Literature review

Novel therapeutic advances in β-thalassemia

"Sapientia maior est quam fatum" (Wisdom is greater than fate)

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Introduction

β-thalassemia is one of the most prevalent single-gene disorders worldwide, caused by mutation in the *HBB* gene.[1] The disease is characterized by a reduction or absence of β-globin synthesis, leading to an imbalance between α- and β-globin and the excess deposition of α-globin in developing erythrocytes. This results in ineffective erythropoiesis, the hallmark of β-thalassemia.[2] Consequently, this pathophysiology leads to various clinical manifestations, including chronic anemia, iron overload and extramedullary hematopoiesis (EMH).[3] The phenotypic presentation of β-thalassemia varies, ranging from asymptomatic to severe forms requiring regular blood transfusion. Recently, the degree of transfusion requirement and clinical severity have been used to classify thalassemia into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT).[4]

For TDT patients, the mainstays of treatment are life-long transfusion and iron chelation therapy. However, the ongoing need for chronic transfusions and iron chelation imposes both physical and emotional burdens, making long-term treatment challenging to sustain. As a result, individuals with TDT, particularly those affected by iron overload and the adverse effects of iron chelators, may experience organ dysfunctions, leading to an increased risk of morbidity and mortality.[5] In contrast, NTDT patients do not require lifelong, regular blood transfusions for survival; however, some may occasionally require transfusions or become transfusion-dependent (*neo*TDT) under certain clinical settings.[6] β-thalassemia is one of the best-characterized genetic disorders at the molecular level. Accordingly, several curative and novel therapeutic strategies for thalassemic patients have been rapidly developed in recent years. Here, we provide an overview of pathophysiology of β-thalassemia, along with disease modifiers that contribute to the potential disease-modifying targets and novel therapeutic approaches.

Pathophysiology, genetic modifiers and potential therapeutic targets in β-thalassemia

In β-thalassemia, the pathophysiology primarily involves a reduction in β-globin chain production causing an excess of unpaired α-globin chains. These excess β-globin chains denature and form highly insoluble aggregates called hemichromes, which precipitate and induce oxidative stress in immature erythroid cells, leading to apoptosis and contributing to anemia.[7] Moreover, alterations in the cell membranes are thought to affect membrane permeability causing inadequate ATP production and decreased overall red blood cell (RBC) lifespan, resulting in increased hemolysis.[8,9] This anemia leads to elevated erythropoietin (EPO) levels as a compensatory response to tissue hypoxia. This, in turn, stimulates the Janus kinase 2 (JAK2)-dependent phosphorylation cascade, promoting the expansion of erythroid precursors. Under the pathologic condition of thalassemia, erythroid cells in bone marrow fail to effectively respond to erythropoietin, leading to com-

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pensatory erythroid hyperplasia combined with impaired erythroid differentiation. This is driven by overactivation of transforming growth factor beta (TGF-β) superfamily ligands and elevated suppressor members against decapentaplegic (SMAD) 2/3 signaling pathway. Finally, these underlying mechanisms cause inadequate functional RBC production, so called ineffective erythropoiesis, the hallmark of β-thalassemia.[10-12] Beyond inefficient RBC production, persistent ineffective erythropoiesis may contribute to hepatosplenomegaly, EMH in other parts of the body, osteoporosis and bone deformities.[13] Moreover, sustained elevated erythropoietin levels stimulate erythroid precursors to release erythroferrone (ERFE), which suppresses hepcidin expression-the main systemic iron regulator in the body- resulting in increased intestinal iron absorption.[14] Ultimately, ineffective erythropoiesis, together with extensive erythroid expansion and increased iron absorption, leads to the clinical features of β-thalassemia, including chronic anemia, EMH and iron overload.[13]

