Mediterranean, through the Arabian peninsula, Iraq, Iran, the Indian subcontinent and southeast Asia, to the Pacific coast of China (Figure 1.1).[3] Both sexes are equally affected.

Precise incidence and prevalence data are not available, but it is estimated that approximately 1.5% of the world's population carries a single β-thalassemia mutation, amounting to 80–90 million individuals, most of whom live in Asia.[4] Around 46 000 individuals

are born each year with both β-globin genes affected with thalassemia mutations, and approximately half of these individuals will be transfusion dependent.[5] It is estimated that 20% of people in Asia, mostly southeast Asia and China, and approximately 5% of the African population are carriers for α-thalassemia, and around 1 million individuals globally have some form of α-thalassemia. Widespread migration over the past 100 years or so has ensured that the thalassemias are now prevalent globally, though β-thalassemia is still not particularly prevalent in most of sub-Saharan Africa.[6–9]

Like sickle cell anemia, thalassemia is most common in areas where malaria has been endemic. Thalassemia gene frequency is high and fixed in populations that are chronically exposed to malaria. It is thought that individuals who are heterozygous for thalassemia have a selective survival advantage in malaria-endemic areas, experiencing milder infections and with malaria having less impact on reproductive fitness.[10,11]

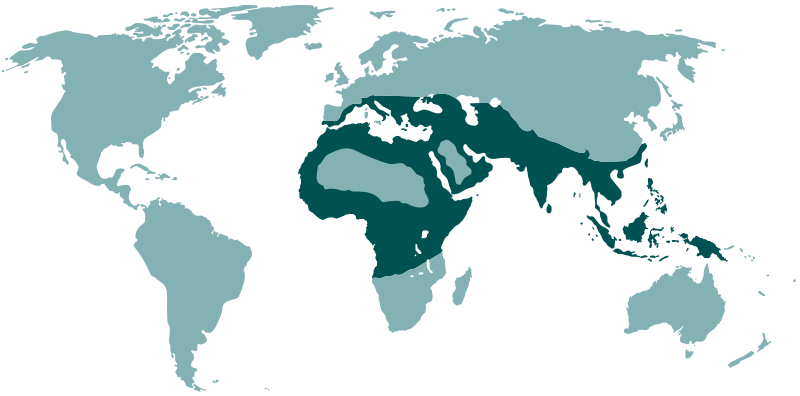

Figure 1.1 Regions of the world where thalassemia is endemic. Adapted from Weatherall 1997.[3]

Key points – what are the thalassemia syndromes?

- The thalassemia syndromes are a heterogeneous group of inherited disorders of Hb with the common theme of disordered globin chain biosynthesis.
- Thalassemias are most prevalent in Asia, but migration over the past 100 years has resulted in worldwide occurrence.
- α- and β-thalassemias are the most common thalassemia syndromes; γ-, δ- and other thalassemias are much less common.
- Thalassemia gene frequency is high and fixed in populations that are chronically exposed to malaria and individuals who are heterozygous for thalassemia appear to have a selective survival advantage in malaria-endemic areas.

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Hematology

2 Molecular understanding and classification

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Molecular basis

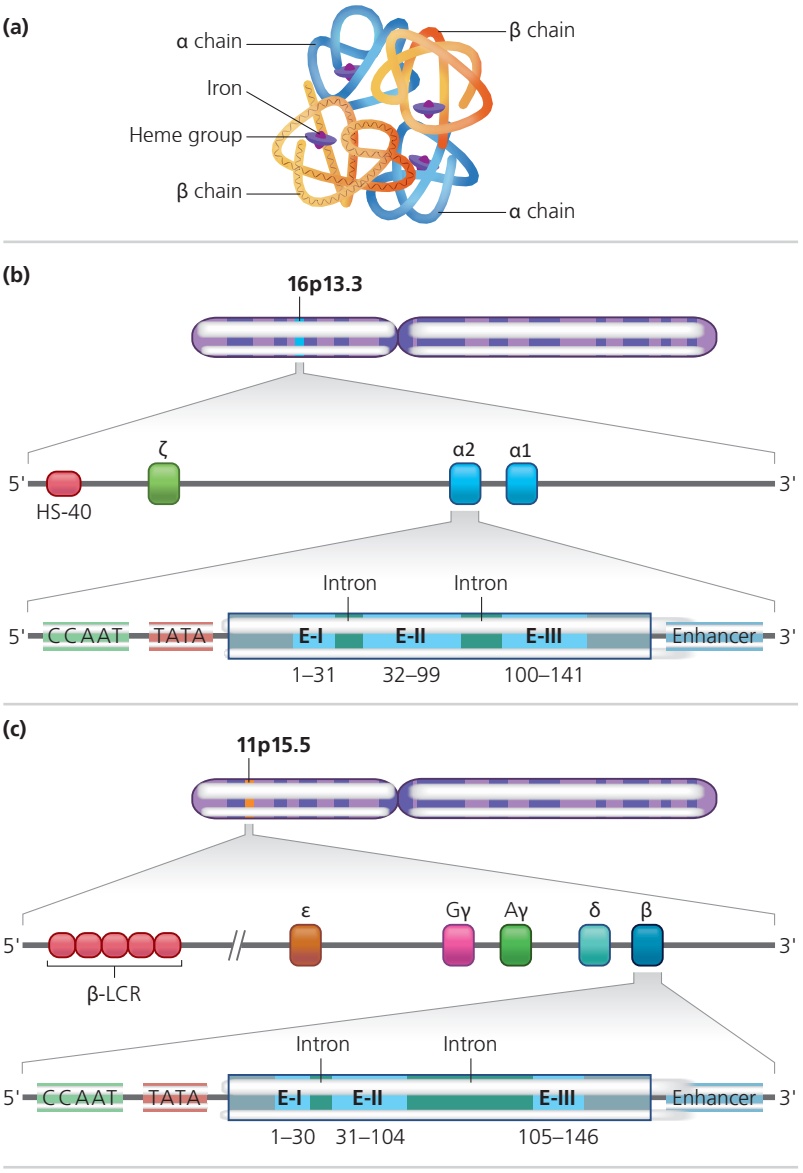
The Hb molecule is a heterodimer comprising four globins (heme-containing globular proteins): two globins are synthesized as a result of the expression of genes from the α-globin gene cluster on chromosome 16 and two globins are produced as a result of the expression of genes from the β-globin gene cluster on chromosome 11 (Figure 2.1).[1]

The α-globin gene cluster consists of one functional zeta (ζ) gene and two α genes (α_2 and α_1) (see Figure 2.1b). The exons of both α genes are the same but they differ in the second intron. The amount of mRNA produced by α_2 expression is 1.5–3 times greater than that produced by α_1 expression. The β-globin gene cluster consists of one functional ε gene, a $^{G}\gamma$ gene, an $^{A}\gamma$ gene (position 136 in some γ-globin chains is occupied by glycine and in others by alanine; these are designated $^{G}\gamma$ and $^{A}\gamma$, respectively), a δ gene and a β gene (see Figure 2.1c).

Expression of the globin genes is regulated by complex control mechanisms involving the interaction of upstream control sequences in each globin gene cluster with an immediate upstream local gene promoter.

All normal adult human Hb molecules have one pair of α-globin chains. The two α-globin chains can combine with two β-globin chains ($\alpha_2\beta_2$) to form HbA (see Figure 2.1a), two δ-globin chains ($\alpha_2\delta_2$) to form HbA_2, or two γ-globin chains ($\alpha_2\gamma_2$) to form HbF. In adults, approximately 97% of Hb is HbA, less than 3.5% is HbA_2, and less than 2% is HbF.[1]

Figure 2.1 (a) All Hb molecules comprise four globin chains: two α globins and two other globins that vary depending on the type of Hb molecule. (b) The two α globins are synthesized from the α-globin gene cluster, which is located on the short arm of chromosome 16 (16p13.3) and includes three protein-coding genes (α_1, α_2 and ζ). The α_1 and α_2 genes are highly homologous, only differing in the length of the second intron (149 nucleotides in α_1 versus 142 nucleotides in α_2). Upstream of the ζ gene, HS-40 is the major regulatory element of the α-globin locus. (c) The other two globins are synthesized from the β-globin gene cluster, which is located on the short arm of chromosome 11 (11p15.5) and includes five protein-coding genes (ε, $^{G}\gamma$, $^{A}\gamma$, δ and β). Upstream of the ε gene, the β-globin locus control region (β-LCR) is the main regulatory element of the β-globin locus.


