Beta Thalassemia

Monitoring and New Treatment Approaches

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KEYWORDS

• Beta thalassemia • Iron overload • Chelation • Gene therapy

KEY POINTS

- Individuals with thalassemia should be monitored for disease and treatment-related complications, adequacy and safety of transfusions, iron overload, and adverse effects of iron chelation.
- Ongoing preclinical and clinical trials target globin chain imbalance, fetal hemoglobin reactivation, signaling pathways in ineffective erythropoiesis, and mechanisms of iron overload.
- In this article, the authors discuss recommendations for monitoring of individuals with thalassemia, as well as ongoing preclinical and clinical trials of therapies targeting different aspects of thalassemia pathophysiology.

INTRODUCTION

Beta thalassemias represent a class of disorders with a high global prevalence and significant health and economic impact.[1] Since the elucidation of the molecular mechanism in the 1960s, there has been significant progress in treatment of disease complications. With increasing use of transfusion therapy, iron overload has become a pressing problem, and chelation therapy is a key component of treatment. The first part of this review focuses on monitoring of disease complications, transfusion therapy, iron overload, and chelator toxicity. In the second part, the authors review new developments in therapy for beta thalassemia.

BETA THALASSEMIA PATHOPHYSIOLOGY

Thalassemias are a class of disorders caused by imbalance of the alpha (α) and beta (β) globin chains that make up the principal adult oxygen transporter hemoglobin

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A ($\alpha_2\beta_2$). Beta thalassemias result from a relative excess of α chains due to reduced production of β globin chains and, in some instances, increased dosage of α globin genes.[2] In addition to reduced functional hemoglobin production, red blood cells (RBCs) and their precursors are damaged by α globin, which is unstable in the absence of a binding partner. Free α globin forms precipitates, leads to formation of reactive oxygen species, and damages RBC membranes leading to hemolysis and abnormal erythroid maturation. The beta thalassemia phenotype is determined by the degree of the imbalance and ranges from minimal effects in beta thalassemia trait to severe transfusion-dependent anemia.

Symptoms in beta thalassemia are due to a combination of anemia and ineffective erythropoiesis. Increased erythropoietin levels due to anemia drive erythroblast proliferation through JAK2-STAT5 signaling; additional RBC extrinsic and intrinsic factors have been implicated in this process and are reviewed elsewhere.[3] Complications of beta thalassemia are numerous and include growth failure, bone disease, cardiac abnormalities (pulmonary hypertension, heart failure, arrhythmias), predisposition to thrombosis, extramedullary hematopoiesis (splenomegaly, masses with compression), and a broad range of endocrinopathies.

BETA THALASSEMIA THERAPY

Beta thalassemias were previously classified as thalassemia major, thalassemia intermedia, and thalassemia minor (trait), but a more useful classification is one of transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT). The decision to initiate regular transfusions includes objective laboratory data as well as clinical findings. Transfusions are recommended if the steady-state hemoglobin level is less than 7 g/dL. Poor growth, the development of frontal bossing or maxillary hyperplasia or other symptoms of anemia and ineffective erythropoiesis, even in the absence of severe anemia, should prompt initiation of transfusions. The goals of regular RBC transfusion therapy are relief of anemia symptoms (allowing for normal growth) as well as suppression of endogenous ineffective erythropoiesis. This generally is accomplished by administering transfusions every 3 to 5 weeks to maintain the hemoglobin level greater than 9.5 g/dL before transfusion.[4]

IRON OVERLOAD AND CHELATION

Beta thalassemia is characterized by abnormal iron metabolism through increased erythroferrone production by erythroid precursors and downregulation of hepatic hepcidin production, resulting in increased iron absorption.[5,6] Patients with NTDT can develop iron overload even in the absence of transfusions through increased dietary absorption, whereas TDT patients invariably have more rapid iron loading because of the high content of iron within transfused cells. Iron deposition in the liver, heart, and endocrine organs causes the most significant morbidity. Heart disease, the leading cause of death from iron overload,[7] includes left ventricular dysfunction, heart failure, and arrhythmias. Iron deposition in endocrine organs causes hypothyroidism, hypoparathyroidism, growth failure, delayed puberty, and diabetes. Hepatic fibrosis, cirrhosis, and hepatocellular carcinoma related to cumulative iron exposure usually do not manifest until later in life. Monitoring and management of iron overload are therefore an essential part of thalassemia treatment.