The clinical manifestations of β-thalassemia are highly diverse, ranging from asymptomatic case to severe anemia requiring regular transfusion. Although the clinical severity of β-thalassemia is primarily associated with genotypic mutations, several studies have shown that phenotypic variability cannot be fully explained among individuals with the same genotype.[15] For this reason, clinical variability in the β-thalassemia syndromes has led researchers to identify factors that influence disease severities. In addition to β-globin gene mutations, known as primary genetic modifiers, several secondary and tertiary genetic modifiers have been identified that can mitigate disease severity. Secondary genetic modifiers involve genetic variations that affect the α-/β-globin chain imbalance, particularly α-thalassemia co-inheritance and conditions associated with increased fetal hemoglobin (HbF) synthesis.[16] For instance, the co-inheritance of α-thalassemia may ameliorate symptoms, while the presence of a triplicated or quadruplicated α-genotype can lead to excessive α-globin chain production, exacerbating the α-/β-globin chain imbalance and worsening disease severity.[17] Polymorphisms in *XmnI*, *BCL11A*, *KLF1* and *HBS1L-MYB* genes, which result in elevated HbF levels, as well as genetic variants associated with the persistence of HbF such as hereditary persistence of fetal hemoglobin (HPFH), have been linked to a reduced burden of β-thalassemia.[17,18] Lastly, tertiary genetic modifiers refer to mutations outside the globin genes that influence phenotypic complications of β-thalassemia syndromes such as hyperbilirubinemia, gallstone formation, bone disease, thrombophilia and cardiomyopathy.[19]

With advances in understanding the pathophysiology and disease modifiers of β-thalassemia, an increasing number of novel therapeutic strategies have been developed to target the different underlying mechanisms of the disease, such as correcting the globin chain imbalance and addressing the ineffective erythropoiesis (Figure 1). Similarly, to improve chronic anemia in thalassemic patients, several novel treatments aimed at correcting iron dysregulation, including hepcidin mimetics, ferroportin inhibitors, transmembrane serine protease 6 (TMPRSS6) inhibitors and apotransferrin, have been progressively developed.[3,14] While therapies targeting iron overload in β-thalassemia are not covered in this review, they may be explored in future reviews. In the following sections, we present novel therapeutic approaches and current ongoing treatments for β-thalassemia, with a focus on targeted mechanisms of the disease, particular in retention to chronic anemia.

Curative approaches for β-thalassemia

While regular blood transfusions can temporarily ameliorate the clinical severity of patients with TDT, these patients might be consequently affected by iron overload and subsequent organ dysfunction requiring iron chelation therapy imposing a burden on patients and can be challenging to sustain long term treatment. Additionally, as aging thalassemic patients, comorbidities further increase the therapeutic cost of lifelong patient

care and are associated with a reduced health-related quality of life.[20,21] For these reasons, curative treatments or novel therapies that reduce the requirement for blood transfusion are intensely needed.

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) is considered the first curative therapy for β-thalassemia. The principle of HSCT is to replace the defective thalassemic hematopoietic stem cells (HSCs) with normal HSCs from a healthy donor through allogeneic stem cell transplantation (alloSCT) (Figure 2). As a result, the infused donor HSCs can produce normal beta-globin and restore effective erythropoiesis, eliminating the need for lifelong blood transfusions and iron chelation.[22,23] A study from the European Bone Marrow Transplant (EBMT) registry of thalassemia major patients who underwent alloSCT from human leukocyte antigen (HLA)-matched sibling donor (MSD) has shown excellent overall survival and event-free survival. However, alloSCT is most effective when performed in patients younger than 14 years, with inferior outcomes in adults, primarily due to more advanced disease, iron overload-associated organ dysfunction, and transplant-related mortality.[24] Therefore, it is recommended that patients with thalassemia major, particularly young patients without severe iron overload and organ dysfunctions, should consider HSCT if they have MSD donors.[25]

Although an MSD is the preferred option for alloSCT, the probability of finding an MSD within the family is less than 30%.[26] The use of alternative HSCT donors for thalassemic patients has been increasingly investigated in recent years employing HSC from unrelated, HLA-mismatched and haploidentical donors to expand donor availability.[27-29] Previously, the increased risk of graft rejection, graft-versus-host disease (GVHD) and transplant-related mortality (TRM) raised concern for thalassemic patients undergoing non-MSD transplantation. However, recent studies have reported successful transplantation from alternative donors using novel transplant strategies.[30,31] The key success factors consist