(a)
α chain
β chain
Iron
Heme group
β chain
α chain
(b)
16p13.3
ζ
α2
α1
5'
3'
HS-40
Intron
Intron
5'
CCAAT
TATA
E-I
E-II
E-III
Enhancer
3'
1–31
32–99
100–141
(c)
11p15.5
ε
Gγ
Aγ
δ
β
5'
3'
β-LCR
Intron
Intron
5'
CCAAT
TATA
E-I
E-II
E-III
Enhancer
3'
1–30
31–104
105–146

Developmental aspects

Different forms of Hb are produced at different stages of development (Figure 2.2).[2,3] Three types of Hb are produced during embryonic stages: Hb Gower 1 ($\zeta_2\varepsilon_2$), Hb Gower 2 ($\alpha_2\varepsilon_2$) and Hb Portland ($\zeta_2\gamma_2$). HbF ($\alpha_2\gamma_2$) predominates during fetal development, as globin gene expression switches from ζ to α and from ε to γ. γ Globin is produced at high levels early in fetal development but starts to decline at

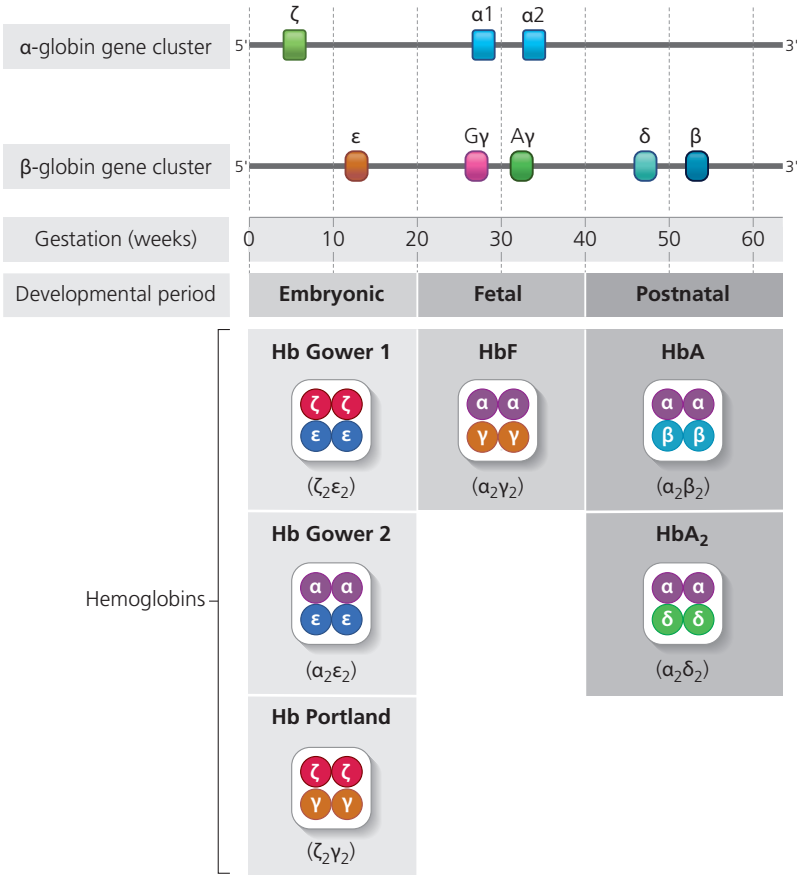


Figure 2.2 Changes in the types of Hb produced over the course of embryonic and fetal development through to postnatal forms of Hb. Adapted from Hofmann et al. 1995 and Nathan and Oski 1992.[2,3]

around 36 weeks of gestation. Shortly after birth, γ-gene expression switches to β- and δ-gene expression, resulting in the formation of HbA ($\alpha_2\beta_2$) and HbA_2 ($\alpha_2\delta_2$).[4]

At full term, production of β globin and γ globin is approximately equal, but Hb composition is 80–90% HbF and 10–20% HbA. By 1 year of age, γ-globin production is less than 1% of total non-α-globin production. The switch from fetal Hb production to postnatal ('adult') forms of Hb is controlled by erythroid Kruppel-like factor (KLF1), a transcription factor that activates both human β-globin gene expression and the *BCL11A* gene. KLF1 is a key repressor of the γ-globin gene.[4]

Genotypes

Approximately 350 thalassemia mutations have been described across all of the globin genes. The thalassemia syndromes are inherited in an autosomal recessive manner and both sexes are affected equally. The genotypes of the common and some less common thalassemia syndromes are summarized in Table 2.1 and discussed in more detail below.[5]

α-Thalassemias are usually caused by the deletion of one or more of the four α genes (two α genes per α-globin gene cluster on each haploid chromosome).[6] If one of the two α loci on chromosome 16 is deleted, the thalassemia is designated α–. If both are deleted, the designation –– is used. A patient with two α-locus deletions has α-thalassemia trait, which is designated α–/α– (*trans*) or αα/–– (*cis*) depending on the arrangement of the deletions on the chromosomes.

α-Thalassemias also arise via a variety of other mechanisms, such as an elongated α chain resulting from a mutated stop codon (this produces a variant of Hb known as Hb Constant Spring [HbCS]) or missense or nonsense mutations.

HbH disease occurs when three of the four α genes are affected. With only one normal α gene, the synthesis of α-globin chains is markedly reduced and tetramers of β globin called HbH form. The genetic abnormalities can be deletional (α–/– –) or non-deletional (for example, $\alpha\alpha^{CS}$/––).

TABLE 2.1

Genotypes of common and less common thalassemia syndromes

Thalassemia syndrome	
Deletional α-thalassemia	Non-deletional α-thalassemia
• αα/α–	• e.g. $\alpha\alpha^{CS}$/––
• α–/α– or αα/––	
• α–/––	
• ––/––	
β-Thalassemias	
• β^0/β^0	
• β^0/β^+	
• β^+/β^+	
• Compound heterozygote	
– HbE/β^0 or HbE/β^+	
• β/β^+ or β/β^0	
δβ-Thalassemia	δ-Thalassemia
• $(\delta\beta)^+$	• δ^0
• $(\delta\beta)^0$	• δ^+
• $(^A\gamma\delta\beta)^0$	
Hereditary persistence of fetal hemoglobin	
Deletional	Non-deletional
• $(\delta\beta)^0$ or $(^A\gamma\delta\beta)^0$	• Linked to β-globin genes
	– $(^G\gamma\beta^+)$ or $(^A\gamma\beta^+)$
	• Unlinked to β-globin genes

β^0, no expression; β^+, some expression; cs, Constant Spring; HbE, a structural variant of β hemoglobin.
Adapted from Sheth and Thein 2021.[5]

If all four genes are deleted (––/––), this gives rise to α-thalassemia major and results in hydrops fetalis since neither HbF nor HbA can be formed.

β-Thalassemias. The worldwide distribution of the different β-thalassemia mutations is variable and mutations can be traced back

to their origin based on ethnicity and genetic background. There are two main types of β-thalassemia mutation:[7]

- mutations leading to a total absence of β-globin production (β^0-thalassemia)
- mutations leading to a partial deficiency of β-globin production (β^+-thalassemia).

Broadly, β-thalassemias are classified as β^0/β^0 or non-β^0/β^0.

The clinical manifestations of β-thalassemias vary based on the amount of β globin produced, which alters the ratio of α to β globins and thus the efficacy of erythropoiesis (see Chapter 3) and the amount of HbA produced.

The hallmark of the common forms of β-thalassemia is an increased proportion of HbA_2 and often HbF as a percentage of total Hb. This is seen in heterozygotes as well, and makes diagnosis easier. Compound heterozygotes with mutations for both thalassemia and HbE (a structural variant of β globin) also have manifestations of β-thalassemia.

Silent mutations have been described in the β-globin gene wherein heterozygotes have a completely normal hematologic picture and no increase in HbA_2 or HbF. When inherited along with another β-thalassemia mutation, this may result in a clinically severe β-thalassemia syndrome. Dominant β-thalassemia mutations have also been described, though these are rare.[8]

δβ-Thalassemias are heterogeneous. In some cases, no δ or β globin is produced; in others, the non-α chains are fusion δβ globins with the N-terminal residue of the δ chain fused to C-terminal residues of the β chain. Fusion variants are called lepore hemoglobins. Levels of HbF are elevated in individuals with δβ-thalassemias, but levels of HbA_2 are not.[9]

Hereditary persistence of fetal hemoglobin (HPFH) is a genetically heterogeneous condition with deletional and non-deletional forms. It is characterized by the persistence of HbF in adult life. As in δβ-thalassemias, HbF levels are elevated but HbA_2 levels are not.

Genotype–phenotype correlation

Overall, there is some (but not perfect) correlation between the severity of the genotype and the clinical manifestations of each thalassemia syndrome. The correlations for α- and β-thalassemia are summarized in Table 2.2.