Iron chelation therapy is administered with the goal of providing as much chelator exposure per 24-h period as possible to reduce the toxic effects of non-transferrin bound iron (NTBI). Three iron chelators are approved for use in the United States—deferoxamine, deferasirox, and deferiprone. Deferoxamine, the first approved agent,

is administered by intravenous or subcutaneous infusion. Deferiprone and deferasirox are administered orally, and both have pediatric-friendly forms. Deferiprone is available in tablet and solution forms, with 3 times daily administration. Currently, it is approved as a second-line agent in the United States. Deferasirox, available as a dispersible tablet, film-coated tablet, and granule formulation, provides good chelation coverage with once daily dosing. Combination therapy may be beneficial for some patients with severe iron overload. The properties of iron chelators have been extensively reviewed elsewhere.[8]

Initiation of iron chelation in children is usually delayed until after age 2 years and when there is evidence of significant iron loading (10–20 transfusions, ferritin >1000 ng/mL and liver iron concentration >5–7 mg/g dry weight [dw]). This practice stems from concerns about growth delay and bone toxicity when deferoxamine was used in young children with low iron burden, which might not apply to the newer oral chelators. A recent pilot study assessed the earlier use of low-dose deferiprone in infants and toddlers with serum ferritin levels of 400 to less than 1000 ng/mL.[9] This approach delayed development of iron overload and reduced exposure to toxic labile plasma iron without unexpected, serious, or severe adverse effects. Larger confirmatory studies, and studies with deferasirox, are needed before routinely recommending earlier chelation initiation.

ALLOGENEIC STEM CELL TRANSPLANT

Correction of the underlying genetic defect through stem cell transplantation is currently the only curative therapy for beta thalassemia (reviewed elsewhere[10]). Unfortunately, access to this therapy is limited by the resources of the local medical system as well as by availability of optimal donors. In addition, the patients who benefit most are younger patients whose transfusion and chelation have been well managed and who have good organ function. Outcomes are better in younger patients (preferably under 14 years old), and predictors of increased transplant mortality have been described, including hepatomegaly, portal fibrosis, and history of inadequate iron chelation therapy.[11] A large part of transplant-associated toxicity is due to myeloablative conditioning regimens; as mixed donor-recipient chimerism can still produce transfusion independence, reduced intensity conditioning is currently being explored to improve outcomes.[12] Transplant outcomes are best with a fully HLA-matched related donor; cord blood from a matched related donor can also potentially provide good outcome in young patients.[13,14] HLA-matched unrelated donors are available for approximately 40% to 50% of patients of caucasian background, and although in past studies these patients had worse outcomes than with matched related donors, this has improved with better matching, donor cell processing, and graft versus host disease prophylaxis. Finally, HLA haploidentical parent donors are a more easily available option, but with higher risks of graft-versus-host disease, mortality, and graft failure, and currently should be used in clinical trial setting only. Therefore, it is important to carefully weigh the risks associated with the transplant versus the known complications of thalassemia.

MONITORING

Monitoring recommendations can be divided into several categories. Assessment for known complications of thalassemia due to anemia and ineffective erythropoiesis allows for appropriate decision-making regarding the need for and efficacy of transfusions (**Table 1**). For patients with TDT, monitoring is necessary to optimize transfusion therapy and to evaluate for infectious, immunologic, and iron-related complications of

Table 1
Routine monitoring for individuals with thalassemia

Test	Frequency of Monitoring
Alpha and beta globin genotyping	Once at diagnosis
High-resolution HLA typing	At diagnosis, when transplant is being considered
Growth parameters: height, truncal height, weight, head circumference, growth velocity	Every 3–6 mo[a]
Pain assessment	Every 3–6 mo
CBC with differential	Every 6 mo if no transfusions[b]
Comprehensive metabolic panel	Every 6 mo
Iron panel	Every 6 mo
Ferritin	Every 3 mo (monthly if on intensive chelation or low iron burden)
Echocardiogram and EKG	Annually starting at 10–14 y
Tanner staging and menstrual assessment	Every 6 months starting at 8–10 y.
TSH and free T_4	Annually starting at 6 y
FSH, LH, estradiol, prolactin (women) Testosterone (men)	Annually starting at 10 y
Vitamin D	Annually
PTH	Annually, starting at age 10 - 12 y
Bone density by DXA scan	Annually starting at 10 y
Fasting glucose, fructosamine	Annually starting at 10 y