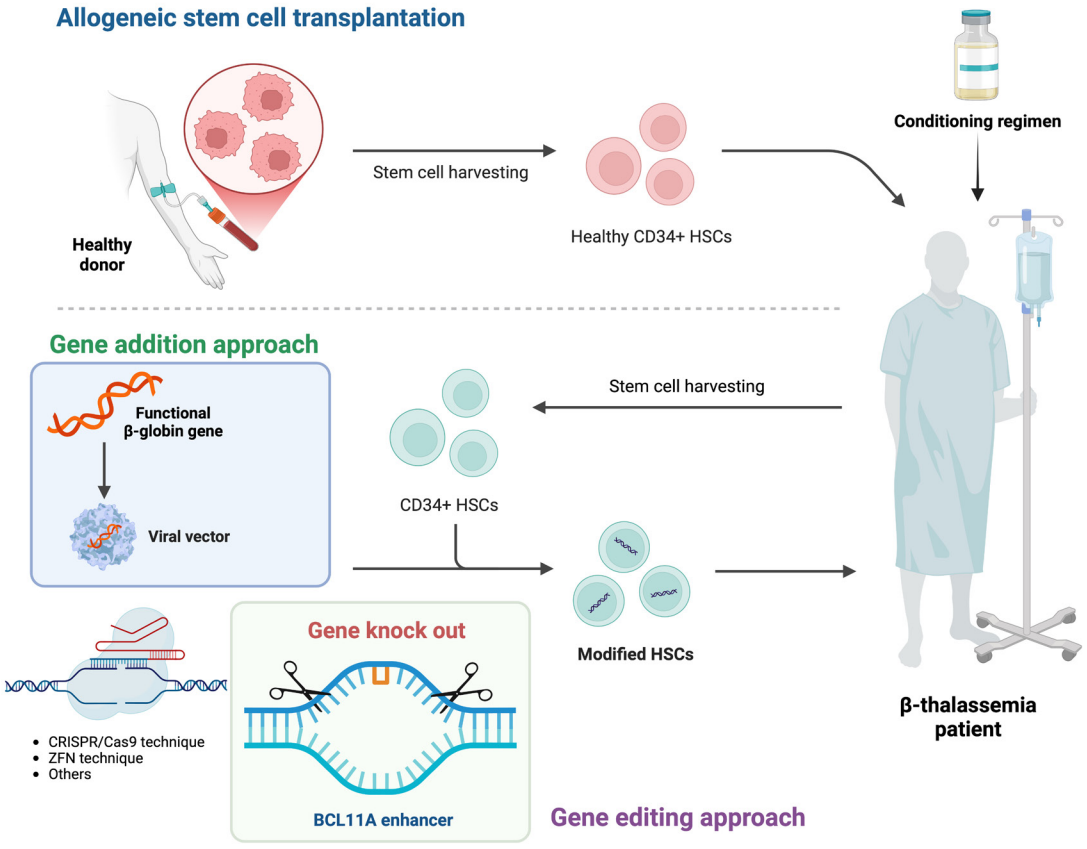

Figure 1 Pathophysiology, disease modifiers and potential therapeutic targets in β-thalassemia