Genetic modifiers of severity

Co-inheritance of α-globin mutations may mitigate the severity of β-thalassemia and vice versa, by making the α- to β-globin ratio more balanced. Similarly, persistence of HbF may ameliorate ineffective erythropoiesis (IE) (see Chapter 3) and reduce anemia, resulting in a milder form of β-thalassemia. Concomitant mutations of other genes such as those for Gilbert's syndrome or hereditary hemochromatosis may worsen clinical disease by exacerbating hepatic disease and iron overload, respectively.[10]

TABLE 2.2

Correlation between genotype and clinical presentation of α- and β-thalassemia

Genotype	Phenotype	Clinical presentation
α-Globin gene abnormalities		
αα/α–	α-Thalassemia trait/silent carrier	Asymptomatic
α–/α– (*trans*) or αα/–– (*cis*)	α-Thalassemia trait	Mild anemia
α–/––or $\alpha\alpha^{CS}$ /– –	HbH disease or HbH CS	Anemia, hemolysis
––/––	α-Thalassemia major	Hydrops fetalis
β-Globin gene abnormalities		
β/β^0 or β/β^+	β-Thalassemia trait (minor)	Asymptomatic
β^+/β^+ or β^0/β^+ HbE/β^0 or HbE/β^+	β-Thalassemia intermedia	Mild/moderate anemia
β^0/β^0 or β^0/β^+	β-Thalassemia major	Severe anemia

Key points – molecular understanding and classification

- The α-globin gene cluster is located on chromosome 16 and comprises one functional ζ gene and two α genes (α_2 and α_1).
- The β-globin gene cluster is located on chromosome 11 and comprises one functional ε gene, a $^{G}\gamma$ gene, an $^{A}\gamma$ gene, a δ gene and a β gene.
- Normal adult human Hb is a heterodimer comprising two α-globin chains and two other globin chains produced as a result of the expression of genes in the β-globin gene cluster.
- The predominant form of Hb in adult humans is HbA ($\alpha_2\beta_2$), which comprises up to 97% of Hb.
- The thalassemia syndromes are inherited in an autosomal recessive manner.
- α-Thalassemias are usually caused by the deletion of one or more of the four α genes (two α genes per haploid chromosome).
- β-Thalassemia mutations result in either reduced production of β globin (β^+-thalassemia) or no β-globin production (β^0-thalassemia).
- The clinical manifestations of disease vary, based on the number and types of mutations present.

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Hematology

3 Pathophysiology and disease manifestations

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Normal erythropoiesis

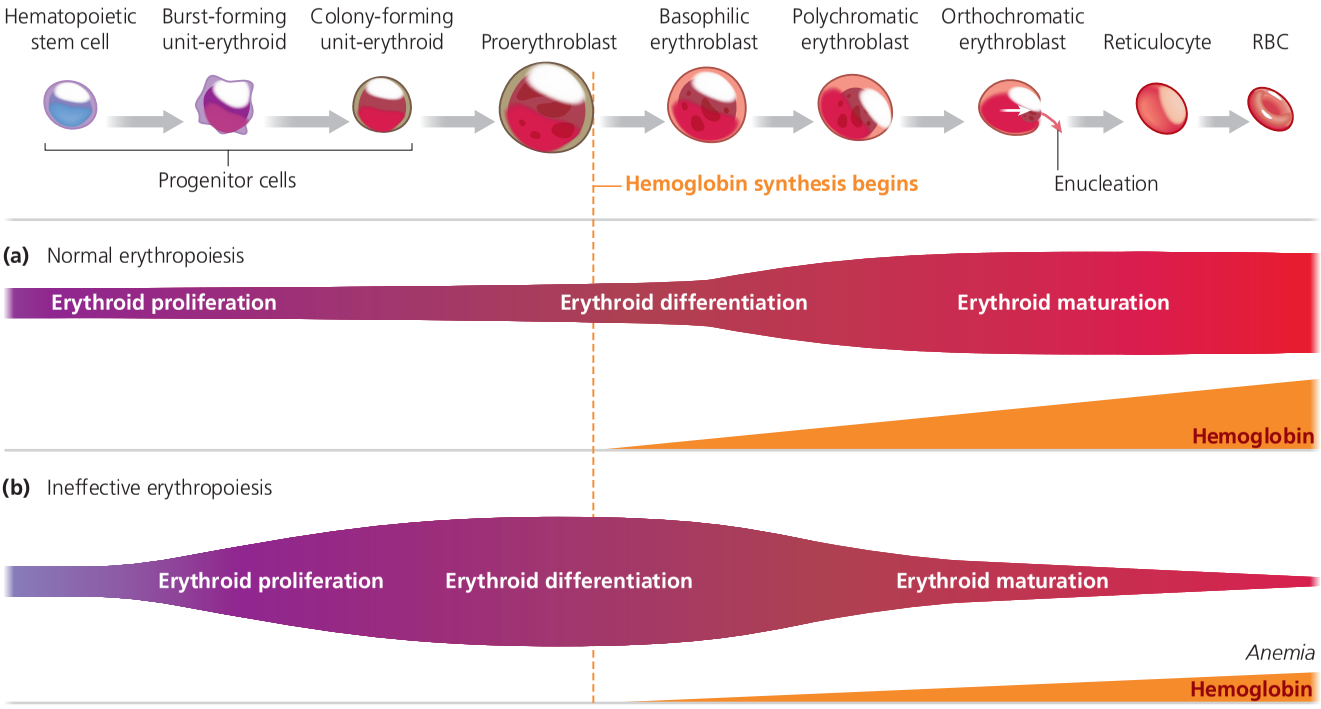
Erythropoiesis occurs in two main phases, the proliferative phase and the differentiation and maturation phase (Figure 3.1). During the proliferative phase hematopoietic stem cells, under the primary influence of erythropoietin, become burst-forming and colony-forming units, which are progenitor cells committed solely to the erythroid lineage, and eventually form a vast number of proerythroblasts. The proerythroblasts then go through a series of differentiation and maturation steps, which include the formation of Hb, a progressive decrease in cell size and the eventual extrusion of the nucleus, to form reticulocytes. Further maturation of the reticulocytes results in the formation of red blood cells (RBCs) which are released into the circulation. Erythropoiesis is regulated by ligands belonging to the transforming growth factor-β (TGF-β) family, primarily growth and differentiation factor 11 (GDF11).

Pathophysiology

The pathophysiological basis of disease in the thalassemias is the underlying imbalance between α- and β-globin production.[1–4] In α-thalassemia, there is an excess of β globin, whereas in β-thalassemia, there is an excess of α globin. The differences between these conditions arise mainly because of the different effects of tetramers of α globin and β globin.

Ineffective erythropoiesis is the hallmark of β-thalassemia.[5,6] α-Globin tetramers formed in β-thalassemia precipitate immediately in the developing erythroid precursors in the bone marrow. This leads to the formation of hemichromes along with iron, which cause oxidative damage to the precursor cells, leading to premature apoptosis (Figure 3.2).[7] As a result, fewer RBCs can be released and those that

Figure 3.1 (a) Normal versus (b) ineffective erythropoiesis.


Hematopoietic stem cell
Burst-forming unit-erythroid
Colony-forming unit-erythroid
Proerythroblast
Basophilic erythroblast
Polychromatic erythroblast
Orthochromatic erythroblast
Reticulocyte
RBC
Progenitor cells
Hemoglobin synthesis begins
Enucleation
(a) Normal erythropoiesis
Erythroid proliferation
Erythroid differentiation
Erythroid maturation
Hemoglobin
(b) Ineffective erythropoiesis
Erythroid proliferation
Erythroid differentiation
Erythroid maturation
Anemia
Hemoglobin

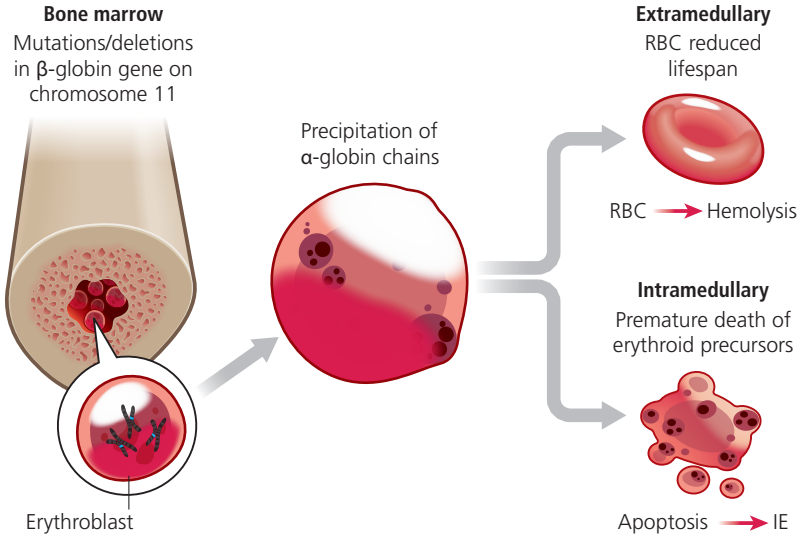


Figure 3.2 Pathophysiology of β-thalassemia.

are released are significantly abnormal, with a reduced lifespan in the circulation.