Abbreviations: CBC, complete blood count; DXA, dual enegy x-ray absorptiometry; EKG, electrocardiogram; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PTH, parathyroid hormone; TSH, thyrotropin; T4 thyroxine.

[a] Endocrine evaluation should be undertaken if there is a fall-off on the growth curve or decreased height velocity.

[b] At least monthly CBC should be performed in infants when determining if regular transfusions are indicated.

transfusion therapy (Table 2). Both TDT and NTDT patients must be routinely evaluated for degree of iron overload to guide the need for chelation, as well as to assess the efficacy of ongoing chelation therapy (Table 3). Finally, patients receiving chelation therapy must be assessed for known chelator toxicities (Table 4).

Monitoring for Complications of Thalassemia and Iron Overload

The clinical complications of thalassemia have been extensively outlined previously.[7,15] The first step is to determine whether a patient will need transfusions; a combination of molecular genotyping, serial hemoglobin levels, and close monitoring of growth parameters, symptoms of anemia, and signs of ineffective erythropoiesis are necessary to make this decision.

Growth and bone health

In pediatric patients, height, weight, head circumference, and growth velocity should be assessed at least annually, and ideally every 3 months. Truncal (sitting) height should be obtained in older children. Osteopenia and osteoporosis are common in TDT and NTDT, and develop early, with spine bone mineral density *Z* score less than –2 SD reported in 9% of 6 to 10 year olds and 44% of 11 to 19 year olds.[16] Annual dual energy x-ray absorptiometry scan is recommended beginning by age 10 years old.

Table 2
Monitoring of transfusions and related complications

Test	Frequency of Monitoring
Red blood cell genotype/phenotype	Once at start of transfusions
Pre-transfusion CBC	Every 3–5 wk
Transfusion history assessment (volume transfused, presence of red cell antibody, IV access)	Every 3–5 wk
Hepatitis A, B, C serology (PCR as indicated) HIV testing	Annually

Abbreviations: CBC, complete blood count; HIV, human immunodeficiency virus; IV, intravenous; PCR, polymerase chain reaction.

Endocrine

Iron deposition in the pituitary, pancreas, and other endocrine organs leads to significant morbidity in TDT. The prevalence of hypothyroidism and hypoparathyroidism is about 10% and 2%, respectively.[17] Patients should undergo annual screening for thyroid function (thyrotropin and thyroxine), and 25-hydroxyvitamin D levels, and possibly also parathyroid hormone levels and urinary calcium. North American TDT patients have a 14% prevalence of diabetes mellitus.[17] At a minimum, patients should be screened annually for fasting levels of glucose and fructosamine (hemoglobin A1C is unreliable in transfused patients) beginning around adolescence. Several groups also recommend annual oral glucose tolerance tests. Hypogonadism is common, occurring in about 50% to 60% of patients with TDT.[17] Adolescent patients should be screened for onset of menarche and progression through puberty with careful history and Tanner staging every 6 months. Serum gonadotropins should be monitored annually for male and female adolescents. If abnormalities are detected, early referral to an endocrinologist is recommended.

Pain and quality of life

Pain is a frequent symptom in both TDT and NTDT, and negatively affects quality of life. In the Thalassemia Longitudinal Cohort, 69% of adolescents and adults and 56% of children reported pain over a 4-week period, most commonly in the back, knees, and head and neck.[18] Importantly, pain reports were similar in TDT and

Table 3
Monitoring for Iron overload

Test	Frequency of Monitoring	Starting Time
Serum ferritin	Every 3 mo (TDT) Monthly for TDT with low iron burden or on aggressive chelation At least annually (NTDT)	With start of transfusions in TDT
Liver iron concentration by MRI	Annually in TDT Every 6 mo if LIC >10 mg/g dw	1–2 y after start of transfusions or at start of chelation in TDT[a] When ferritin level reaches 500 ng/mL in NTDT
Cardiac T2* MRI	Annually Every 6 mo if T2* <10 ms	By 10 y of age if appropriately chelated

[a] For young children, risks of sedation need to be weighed against the value of the information to be obtained. Initial MRI may be deferred if transfusion and chelation history are known and ferritin is well controlled.