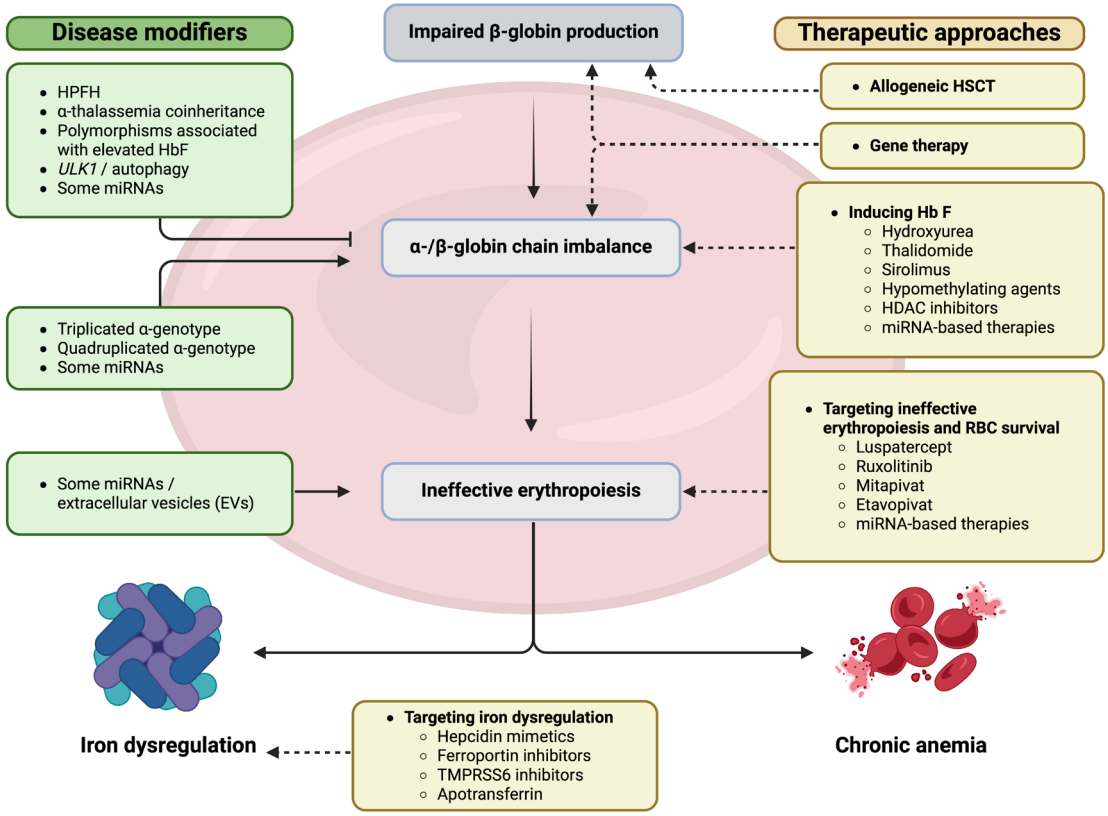

Figure 2 Principles of hematopoietic stem cell transplantation, gene addition and gene editing approaches

of stringent immunogenetic compatibility testing, an initial pharmacologic pretransplant immune suppression phase and in vivo T cell-depleted GVHD prophylaxis. These novel comprehensive strategies have demonstrated promising survival outcomes and decreased the rate of transplant-related toxicity, TRM and graft failure yielding comparable outcomes to MSD transplantation.[32-35]

In addition to the selection of suitable donors, the conditioning regimen also has significantly impacted treatment outcomes. Historically, the myeloablative conditioning regimen (MAC) has been considered as the standard conditioning regimen contributing adequate immune suppression and reducing the risk of graft rejection. However, MAC is associated with high TRM, for example, infection, veno-occlusive disease (VOD) and secondary malignancy which remain the challenging issues in alloSCT. There has been a gradual improvement in supportive care and modified conditioning regimen strategies such as a reduced intensity regimen, over the past few years resulting in achieving transplantation in thalassemia.[36,37]

Despite the advances in HSCT strategies, transplant-related complications, especially GVHD, short and long term toxicity from conditioning regimens and graft failure remain the major limitations leading to inferior outcomes for this therapeutic strategy.[38,39] Accordingly, limited availability of donors and adverse risks associated with alloSCT have led to the interest in developing other novel curative approaches for β-thalassemia.

Gene Therapy

To overcome the limitations of HSCT, "gene therapy", the transplantation of a therapeutic gene using autologous, self-HSCs has been rapidly developed as a potential curative approach for β-thalassemia. Theoretically, the use of autologous HSCs eliminates the risk of GVHD and immune-mediated graft rejection, as well as no need for post-transplant immunosuppression, resulting in lower TRM.[40] In recent years, several gene therapies using gene addition or gene editing approaches have been developed to address the underlying mechanisms of β-thalassemia, which has the potential to be a curative treatment for the disease.[41]

Gene addition approach

Given that reduced or absent β-globin synthesis leads to equation β imbalance and consequent ineffective erythropoiesis, gene addition therapy aims to restore β-globin production by introducing the functional β-globin gene into the autologous HSCs, which are then transferred back into the thalassemic patient (Figure 2). The first successful gene addition therapy for β-thalassemia, betibeglogene autotemcel (Beti-cel), was developed using autologous HSCs transduced with the BB305 lentiviral vector, which encodes a modified functional β-globin, $\beta^{A(T87Q)}$, capable of producing Hb A^{T87Q}.[42,43] A clinical trial demonstrated promising outcomes, with approximately 90% of patients with non-β^0/β^0 genotypes achieving transfusion independence and significantly improved hemoglobin levels. However, the limitation of gene addition has been reported, particularly in achieving transfusion independence for most patients with the β^0/β^0-genotype.[44] As a result, the suitability of gene addition approaches for patients with β^0/β^0 genotypes is currently being evaluated. Recently, a modified BB305 vector was introduced to enhance efficacy and reduce variability in the Northstar-3 study (NCT03207009), demonstrating that nearly 90% of patients achieved transfusion independence with a durable response. Another ongoing study is evaluating a GLOBE lentiviral vector harboring wild-type β^A (NCT02453477), though full results have not yet been reported.[45-47]