When erythropoiesis is ineffective, the resulting anemia leads to increased erythropoietin release from the kidneys. The erythropoietin stimulates the proliferative phase, but because Hb cannot be formed the precursors do not mature normally, leading to developmental arrest at the proerythroblast stage followed by apoptosis (see Figure 3.1b). As a result, the bone marrow shows hyperplasia of the erythroid lineage, with maturational arrest, and accumulation of TGF-β ligands.

IE not only leads to expansion of the bone marrow, but also to extramedullary hematopoiesis, with the proliferation of erythroid precursors in the liver and spleen. This causes enlargement of the organs, as well as the formation of nodules of erythropoietic tissue extruding from vertebral bodies.

IE also leads to dysregulation of iron metabolism.[8,9] Increased production of the hormone erythroferrone in turn suppresses

hepcidin production. Low levels of hepcidin lead to increased gastrointestinal absorption of iron and increases in circulating iron, which deposits in organs and can cause toxicity and organ dysfunction.

In contrast, in α-thalassemia (excluding α-thalassemia major in which all four α-globin genes are deleted), tetramers of β globin form HbH. HbH is soluble and does not precipitate in the precursor cells, so it is present in RBCs as they mature and are released into the circulation. In deletional HbH disease, where three α-globin genes are deleted, IE is not a prominent feature. However, in non-deletional HbH disease, such as HbH Constant Spring, where two α-globin genes are deleted and one is mutated with a Constant Spring mutation, unstable α globin is formed, which precipitates in the precursor cells and causes apoptosis, leading to more prominent IE.[10,11]

Fetal health and newborn screening

Fetal health. When all four α-globin genes are affected, the developing fetus becomes progressively more anemic because HbF ($\alpha_2\gamma_2$) cannot be formed. This leads to hydrops fetalis, which is characterized by heart failure, anasarca and hepatosplenomegaly, ultimately resulting in fetal demise unless intrauterine blood transfusions are initiated. All other α- and β-thalassemias are associated with relatively uncomplicated pregnancies and live births, with varying degrees of anemia at birth.

Newborn screening. Where available, newborn screening will identify infants with some of the thalassemia syndromes.[11] A healthy newborn screen after birth usually detects 80–90% HbF and 10–20% HbA. The detection of hemoglobin Barts (Hb Barts), a γ-globin tetramer (γ_4) that can be present in varying proportions, suggests α-thalassemia. The level of Hb Barts increases as the number of functional genes declines. Infants with β^0/β^0-thalassemia will show a complete absence of HbA, and those with β^+-thalassemia will have decreased HbA.[12]

Postnatal disease manifestations

Spectrum of disease severity. Postnatally, disease manifestations occur at different rates depending on the severity of the disease – that is, the degree of imbalance between the amounts of globin chains produced. At one end of a wide spectrum of disease there are individuals with a thalassemia trait, who are generally asymptomatic and have a mild microcytic anemia without the consequences of IE. They have a normal life expectancy. At the other end of the spectrum there are individuals with severe anemia who require regular blood transfusions early in life (or before birth in the case of α-thalassemia major) for survival. Between these two extremes is a disease of intermediate severity, where occasional transfusions may be required for severe anemia.[13] HbH disease, for example, has a variable presentation and an intermediate phenotype, but is rarely transfusion dependent. Overall, individuals are broadly classified as having non-transfusion-dependent thalassemia (NTDT; also called thalassemia intermedia) or transfusion-dependent thalassemia (TDT; also called thalassemia major) (Table 3.1).

Clinical features. If not treated optimally, IE leads to bone marrow hyperplasia and extramedullary hematopoiesis, which cause many

TABLE 3.1

Spectrum of disease severity

Mild	Non-transfusion dependent	Transfusion dependent
Very mild to low end of normal anemia	Moderate anemia	Severe anemia
• α-Thalassemia trait/ silent carrier	• α-Thalassemia intermedia/HbH	• α-Thalassemia major/Hb Barts
• β-Thalassemia trait/ minor	• β-Thalassemia intermedia	• β-Thalassemia major
	• Dominant β-thalassemia	• Severe HbE β-thalassemia
	• HbH Constant Spring	• Severe HbH Constant Spring
	• HbE β-Thalassemia	

of the clinical manifestations of thalassemia (Figure 3.3). Bone marrow expansion and cortical thinning lead to bone deformities and pathological fractures.[14] Bone deformities in the skull include frontal and parietal bossing as the diploic space expands, limited by the suture lines. Prominence of the maxilla may also occur due to non-formation of the sinuses, which are replaced by active erythroid marrow.[15] Extramedullary hematopoiesis, with enlargement of the liver and spleen, is a prominent feature early in the disease course, with the development of para- or prespinal nodules of erythropoietic tissue giving rise to potential neurological complications such as sciatica or nerve root compression and pain.[16]

Increased iron absorption leads to iron overload, which is worsened by blood transfusions, both intermittent and regular. The quantity of iron added by transfusions far exceeds loading from increased intestinal absorption. Complications from iron overload include liver fibrosis progressing to cirrhosis with a risk of hepatocellular carcinoma, multiple endocrinopathies and heart disease, including contractile and electrical dysfunction.[17] Heart failure from iron overload remains the leading cause of death among patients with TDT. Iron overload also contributes to increased susceptibility to infections, something that is compounded in splenectomized individuals.[18]

Thalassemia also results in a vasculopathy, with a complex pathophysiology that includes damage to the vascular endothelium, and a hypercoagulable state caused by abnormal RBCs and thrombocytosis following splenectomy.[19] This can lead to complications such as pulmonary hypertension,[20] silent cerebral infarcts and venous thromboembolism.

Natural history

As discussed above, without transfusions to sustain the fetus, α-thalassemia major causing hydrops fetalis results in intrauterine death. Transfusions must be continued indefinitely postnatally.

Without transfusional support, individuals with β^0/β^0-thalassemia will die in the first 2 years of life from heart failure caused by severe anemia. With regular transfusions, growth and development may be fairly normal, provided the excess iron is chelated and endocrine complications such as growth retardation and delayed puberty do not

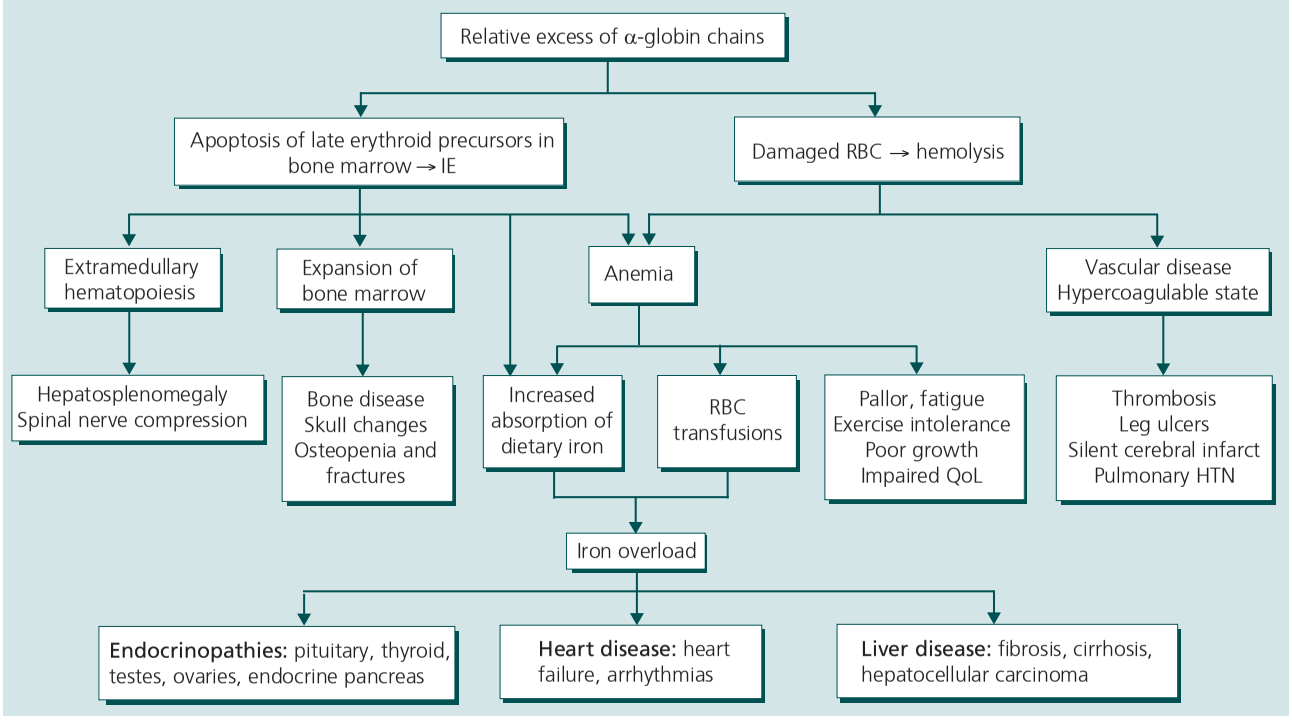


Figure 3.3 Pathological cascade leading to the manifestations of β-thalassemia. Ineffective erythropoiesis (IE) is generally more severe in β-thalassemia than in α-thalassemia, hence the description of the pathophysiology of β-thalassemia here. HTN, hypertension; QoL, quality of life.

occur.[21] Individuals with milder forms of thalassemia (NTDT) may tolerate the moderate anemia but may require regular transfusions if they experience growth or developmental issues or bone deformities caused by erythroid hyperplasia.[22] The spectrum of disease is not static, and as complications occur, individuals with NTDT may become transfusion dependent (Figure 3.4).[23,24] It is therefore important to monitor these individuals carefully and start regular transfusions if required.