Table 4
Monitoring for adverse effects of iron chelation

Test	Frequency of Monitoring
All chelators	
Visual acuity and dilated ophthalmology examination	Annually
Audiology examination	Annually
Vitamin C level	Annually
Zinc level	Annually
Deferasirox	
Urinalysis for proteinuria	Every 3 mo
Liver function testing	Every 2 wk × 2 after initiation, then monthly
Renal and tubular function—creatinine, potassium, phosphorus, bicarbonate	Monthly
Deferiprone	
Complete blood count with differential	Weekly
Liver function testing	Every 3 mo

NTDT. The cause of the pain is unclear but may be related to intramedullary or extramedullary hematopoiesis or bone pathology. Patients should therefore be assessed for pain at all visits.

Monitoring Patients on Chronic Transfusions

Patients on chronic transfusions must be regularly assessed both to optimize transfusion therapy and to minimize transfusion risks. At initiation of transfusions, an RBC antigen profile (genotype or phenotype) should be obtained to facilitate appropriate RBC product choice and evaluation of any new RBC antibodies that develop. Alloantibodies in patients with hemoglobinopathies are most commonly directed toward C, E, and Kell, and, at a minimum, these antigens should be matched beyond the typical ABO and RhD matching.[19] A goal pre-transfusion hemoglobin of 9.5 to 10.5 g/dL usually is recommended because this level suppresses ineffective erythropoiesis[4] and relieves symptoms of anemia, allowing for normal growth. Typically this can be achieved with every 3- to 5-week transfusions. Higher pre-transfusion hemoglobin levels may be needed in the setting of heart disease or other complications. Patients should be asked about symptoms before transfusion to determine if a higher hemoglobin goal is appropriate. Pre-transfusion hemoglobin levels and volume of RBCs administered should be routinely tracked to allow adjustment of transfusion regimens and assessment of iron loading. Unexpectedly low pre-transfusion hemoglobin levels may indicate a hemolytic transfusion reaction or hypersplenism. Although the rates of infection in modern blood-banking systems are low, patients should have annual testing for human immunodeficiency virus, hepatitis A, hepatitis B, and hepatitis C.

Iron Overload Monitoring

Monitoring of iron overload is an essential aspect of care and several different tests are used. Initial observational studies in TDT showed higher rates of complications and mortality in patients with severe iron overload, indicated by serum ferritin greater

than 2500 ng/L or liver iron concentration (LIC) greater than 15 mg/g dw.[20,21] Although serum ferritin is a readily available and inexpensive test, it has multiple limitations. Ferritin provides a global reflection of iron stores and trends are helpful, but it does not correlate well with organ-specific iron deposition.[22,23] NTBI and labile plasma iron may provide alternative serum markers to ferritin but are not routinely available or fully validated. The LIC provides a reliable estimate of total body iron stores[24]; use of MRI techniques has generally replaced the more invasive liver biopsy. Cardiac iron estimation by MRI allows accurate prediction of risk of heart failure and arrhythmias.[25] Liver and cardiac iron imaging by MRI are the standard of care, although iron quantification in other organs is currently under investigation.

Spin echo (R2) and gradient echo (R2*) are the 2 most widely used validated MRI techniques for LIC. These methods report iron loading as mg iron per gram dry weight, as they are based on correlation curves with liver biopsy iron quantification. R2 and R2* measurements generally correlate well, but variability in quantification between methods exists and most validation has been done on LIC values less than 30 mg/g dw.[26,27] Although both methods have good accuracy and reproducibility, individual patients ideally should be monitored with the same method longitudinally.