Gene editing approach

The gene editing approach represents the promising therapeutic approach for β-thalassemia, directly targeting the underlying pathophysiological mechanisms of the disease.[48] Briefly, the gene editing strategies use targeted nucleases to introduce sequence-specific double-stranded DNA breaks (DSBs) within the gene of interest, which are subsequently repaired by endogenous DNA repair mechanisms. The two major cellular repairs of DSBs include nonhomologous end joining (NHEJ), which introduces spontaneous insertions/deletions (Indel) mutations leading to the gene inactivation, and homology-directed repair (HDR), which introduces a donor template to the DSBs region.[49] Currently, advances in gene-editing technologies have enabled precise modifications in the β-globin gene, β-regulatory elements or other targets such as the β-globin gene, providing a potentially curative treatment for thalassemic patients. Several editing strategies have been proposed. The first strategy involves directly correcting specific mutations in the *HBB* gene using HDR-based gene editing to restore normal β-globin production. This strategy introduces a normal β-globin gene template to correct an individual mutation point of the β-globin gene in a precise manner. However, the heterogeneous spectrum of mutations in β-thalassemia and the low efficiency of HDR technique remain substantial challenges. Another gene editing strategy focuses on reducing the equation β-globin imbalance through specific gene inactivation using NHEJ, which offers high on-target efficiency. Accordingly, this approach has been increasingly developed.[48,50] *BCL11A* is considered as one of the most crucial regulators of hemoglobin switching, for which reduction in the enhancer region of the *BCL11A* gene can potentially allow the significant production of HbF in adults, thereby improving equation β-globin imbalance (Figure 2). Consequently, *BCL11A* has emerged as one of the most extensively investigated and promising targets for gene editing in patients with β-thalassemia.[51]

Several gene editing approaches disrupting the *BCL11A* enhancer have been investigated in clinical trials for β-thalassemia using various designed nuclease techniques. The most notable clinical studies involving gene editing in TDT β-thalassemia utilize autologous HSCs with disruption of an erythroid-specific enhancer of the *BCL11A* gene. These include the Thales study (NCT03432364), which employs zinc finger nuclease (ZFN) technology with ST-400, and the CLIMB THAL-111 study (NCT03655678), which uses cluster regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology with exagamglogene autotemcel (Exa-cel, CTX001).[46] The Phase 3 study of

Exa-cel, which includes both β^0/β^0 and non-β^0/β^0 genotype, has shown promising results, with most patients achieving transfusion independence, highlighting the potentially curative nature of gene editing treatment.[52] Another approach to reducing equation β globin imbalance involves decreasing equation gene expression by targeting *HBA* gene enhancers, particularly MCS-R2. Preclinical studies have demonstrated that CRISPR/Cas9-mediated deletion of MCS-R2 significantly reduces equation chain levels, thereby correcting the equation β-globin imbalance. The finding suggests a potential gene editing strategy for treating β-thalassemia by modifying the *HBA* gene; however, clinical studies are still required to validate this approach.[53,54]

Recently, gene therapy strategies, including beti-cel and exa-cel, have been approved for the treatment of β-thalassemia, supported by data from multicenter Phase 3 studies. Since 2022, the US Food and Drug Administration (USFDA) has approved beti-cel for adult and pediatric patients with transfusion-dependent β-thalassemia who are eligible for HSCT but lack a suitable donor. More recently, exa-cel, a CRISPR-based gene editing therapy, has been approved for transfusion-dependent β-thalassemia patients aged 12 years and older who are eligible for HSCT but do not have an HLA-matched related donor. Although genetically modified autologous HSCs eliminate the risk of GVHD and graft failure while overcoming donor availability, several challenges remain to be addressed. These include therapy-related toxicities from potential off-target effects and conditioning regimens, high costs and large-scale accessibility. Additionally, long-term efficacy and safety profiles, particularly the potential risk of mutagenesis, need to be carefully monitored.[55-57]