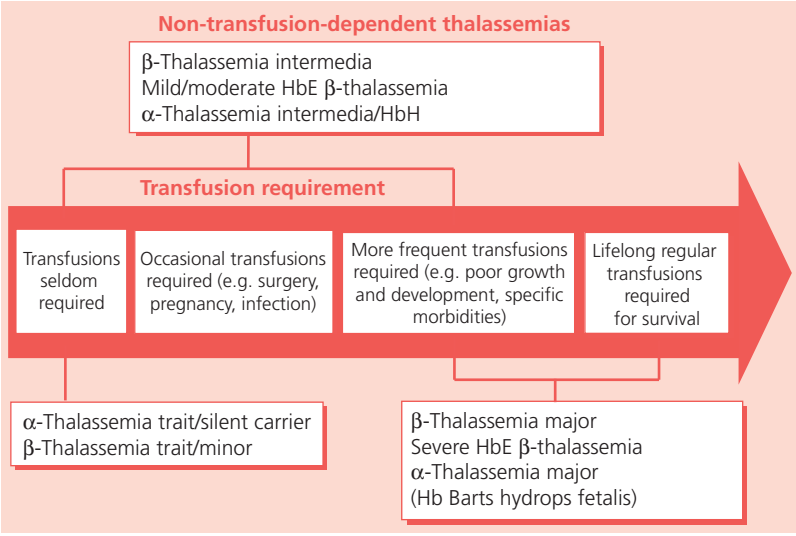


Figure 3.4 Progression of the thalassemia disease spectrum, showing transfusion requirements in different forms. From Musallam et al. 2013, reproduced under CC BY 4.0 license.[23]

Key points – pathophysiology and disease manifestations

- The pathophysiological basis of the thalassemia syndromes is the underlying imbalance between α- and β-globin production.
- The hallmark of β-thalassemia is IE, leading to anemia, bone marrow expansion, extramedullary hematopoiesis and dysregulation of iron metabolism.
- In α-thalassemia, IE is not a prominent feature in individuals with three deleted α-globin genes (deletional HbH) but it is more prominent in those with non-deletional HbH disease.
- Individuals with thalassemia are broadly classified into those who have NTDT and those who have TDT.
- There is a wide spectrum of disease severity from asymptomatic mild microcytic anemia through to severe anemia that requires regular lifelong blood transfusions.
- Disease manifestations include bone deformities in the skull, hepato- and splenomegaly, neurological and hepatic complications, multiple endocrinopathies, vasculopathy, heart disease and increased risk of infection.
- As complications progress, individuals who have NTDT may require regular transfusions and become transfusion dependent.

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Hematology

4 Clinical complications and their management

HEALTHCARE

General principles

Given the heterogeneity of disease manifestations (see Figure 3.3), the management of thalassemia can be complex and require expertise beyond that of pediatric and adult hematologists, necessitating a multidisciplinary management approach at treatment centers. Treatment decisions rely heavily on expert opinion, as data from randomized clinical trials to support various aspects of management remain limited. Beyond the conventional therapeutic options discussed below, all patients should also receive appropriate vitamins and other supplements to support hematopoiesis and should be considered for psychosocial support.

The transition of care from pediatric to adult providers and facilities should always be organized and systematically applied. Survival has greatly improved for individuals with thalassemia over the past few decades, which means that adult and even elderly patients should now also be considered at risk of common diseases affecting the general population, such as cancer and cardiovascular disease.[1–3]

Ineffective erythropoiesis and chronic anemia

Chronic anemia in patients with thalassemia is a marker of IE which can lead to a variety of complications secondary to hemolysis and hypercoagulability, primary iron overload (see pages 40–3), or the anemia and tissue hypoxia itself. Anemia has been associated with growth and developmental delay, fatigue and exercise intolerance, mental health problems and chronic organ failure in adolescents and young adults.[4–6] Marrow expansion leading to bone changes, pain and deformity, and extramedullary hematopoiesis leading to hepatosplenomegaly or pseudotumors are also characteristic of patients with severe IE and anemia.[4,6]

The hemolytic component of chronic anemia may lead to acute crisis in patients with α-thalassemia and to hypercoagulability and secondary venous and arterial thrombosis, pulmonary hypertension and cerebrovascular events, including silent infarcts, especially in splenectomized and older adults.[7–9]

Splenectomy. Although splenectomy has been used in the treatment of thalassemia to raise Hb levels, it is associated with higher rates of vascular disease and infections and should now be reserved for cases of hypersplenism or symptomatic splenomegaly.[1,2,8,9]

Transfusions

In patients with NTDT, the severity of anemia (each 1 g/dL decrease in Hb level) correlates with the risk of morbidity and mortality, especially for Hb levels below 10 g/dL.[10,11] Until recently, transfusions were the only option for addressing anemia in NTDT. They tend to be used occasionally in cases of acute stress/bleeding, or more frequently, but for defined periods of time, to promote growth and development in childhood or to manage certain complications in adulthood (Table 4.1).[2,9,12]

The risk of alloimmunization should be considered in transfusion-naive, older, splenectomized and pregnant patients. Recent evidence shows that patients with NTDT who receive frequent transfusion therapy have more favorable survival than those who do not.[13] However, lifelong regular transfusion therapy is not recommended for

TABLE 4.1

Common uses of transfusion in NTDT[2]

Occasional
• Pregnancy
• Surgery
• Infections
Frequent
• Declining Hb level in parallel with profound enlargement of the spleen
• Growth failure
• Poor performance at school
• Diminished exercise tolerance
• Failure of secondary sexual development in parallel with bone age
• Signs of bony changes
• Frequent hemolytic crisis (HbH disease)
• Poor quality of life
• Prevention or management of:
– Thrombotic or cerebrovascular disease
– Pulmonary hypertension with or without secondary heart failure
– Extramedullary hematopoietic pseudotumors

such patients because of the risk of secondary iron overload. Patients may be considered for novel therapies aiming to improve anemia in NTDT (see Chapter 5).[6,14]

In patients with TDT. Most of the morbidities encountered in patients with NTDT occur less frequently in patients with TDT since transfusion therapy ameliorates the anemia and IE (Figure 4.1).[12] Lifelong regular transfusions are administered in individuals with TDT to achieve target pretransfusion Hb levels of 9–10.5 g/dL (11–12 g/dL in patients with heart disease) after appropriate compatibility testing, preparation and storage of an RBC product.[1] Transfusions bring their own side effects, including secondary infections, alloimmunization and, most importantly, secondary iron overload. There is a correlation between the amount of blood transfused in patients with TDT and healthcare resource utilization.[15]

Luspatercept, a novel erythroid maturation agent, is now approved for the treatment of anemia in adults with TDT (USA/EU) and NTDT (EU). See Chapter 5 for more information.