Cardiac iron is typically measured with T2* images with electrocardiogram gating, and this technique is widely available and highly reproducible.[28–30] Results are usually presented in milliseconds, with T2* of greater than 20 ms being adequate. Mild cardiac iron overload is defined as T2* of 10 to 20 ms, and severe iron overload is less than 10 ms. In a large study, nearly half of patients with T2* less than 6 ms developed heart failure within 1 year.[25]

Guidelines for monitoring of iron overload are shown in **Table 3**. Serum ferritin should be assessed at least every 3 months in TDT, with more frequent monitoring in the setting of intensive chelation or with ferritin levels less than 1000 ng/mL to avoid overchelation. Most centers aim to keep the ferritin in a range of 500 to 1500 ng/mL in TDT. LIC should first be measured after 1 to 2 years of transfusions in TDT. For young children, the value of information gained from MRI should be weighed against the risks of sedation, and imaging may be deferred if the serum ferritin is in an appropriate range. Annual liver MRI is generally recommended; imaging every 6 months is useful in patients with high LIC undergoing aggressive chelation. Cardiac iron monitoring should begin by 10 years of age based on studies of timing of cardiac iron loading, but, as one study reported, 5% of 2- to 5.5-year-old children had T2* of less than 20 ms, an earlier start is reasonable, especially if chelation is inadequate or history unknown.[27,31] Cardiac MRI should then be monitored annually; high-risk patients should have T2* assessed every 6 months, whereas well-chelated, low-risk patients may have assessment spaced to every 2 years.

Monitoring for Complications of Chelator Therapy

Iron chelators have varying potential adverse effects, depending on the agent used. Patients receiving chelation should be regularly assessed for symptoms and laboratory signs of these toxicities to select the best chelator, reduce barriers to adherence, and minimize potential for irreversible damage (see **Table 4**). Iron chelation may cause ophthalmologic and audiologic adverse effects, including reduced visual acuity, impaired color vision, night blindness, tinnitus, and high-frequency sensorineural hearing loss.[32] These risks are greatest with deferoxamine, particularly when the chelator dose is high relative to the total body iron load.[32,33] Patients should be assessed for these symptoms at all visits and should undergo annual ophthalmology and audiology evaluations. Zinc deficiency can develop with any of the 3 chelators, and zinc levels should be checked annually. Iron overload can cause vitamin C deficiency, which

adversely affects iron excretion and bone health so levels should be routinely checked.[34]

Patients on deferoxamine should be regularly assessed for adherence and any potential barriers, such as injection site reactions. Bone dysplasia and growth failure can occur in young children,[35] and all patients should be monitored for growth velocity.

Gastrointestinal side effects such as abdominal pain, emesis, and diarrhea occur in approximately 16% of pediatric patients receiving the dispersible tablet form of deferasirox and may limit adherence.[36] These adverse effects may be lessened with the newer film-coated tablet and granule forms, which do not contain lactose, which is thought to contribute to this effect. Renal side effects including elevated creatinine and proteinuria occur in 8.8%; renal Fanconi syndrome occurs less commonly.[36] Risk factors for renal complications include pre-existing kidney disease, dehydration, ferritin less than 1000 ng/mL, and concomitant use of nephrotoxic agents; more frequent monitoring of renal function and appropriate dose adjustment are recommended in these situations. Elevation of liver transaminases also can commonly occur. In one large 5-year study of deferasirox, 3.4% of patients had a serious adverse event, 21% of patients experienced an elevation in alanine aminotransferase, and 3.8% of patients had an elevation in creatinine.[37] All patients on deferasirox should have a monthly complete metabolic panel and urinalysis checked.

The most concerning adverse event of deferiprone is reversible agranulocytosis (absolute neutrophil count <500/μL), with an incidence of 0.2 to 0.43 per 100 patient-years, most commonly in the first year of therapy; milder, often reversible, neutropenia also occurs.[38–40] A complete blood count with differential should be assessed weekly to monitor for neutropenia/agranulocytosis. The drug should be held and a complete blood count with differential checked for all febrile illnesses. Elevation in serum alanine aminotransferase are reported in 2.8% to 10.4% of patients,[38–40] and this should be monitored every 3 months. Arthralgia and arthropathy also are common, occurring in 3.9% to 11.8% of patients and leading to drug discontinuation in 2% to 8.4%, so all patients should be asked about these symptoms.[38,39]