Pharmacological approaches for β-thalassemia

Advances in understanding the molecular pathophysiology of β-thalassemia have led to the exploration of the novel therapeutic landscape that targets specific pathological processes, providing more precise and effective treatments. This section reviews pharmacological approaches for β-thalassemia, focusing on strategies that target ineffective erythropoiesis, prolong RBC survival, induce HbF and explore the potential roles of microRNA-based therapy.

Targeting Ineffective Erythropoiesis Approaches

Erythroid maturating agent (EMA)

The TGF-β superfamily ligands, including growth differentiation factors and activins, are associated with the failure of late-stage erythropoiesis. Dysregulation of TGF-β superfamily signaling and elevated SMAD2/3 pathway activity in β-thalassemia may suppress late-stage erythroid differentiation, leading to the accumulation of erythroid precursors and ineffective erythropoiesis.[58,59] With an advanced understanding of this molecular mechanism, several therapeutic approaches targeting the TGF-β superfamily have been investigated to enhance late-stage erythroid maturation and improve chronic anemia.[60]

- **Luspatercept**

Luspatercept is the first disease-modifying pharmacological therapy for β-thalassemia, currently approved by the USFDA and the European Medicines Agency (EMA) for the treatment of TDT. The drug is a recombinant fusion protein that acts as the activin receptor type IIB ligand trap binding to the TGF-β superfamily ligands. Consequently, the reduction of the TGF-β signaling and SMAD2/3 signaling promotes late-stage erythroid differentiation mitigating ineffective erythropoiesis and disease complications in β-thalassemia.[60,61] Following encouraging clinical results[62], a Phase 3, randomized controlled study (BELIEVE study, NCT02604433) evaluating the efficacy and safety of luspatercept in patients with TDT and a Phase 2 randomized controlled study (BEYOND study, NCT03342404) in the setting of NTDT have been conducted. The BELIEVE study demonstrated a significant reduction in the transfusion burden among patients with TDT compared to the placebo group. Additionally,the extended phase study has shown continued improvement in transfusion burden and

iron overload among patients receiving luspatercept.[63,64] Similarly, the BEYOND study met its primary endpoint, with nearly 80% of patients achieving an increase in hemoglobin levels of ≥ 1 g/dL from baseline, a statistically significant compared to the placebo group[65] (Table 1). These related clinical studies have demonstrated the promising efficacy of luspatercept in both TDT and NTDT patients, offering a novel therapeutic approach that directly targets the underlying pathophysiology of ineffective erythropoiesis in β-thalassemia. Due to the complexity of determining meaningful responses and the limitation of local resources, some experts have proposed criteria for treatment prioritization to maximize clinical benefit and cost efficiency. TDT patients prioritized for luspatercept treatment include those with non-β^0/β^0 genotypes, those receiving RBC transfusion ≤ 4 units/month, splenectomized patients or those unable to sustain regular transfusion to maintain a targeted hemoglobin level. Patients with progressive iron overload who have intolerance or a poor response to iron chelation therapy are also preferred candidates for luspatercept use.[66]

Regarding the safety profile, adverse events include bone pain, arthralgia, dizziness, hypertension and hyperuricemia. Rare cases of thromboembolic events have been reported, particularly in the splenectomized patients.[63] Previously reported case series of TDT patients treated with luspatercept have mentioned the occurrence of EMH during treatment, particularly among patients with EMH risk factors such as massive splenomegaly and brisk reticulocytosis. However, due to the small numbers of cases and the absence of baseline surveillance, the exact timing of EMH development remains unclear.