Hydroxyurea. Data from small clinical trials have shown improvements in anemia and transfusion requirement in patients with β-thalassemia following treatment with hydroxyurea, but the magnitude of benefit was often modest and non-durable, or limited to patients harboring specific polymorphisms.[16]

Hematopoietic stem cell transplantation (HSCT) is associated with high disease-free survival rates in children (younger than 12 years old) with TDT and matched sibling donors. Older patients, especially those with iron overload, can also be considered for HSCT if they have a favorable risk profile. Unrelated donor blood, cord blood or haploidentical transplant are also becoming increasingly available for patients at specialized centers.[17]

Iron overload

Iron overload has received the most attention in both patients with NTDT and those with TDT over the past few decades because of its detrimental effects on the morbidity profile throughout the patient journey. Serum ferritin is the most widely used iron index, and serial

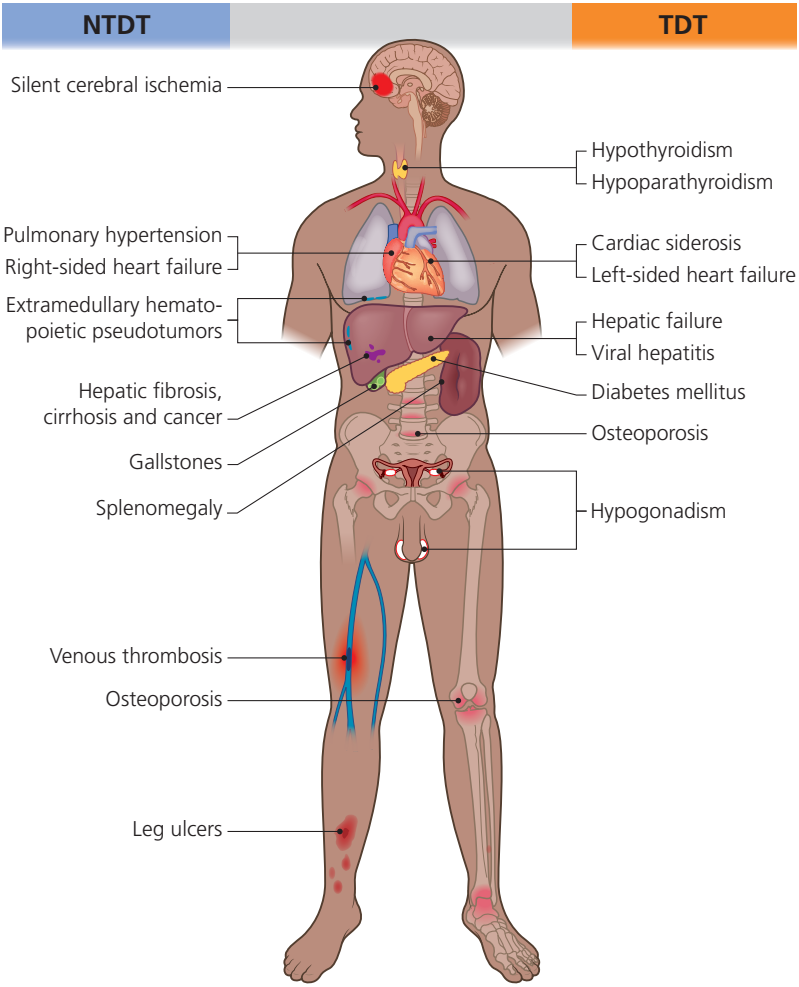

Figure 4.1 Comparison of clinical complications experienced by patients with NTDT and TDT. From Musallam et al. 2013, reproduced under CC BY 4.0 license.[12]

measurements can accurately reflect iron overload status in the absence of inflammation. Indirect measurement of iron levels in the liver and heart is also possible using MRI (R2 and T2*) techniques that have been validated against biopsy measurements to reflect liver iron concentration (LIC) and myocardial iron concentration.[18–20]

Iron chelation in NTDT. Even in the absence of transfusion therapy, IE in patients with NTDT leads to cumulative (primary) iron overload signaled by decreased hepcidin expression and an increase in intestinal iron absorption and its release from the reticuloendothelial system.[21] Iron is preferentially stored in the liver over the heart, and serum ferritin values are usually lower in patients with NTDT than in those with TDT with the same LIC. Chronic LICs greater than 5 mg/g and serum ferritin levels above 800 ng/mL have been shown to increase hepatic disease and malignancy, endocrine and bone disease, and vascular manifestations in patients with NTDT.[2,6,22,23]

It is recommended that patients with NTDT are monitored for iron overload by serum ferritin assessment every 3 months, or by hepatic MRI every 1–2 years from the age of 10 years (or 15 years in patients with HbH disease). Iron chelation therapy should be initiated when serum ferritin levels are 800 ng/mL or more or LIC is 5 mg/g or more, with the aim of reaching target levels of 300 ng/mL or lower and less than 3 mg/g, respectively.[2] Deferasirox (available as dispersible and film-coated tablets) is the only iron chelator specifically approved for iron overload in NTDT.[24] Patients with NTDT who receive iron chelation therapy have lower hepatic disease-related mortality than those who do not.[13]

Iron chelation in TDT. In patients with TDT, (secondary) iron overload manifests through non-transferrin-bound iron, which can cause damage to vital organs such as the heart, liver and endocrine glands. Serum ferritin levels greater than 1000 ng/mL, LIC values above 3 mg/g up to 7 mg/g, and cardiac T2* values lower than 20 ms are usually considered to be clinically significant. Serum ferritin levels greater than 2500 ng/mL, LIC values above 15 mg/g and cardiac T2* values lower than 10 ms are associated with an increased risk of cardiac disease and early mortality.[1,6]

Although mortality is declining in areas with adequate access to new iron chelating agents and organ-specific iron quantification by MRI, elevated iron levels and long-term morbidity and mortality from hepatic iron overload continue to be observed.[25]

In TDT, iron chelation therapy is recommended after receipt of ten packed RBC units or when serum ferritin is 1000 ng/mL or more. Patients should be readily and regularly monitored for iron overload using available iron indices (serum ferritin, and hepatic and cardiac MRI) at frequencies that are commensurate with the severity of existing iron levels.[1,6] Three iron chelators are available for managing iron overload in TDT patients.

- Subcutaneous deferoxamine, 30–60 mg/kg/day, delivered for 8–12 hours over 5–7 days per week. Common adverse events include ocular and auditory symptoms, bone-growth retardation, local reactions and allergy.
- Oral deferiprone, 75–100 mg/kg/day, given three times daily. Common adverse events include gastrointestinal symptoms, arthralgia, agranulocytosis and neutropenia.
- Oral deferasirox, 20–40 mg/kg/day, given once daily, as a dispersible tablet, or 14–28 mg/kg/day, given as a film-coated tablet. Common adverse events include gastrointestinal symptoms, increased creatinine and increased hepatic enzymes.

All three chelators have adequate data demonstrating their efficacy in reducing systemic, hepatic and (at high doses) cardiac iron overload. Patients with severe cardiac iron overload or heart failure may require combination therapy with deferoxamine and deferiprone, or intravenous deferoxamine. Adherence is better with the oral chelators than with subcutaneously injected deferoxamine, but adherence challenges continue to be noted, especially in adolescents and young adults.[1,4,6]

Management of specific morbidities

The treatment of thalassemia is about more than managing IE, anemia and iron overload. Prompt and regular monitoring of common morbidities is essential to ensure early introduction of preventive measures or management before organ damage becomes irreversible (Table 4.2). Monitoring measures and frequencies may vary according to age and the availability of resources and may be individualized based on patient-specific risk factors.

TABLE 4.2

Monitoring and management of morbidities in thalassemia[4]

Complication	Assessment	Management
Cardiac dysfunction and arrhythmia	• Echocardiography (routine) • Electrocardiogram (routine)	• As per standard care
Pulmonary hypertension	• Tricuspid regurgitant jet velocity (routine) • Right heart catheterization (high risk)	• As per standard care • Sildenafil • Bosentan
Cerebrovascular events	• MRI and MRA (high risk)	• As per standard care • Antiplatelet prophylaxis
Venous thrombosis	• Standard imaging (if suggestive signs and symptoms)	• Anticoagulant therapy • Medical and surgical prophylaxis
Leg ulcers	• Physical examination (routine)	• Topical measures • Pentoxifylline • Hydroxycarbamide • Hyperoxygenation
Viral hepatitis	• Viral serology (routine in transfused patients) • Viral RNA-PCR (if positive serology)	• Hepatitis B vaccination • Antiviral therapy
Hepatic fibrosis, cirrhosis and cancer	• Liver function tests (routine) • Ultrasound (high risk) • Alpha fetoprotein (high risk) • Transient elastography (investigational)	• As per standard care

CONTINUED

TABLE 4.2 CONTINUED

Complication	Assessment	Management
Endocrine disease	• Growth retardation (routine) • Sexual development (routine) • Endocrine function tests (routine) • Bone mineral density (routine)	• As per standard care
Bone disease	• Bone mineral density (routine)	• As per standard care • Bisphosphonates
Pregnancy	• As per high-risk pregnancy	• Revisit iron chelation • Anticoagulation prophylaxis • Maintenance of Hb level and heart function
Extramedullary hematopoietic pseudotumors	• Physical examination and imaging to rule out compression (if suggestive signs and symptoms)	• Hypertransfusion • Radiation • Surgery
Hemolytic crisis (HbH disease)	• Infection screening • Electrolytes	• Adequate hydration • Correction of blood electrolytes • Control body temperature • Antibiotics/antivirals

MRA, magnetic resonance angiography; PCR, polymerase chain reaction.