NEW THERAPEUTIC APPROACHES

Pharmacologic Fetal Hemoglobin Induction

Patients with NTDT may benefit from reactivation of gamma globin expression to produce fetal hemoglobin (HbF, $\alpha_2\gamma_2$) as a replacement for β globin and improve the α-/β-like globin chain imbalance. Hydroxyurea has established efficacy in sickle cell disease in part through induction of HbF through still poorly understood pathways, but no rigorous randomized trials have been done to assess its potential in beta thalassemia. Observational studies have demonstrated some benefit in TDT and NTDT.[41–43] Metformin is a well-tolerated US Food and Drug Administration-approved medication used for management of type 2 diabetes mellitus. Recent preclinical studies have demonstrated that metformin induces expression of fetal hemoglobin in primary erythroid cell cultures and is additive with hydroxyurea.[44] There is currently a phase I study of metformin in sickle cell disease and NTDT (NCT02981329) that includes children 12 years old and older.[45] Both hydroxyurea and metformin may be especially beneficial in resource-poor settings and further studies are warranted.

Gene Therapy

Beta globin replacement

The first human gene therapy trial for beta thalassemia was published in 2010.[46] Using a lentiviral vector expressing β globin with a T87Q mutation that mimics the

anti-sickling properties of gamma globin (HPV569) in a patient with β_E/β_0 thalassemia, transfusion independence was achieved, but this study also demonstrated risks of potential integration-dependent clonal hematopoiesis. Subsequently, a phase 1/2 trial was carried out using the LentiGlobin BB305 vector, which was modified from the HPV569 vector. Following myeloablative conditioning with busulfan, BB305 transduced autologous hematopoietic stem cells were infused. The results of 22 treated patients ranging from 12 to 35 years old, with median follow-up of 26 months recently were published.[47] Of 13 patients with a non-β_0/β_0 genotype, 12 achieved transfusion independence with hemoglobin levels ranging from 8.2 to 13.7 g/dL. Of 9 patients with β_0/β_0 genotypes, 3 became transfusion-independent and the median annual transfusion volume decreased by 73% in the others. Importantly, the authors demonstrated good expression of vector-derived hemoglobin, no clonal predominance in patient hematopoiesis, no evidence of replication-competent virus production, and an adverse event profile no worse than that of routine myeloablative conditioning. Two phase III trials are currently enrolling patients, for TDT with non-β_0/β_0 genotypes (NCT02906202)[48] and with β_0/β_0 genotypes (NCT03207009).[49] Preliminary data from these studies were recently presented and of patients with at least 6 months of follow-up, 7 out of 8 treated with non-β_0/β_0 genotypes were able to stop transfusions.[50]

Preliminary data also were recently reported from another phase 1/2 trial using the GLOBE lentiviral vector expressing human β globin, with myeloablative conditioning, and intraosseus infusion of the modified hematopoietic stem cells.[51] Three adults and 4 children (age 6–13 years) were treated, with no adverse events beyond those expected from an autologous stem cell transplant and no evidence of clonal viral integrations. Outcomes for the pediatric patients were better, with 3 of 4 pediatric patients achieving transfusion independence; the adult patients had a reduction in transfusion requirements but still require transfusions. Enrollment is ongoing (NCT02453477).[52]

An additional phase I trial of a TNS9.3 lentiviral vector with wild-type human β globin following a reduced intensity conditioning regimen has also been initiated, but data are not yet available (NCT01639690).[53]

Fetal globin reactivation

Bcl11a is a recently discovered repressor of fetal hemoglobin that is being studied as a therapeutic target in beta thalassemia[54] as loss of Bcl11a activity leads to robust expression of HbF. Tissue-specific expression of Bcl11a is driven by an erythroid-specific upstream enhancer element.[55] Genetic targeting of this enhancer allows for disruption of Bcl11a specifically in erythroid cells. One approach uses a zinc-finger nuclease that is delivered by lentiviral transduction of stem cells and specifically disrupts the Bcl11a erythroid enhancer; a phase I/II study in adult patients with TDT is ongoing (NCT03432364).[56] Another approach utilizes CRISPR-Cas9 gene editing of the Bcl11a erythroid enhancer, which is being studied in a phase I/II study in adults with non-β_0/β_0 TDT (NCT03655678).[57]