Key points – clinical complications and their management

- IE and chronic anemia lead to a variety of clinical morbidities in patients with thalassemia that require management.
- Hb levels below 10 g/dL are associated with increased morbidity and mortality in patients with NTDT. Until recently (see Chapter 5), there were no approved therapies to address anemia in these patients.
- Iron overload is a common concern in thalassemia, even in individuals with NTDT, and can be readily monitored with serum ferritin levels or through MRI assessment of hepatic and cardiac iron levels.
- Serum ferritin levels greater than 800 ng/mL and chronic LICs above 5 mg/g are associated with increased morbidity in patients with NTDT older than 10 years, and iron chelation therapy with deferasirox is recommended.
- Transfusional iron overload in TDT can lead to increased morbidity and mortality and should be closely monitored and promptly treated.
- All available iron chelators have proven efficacy and safety for iron removal in patients with TDT although at various rates from various organs; hence, treatment choice and dosing may need to be individualized, and ensuring adherence remains key.
- It is essential that common morbidities are monitored promptly and regularly so that preventive measures or management can be introduced before organ damage becomes irreversible.

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Hematology

5 Novel therapies

HEALTHCARE

Unmet needs and development strategies

Unmet needs. Several unmet needs exist for patients with TDT and NTDT. In TDT, lifelong dependence on regular transfusion therapy comes with its own public health burden and increased healthcare resource utilization.[1] In many parts of the world where resources are limited, poor access to transfusions means patients cannot achieve target Hb levels and thus suffer the consequences of chronic anemia.[2] Despite the availability of three iron chelators and modern MRI techniques for detecting iron in target organs, limited access, suboptimal use or poor adherence to iron chelation mean that many patients have high iron levels and subsequent vital organ morbidity.[2–4]

In NTDT, similar challenges around iron chelation exist, but the key concern is the lack of specific therapies approved to target the anemia in these patients.[5] Although transfusion therapy is possible, it exacerbates iron overload and iron chelation needs in patients with NTDT and is not a viable solution for all individuals.[6]

Clinical aims. There has been an astounding amount of work carried out to better understand the pathophysiology of thalassemia and identify targets for therapy. Novel strategies have mainly focused on ameliorating the α-/β-globin chain imbalance through genetic manipulation techniques (with curative intent) or increasing HbF production, targeting IE and RBC pathology, or targeting iron dysregulation (Figure 5.1).[7] Irrespective of the approach, the clinical aims are to reduce the transfusion requirement or abolish it altogether in individuals with TDT, leading to a decrease in iron intake and eventually iron overload and iron chelator needs. For patients with NTDT, the clinical aims are to improve Hb levels and prevent primary iron overload resulting from intestinal iron absorption, subsequently decreasing or abolishing the need for iron chelation. Over the long term, such benefits could decrease the morbidity and mortality associated with the disease.[7] This chapter discusses select novel therapies that had recently been approved or were at advanced stages of clinical development at the time of writing.

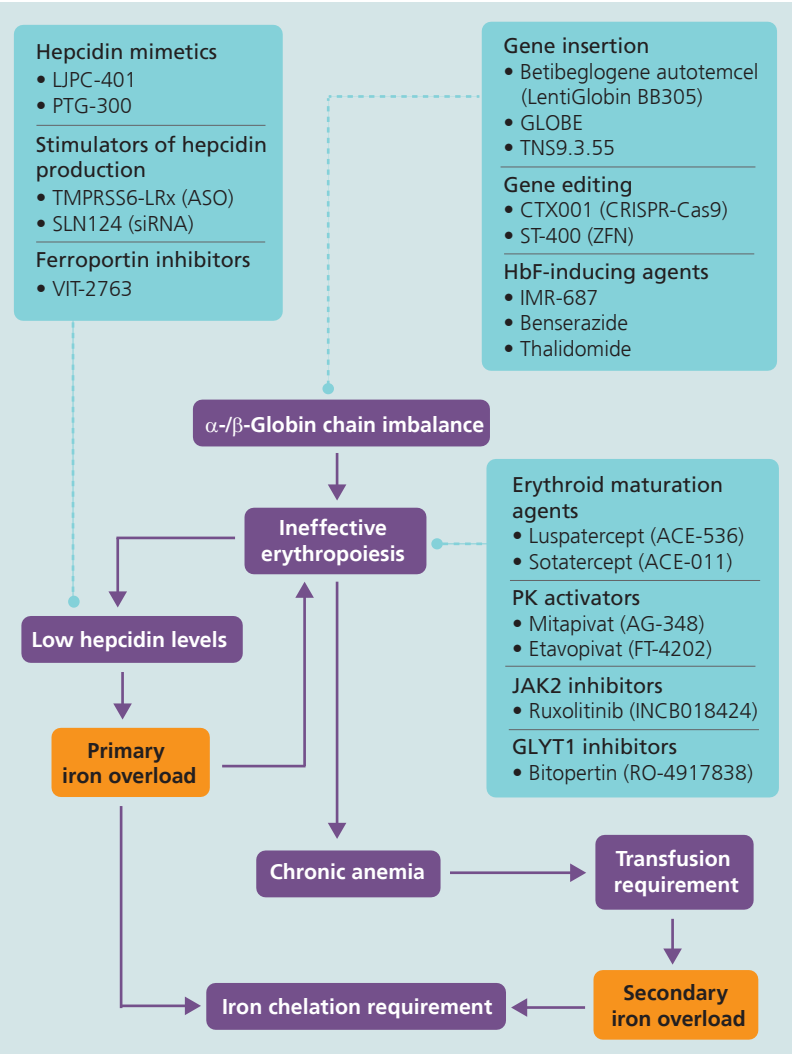

Figure 5.1 Novel therapies and their targets in thalassemia. ASO, antisense oligonucleotides; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases; GLYT1, glycine transporter 1; JAK2, Janus kinase 2; PK, pyruvate kinase; siRNA, small interfering ribonucleic acid; TMPRSS, transmembrane serine protease; ZFN, zinc finger nucleases.

Gene therapy (insertion)

Gene therapy has now become available for patients with β-thalassemia to replace the defective β-globin gene. Hematopoietic stem and progenitor cells are collected from a patient and undergo exogenous β-globin gene insertion using a lentiviral vector. The cells are then reintroduced to the patient through autologous HSCT following conditioning and myeloablation. The procedure requires specialized resources and expertise to ensure highly efficient engraftment and gene transfer/expression with minimal risk of insertional mutagenesis.

The main vector developed in large clinical trial programs is LentiGlobin BB305, which carries a modified β-globin gene with a T87Q amino acid substitution (HbAT87Q) that results in the production of HbA. Results from two Phase I/II studies (HGB-204 [NCT01745120] and HGB-205 [NCT02151526])[8] including 22 patients with TDT aged 12 years or older showed transfusion independence in 12 of 13 patients who had a non-β^0/β^0 genotype, while patients with a β^0/β^0 genotype primarily showed transfusion reduction. Safety was typical of autologous HSCT.

Phase III clinical trials used a more refined transduction process than Phase I/II studies and included younger children. Data from the HGB-207 study (Northstar-2, NCT02906202) confirmed efficacy and safety in achieving transfusion independence in most (20 of 22) patients with non-β^0/β^0 TDT.[9] Final data in β^0/β^0 patients are awaited from the HGB-212 study (Northstar-3, NCT03207009).

The gene therapy product betibeglogene autotemcel was approved by the US Food and Drug Administration (FDA) in August 2022 for adult and pediatric patients with TDT who are non-β^0/β^0, and who are transplant eligible but do not have a matched sibling donor. Approval was granted based on the two Phase III studies discussed above in which 89% of 41 evaluable patients achieved transfusion independence for at least 12 months. The trials were paused in 2021 when cases of malignant transformation were identified in a parallel study of LentiGlobin in sickle cell disease; however, these were eventually deemed to be unrelated to the gene therapy. All patients included in the core trials are enrolled in a 13-year long-term follow-up study (LTF-303, NCT02633943), with preliminary results continuing to demonstrate sustained efficacy and safety. Marketing authorization for betibeglogene autotemcel has been withdrawn in the EU for commercial reasons.

Other gene insertion approaches and vectors are also being evaluated in clinical trials (for example, GLOBE and TNS9.3.55).[10,11]

Gene editing

Effective synthesis of γ-globin chains after birth can ameliorate the underlying α-/β-globin chain imbalance and anemia in β-thalassemia through continued production of HbF. This is evidenced by the observation that patients with thalassemia who have HPFH commonly experience milder disease.[12] More recently, genome-wide association studies of common variations in HbF levels identified the multi-zinc finger-containing transcriptional regulator *BCL11A* as a key regulator of the fetal-to-adult (postnatal) Hb switch and HbF silencing.[13]

Several gene-editing strategies inhibit *BCL11A* expression through the use of enzymes, including clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases (CRISPR-Cas9), transcription activator-like effector nucleases (TALENS) and zinc finger nucleases (ZFN).[14–16] A patient's hematopoietic stem and progenitor cells are mobilized, collected and edited ex vivo using guide RNAs specific for the erythroid-specific enhancer region of *BCL11A*. The product is then infused back into the patient through autologous HSCT following myeloablative conditioning.

Two gene-editing products are being evaluated in Phase I/II trials for their ability to reduce transfusion requirements in patients with TDT. CTX001 (CRISPR-Cas9) is being evaluated in 45 patients with TDT aged 12 years and older in the CLIMB THAL-111 study (NCT03655678) and ST-400 (ZFN) is being evaluated in 6 adults with TDT in the THALES study (NCT03432364). Interim results are encouraging.[17–19]

Luspatercept

Luspatercept (ACE-536) is a first-in-class erythroid maturation agent that neutralizes select TGF-β superfamily ligands to inhibit aberrant Smad2/3 signaling and enhance late-stage erythropoiesis.[20] Phase II data showing that luspatercept reduces transfusion requirements in patients with TDT and improves Hb levels in patients with NTDT[21] encouraged further development for both indications in β-thalassemia (see randomized clinical trial data below), resulting in approvals for the treatment of adults with TDT and NTDT.

BELIEVE (NCT02604433) was a randomized, double-blind, placebo-controlled Phase III trial that included 336 adults with TDT randomized in a 2:1 ratio to receive luspatercept (1.0 mg/kg, titrated up to 1.25 mg/kg) or placebo every 3 weeks for at least 48 weeks, in addition to best supportive care including transfusion and iron chelation therapy. The trial met its primary endpoints by showing that a significantly larger percentage of patients receiving luspatercept achieved a 33% or greater reduction in transfusion burden from baseline during weeks 13–24 compared with placebo (21.4% vs 4.5%). Secondary endpoints of a 33% or greater or 50% or greater reduction in transfusion burden versus baseline at other fixed and 12- or 24-week rolling periods also favored treatment with luspatercept over placebo. Response was observed across all evaluated patient subgroups. Adverse events of transient bone pain, arthralgia, dizziness, hypertension and hyperuricemia were more common with luspatercept than with placebo.[22]

Based on these findings, luspatercept is now approved in the USA (2019) and Europe (2020) for the treatment of anemia in adults with β-thalassemia who require regular RBC transfusions. Long-term follow-up data from BELIEVE are now also starting to show a higher proportion of luspatercept-treated patients shifting to lower serum ferritin levels and trends of decreasing overall iron chelation use.[23] A Phase II trial in pediatric patients is ongoing (NCT04143724).

BEYOND (NCT03342404) is a Phase II, double-blind, randomized (2:1), placebo-controlled, multicenter study evaluating the efficacy and safety of luspatercept in 145 adults with NTDT and a Hb level of 10 g/dL or lower. The trial met its primary endpoint with 74 (77.1%) patients in the luspatercept arm versus no patients receiving placebo achieving a mean Hb increase of at least 1.0 g/dL from baseline over a continuous 12-week interval during weeks 13–24 in the absence of transfusions.[24] The key secondary endpoint was a change in a patient-reported outcome measure of tiredness/weakness specifically developed and validated for patients with NTDT (NTDT-PRO T/W). Improvement in NTDT-PRO T/W was not significant but favored luspatercept over placebo, and correlated with improvement in Hb levels.[24]

Based on these data, in March 2023 the European Commission approved luspatercept for the treatment of anemia in adults with NTDT. It has also been added as a potential treatment option in this setting in the 2023 Thalassaemia International Federation management guidelines.[25]

Mitapivat

Mitapivat (AG-348) is a first-in-class oral, small-molecule, allosteric activator of the RBC-specific form of pyruvate kinase (PK-R). Mitapivat has already shown efficacy and safety in clinical trials of patients with PK deficiency[26–28] and has been approved for this indication in adults in the USA and Europe.

Adenosine triphosphate (ATP) supply in thalassemic RBCs appears to be insufficient to maintain membrane fitness and clearance of globin precipitates. In mouse models of thalassemia, mitapivat increased ATP levels, reduced markers of IE and improved anemia, RBC survival and indices of iron overload. A Phase II, open-label, multicenter study (NCT03692052) evaluated mitapivat in 20 adults with NTDT with a Hb level of 10 g/dL or lower. In total, 16 patients (80%) – 11 of 15 patients with β-thalassemia and 5 of 5 patients with α-thalassemia – met the primary endpoint of a Hb increase of at least 1.0 g/dL at one or more assessment between weeks 4 and 12 of treatment, with favorable changes in markers of erythropoiesis and hemolysis. The most common non-serious adverse events occurring in 25% or more of patients were initial insomnia, dizziness and headache.[29] A long-term extension of the study up to 10 years is ongoing. Improvements in Hb level, hemolysis and IE have been maintained at a median duration of 70.9 weeks, with no treatment-related serious adverse events.[30]

ENERGIZE-T (NCT04770779) and ENERGIZE (NCT04770753) are Phase III, double-blind, randomized, placebo-controlled, multicenter trials evaluating the efficacy and safety of mitapivat (100 mg orally, twice daily) in adults with TDT and NTDT (α- and β-thalassemia). ENERGIZE-T plans to enroll 240 patients with TDT over 48 weeks with an open-label extension for 5 years. The primary endpoint is transfusion reduction response, defined as 50% or greater reduction in transfused RBC units with a reduction of at least 2 units of transfused RBCs in any consecutive 12-week period through week 48 compared

with baseline. ENERGIZE plans to enroll 171 patients with NTDT over 24 weeks with an open-label extension for 5 years. The primary endpoint is Hb response, defined as a 1.0 g/dL or greater increase in average Hb concentration from week 12 through week 24 compared with baseline. Patient-reported outcomes and changes in hemolytic and iron indices will also be assessed.[31]

Agents targeting hepcidin dysregulation

It has been established that IE in patients with thalassemia leads to iron overload through decreased hepcidin production. A bidirectional relationship has also been established where improvements in hepcidin expression and levels lead to amelioration of IE, although the exact mechanism remains unclear.[32] Since initial therapeutic trials with hepcidin mimetics (minihepcidins) in patients with TDT and NTDT were not encouraging,[7] attention has shifted to endogenous stimulation of hepcidin production. This can be accomplished through the downregulation of transmembrane serine protease 6 (TMPRSS6) leading to increased hepcidin levels.[33,34] Antisense oligonucleotides (ASO) and small interfering RNA (siRNA) targeting TMPRSS6 have been used effectively to stimulate hepcidin production, reduce iron burden and improve IE and RBC survival in mouse models of thalassemia.[35,36]

Both approaches have now progressed to clinical trials in patients with thalassemia. The efficacy of TMPRSS6-LRx (subcutaneous ASO) in improving Hb levels by at least 1.0 g/dL is being evaluated in a randomized Phase II trial (NCT04059406) in 36 adults with NTDT and Hb levels of 10 g/dL or lower. SLN124 (siRNA) is being evaluated in a Phase I trial (NCT04718844) in 112 adults with NTDT (α- and β-thalassemia) and myelodysplastic syndromes.

Approaches targeting other members of the iron regulation pathway, such as the iron transporter ferroportin, are also being evaluated. The oral inhibitor VIT-2763 showed potential in restricting iron availability and improving anemia in thalassemia mouse models.[37] After demonstrating safety in a Phase I trial,[38] the VITHAL (NCT04364269) study was initiated as a randomized, double-blind, placebo-controlled, Phase II trial evaluating the efficacy of VIT-2763 in improving Hb levels and iron indices in 36 patients with NTDT aged 12 years or older.

Key points – novel therapies

- Novel therapies in thalassemia are focused on ameliorating the α-/β-globin chain imbalance or on targeting IE or iron dysregulation.
- Gene insertion (through viral vectors) and gene editing (through enzymatic scissors) are now options for replacing the defective β-globin gene or reactivating γ-globin gene expression to produce HbF and ameliorate the α-/β-globin chain imbalance in β-thalassemia. The procedures involve autologous HSCT and myeloablation.
- Luspatercept (subcutaneous), an erythroid maturation agent,is now an approved option for patients with TDT (USA/EU) and NTDT (EU) following data from randomized trials indicating efficacy in reducing transfusion requirement and increasing Hb level, respectively.
- Mitapivat (oral), an allosteric activator of the RBC-specific form of PK, has shown preliminary ability to improve Hb levels in patients with NTDT and is being evaluated in Phase III trials in patients with both transfusion-dependent and non-transfusion-dependent α- and β-thalassemia.
- Several agents targeting the hepcidin dysregulation pathway, to both restrict iron absorption and improve IE and anemia, are in earlier stages of development.

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Useful resources

American Society of Hematology
Hematology.org

Cooley's Anemia Foundation (USA)
thalassemia.org

European Hematology Association
ehaweb.org

National Organization for Rare Disorders (NORD)
rarediseases.org

Thalassemia International Federation (TIF)
thalassaemia.org.cy

Thalassaemia & Sickle Cell Society of NSW (Australia)
thalnsw.org.au

United Kingdom Thalassemia Society (UKTS)
ukts.org

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Index

HEALTHCARE

Hematology

Fast Facts

Thalassemia Syndromes

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