Ineffective Erythropoiesis Signaling Modulators

Signaling molecules in the transforming growth factor beta family, such as BMP4, GDF11, and GDF15 contribute to ineffective erythropoiesis.[58,59] Luspatercept (ACE-536) and sotatercept (ACE-011) target this pathway by competing with the extracellular domain of the activin receptor for circulating ligand; both are produced as human IgG1 Fc domain fusions and are administered subcutaneously every 3 weeks. They were initially developed to treat osteoporosis in postmenopausal women but were

found to transiently increase hemoglobin in a dose-dependent fashion.[60] These findings, as well as clinical safety, were confirmed for both drugs in phase I trials.[61,62] A phase II study of luspatercept (NCT01749540) in adults with beta thalassemia showed a reduction in transfusion burden in TDT patients (by one-third in 83% of patients), and a sustained hemoglobin increase of $\geq$1 g/dL in 50% of NTDT patients in the highest dose group.[63] A phase II study of sotatercept (NCT01571635) showed similar improvements in hemoglobin level and decreased transfusion burden in NTDT and TDT patients, respectively.[64] Luspatercept is being assessed in phase III studies in TDT (BELIEVE, NCT02604433) and phase II in NTDT (BEYOND, NCT03342404).[65,66] Recently presented preliminary data from the BELIEVE trial showed that 21.4% of patients receiving luspatercept achieved the primary endpoint of at least a 33% reduction in RBC transfusion compared to baseline over weeks 13 to 24 versus 4.5% of placebo-treated patients.[67]

Erythropoietin signaling through the Jak2 receptor and the STAT kinases is critical for erythroid precursor survival and maturation. Mice with beta thalassemia intermedia recapitulate ineffective erythropoiesis and have high levels of erythropoietin and activated Jak2 signaling.[68] Jak2 inhibition in this mouse model results in a decrease in spleen size.[69] A single-arm open-label phase IIa study of the Jak2 inhibitor, ruxolitinib, in 30 TDT patients with splenomegaly showed a decrease in spleen size and a modest decrease in transfusion requirements of 5.9% from baseline, and was overall well tolerated.[70] There are no current plans for phase III studies.

Targeting Hepcidin to Reduce Iron Overload

Hepcidin is a 25-amino acid peptide hormone that blocks iron absorption, recycling, and storage through downregulation of the cellular iron exporter ferroportin 1. Hepcidin is produced by the liver in response to inflammation and increased iron stores, and, in the case of thalassemia, is suppressed by erythroferrone produced by erythroid precursors. Ineffective erythropoiesis in thalassemia therefore leads to a state of low hepcidin and high iron absorption.[71] Increasing hepcidin expression ameliorates iron overload in a mouse model of beta thalassemia intermedia.[72] Phase I and II trials are currently ongoing for several classes of hepcidin-targeting drugs, including hepcidin mimetics (LJPC-401, La Jolla Pharmaceutical-NCT03395704[73]; PTG-300, Protagonist Therapeutics), agonists of the hepcidin regulator Tmprss6 (Ionis-TMPRSS6-LRx, Ionis Pharmaceuticals), and ferroportin inhibitors (VIT-2763, Vifor Pharma).

Reducing Cardiac Iron

Animal studies have suggested that iron enters cardiomyocytes through L-type calcium channels.[74] Amlodipine is a calcium channel blocker broadly used in adults and children, and a recent small phase III randomized trial examined whether addition of amlodipine to standard chelation therapy would reduce cardiac iron deposition.[75] This trial enrolled patients from 8 to 49 years old, with a mean age of 22.5 years. In subgroup analysis, patients with high cardiac iron burden had a greater 12-month reduction in cardiac iron with the addition of amlodipine (0.26 mg/g) versus standard therapy alone (0.01 mg/g). Although larger trials are needed for follow-up, this is a potentially promising use of a well-tolerated and studied medication.

SUMMARY

Advances in monitoring and treatment of children and adults with beta thalassemia have led to increased survival and decreased morbidity, but challenges still remain.

New therapies under investigation offer promise of improvements in current standards of care as well as revolutionary curative approaches.

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