Janus Kinase Inhibitors

As mentioned above, the elevated activation of the JAK signaling pathway, mediated by increased EPO levels in response to severe anemia in β-thalassemia, may lead to the expansion of erythroid precursors and erythroid hyperplasia, thereby exacerbating ineffective erythropoiesis.[11,12]

- Ruxolitinib

Preclinical experiments have demonstrated that thalassemic erythroid cells show upregulation of JAK2, along with an abundance of proliferating erythroid cells in the enlarged thalassemic spleen. Studies have also demonstrated that treatment with a JAK2 inhibitor improves ineffective erythropoiesis and reduces splenomegaly in a mouse model. These findings suggest that treatment with ruxolitinib, a JAK1/JAK2 inhibitor, may benefit patients with β-thalassemia.[67,68] A single-arm, multicenter, open-label, Phase 2 clinical study[69] in patients with TDT using ruxolitinib has been conducted. Although the study reported a remarkable reduction in splenic size among patients receiving ruxolitinib, no significant improvement in hemoglobin levels and transfusion requirements, the primary outcome of the study, was demonstrated. Moreover, grades 3 and 4 anemia was reported in 17% of patients receiving ruxolitinib (Table 1). Due to the uncertain clinical benefits, ruxolitinib might be considered in highly selected cases with clinically significant splenomegaly, potentially preventing the need for surgical splenectomy and its long-term complications.[70]

Expanding RBC survival approaches

Pyruvate kinase (PK), a critical enzyme that regulates ATP production via glycolysis, plays a central role erythrocyte energy metabolism. A related in vivo study has demonstrated that PK deficiency may alter erythropoiesis by impairing erythroid precursor differentiation and increasing early apoptosis in the bone marrow. These findings link PK disturbance to ineffective erythropoiesis and altered RBC survival, suggesting that adequate energy metabolism is essential for normal erythropoiesis.[71] Furthermore, reduced ATP in thalassemic RBCs have also been reported, attributed to increased energy demands required to clear globin precipitates and maintain membrane integrity.[72]

This evidence supports the hypothesis that increasing ATP synthesis via PK activation may improve erythropoiesis and thalassemic RBC survival, providing a poten-

Table 1 Summary of pharmacological approaches in β-thalassemia

Drug	Mechanism of Action	Patient Setting	Study Design	Key Outcomes	Study Reference
Luspatercept (BELIEVE trial)	TGF-β superfamily ligand trap	β-thalassemia TDT Aged ≥ 18 years	Phase III Multicenter, randomized, double-blind, placebo-controlled trial	■ Significantly reduced transfusion burden (≥ 33% reduction) compared to placebo	NCT02604433
Luspatercept (BEYOND trial)	TGF-β superfamily ligand trap	β-thalassemia NTDT Aged ≥ 18 years	Phase II Multicenter, randomized, double-blind, placebo-controlled trial	■ Significantly increased Hb levels (≥ 1 g/dL from baseline) and improved fatigue score compared to placebo	NCT03342404
Ruxolitinib	Janus Kinase 1/2 inhibitor	β-thalassemia TDT Aged ≥ 18 years	Phase II Multicenter, single-arm, open-label trial	■ Clinical benefit in splenic size reduction ■ No significantly improved pre- transfusional Hb, and transfusion burden	NCT02049450
Mitapivat (ENERGIZE trial)	Pyruvate kinase activator	β- and α-thalassemia NTDT Aged ≥ 18 years	Phase III Multicenter, randomized, double-blind, placebo-controlled trial	**Preliminary analysis** ■ Significantly increased Hb levels (≥1 g/dL from baseline) and improved fatigue score compared to placebo	NCT04770753
Mitapivat (ENERGIZE-T trial)	Pyruvate kinase activator	β- and α-thalassemia TDT Aged ≥ 18 years	Phase III Multicenter, randomized, double-blind, placebo-controlled trial	**Preliminary analysis** ■ Significantly reduced transfusion burden (≥50% reduction) compared to placebo	NCT04770779
Etavopivat (GLADIOLUS trial)	Pyruvate kinase activator	Thalassemia or SCD Aged 12-65 years **Cohort A** SCD on chronic transfusions **Cohort B** β- and α-thalassemia TDT **Cohort C** β- and α-thalassemia NTDT	Phase II study Multicenter, open-label, trial	**Ongoing study**	NCT04987489
Hydroxyurea	Hemoglobin F inducer	β-thalassaemia TDT Aged ≥ 12 years	Phase II/III Single center, randomized, double-blind, placebo-controlled trial	■ Significantly increased fetal hemoglobin percentage and reduced erythropoietic stress parameters ■ compared to placebo ■ **NO** significantly reduced transfusion burden compared to placebo	SLCTR/2018/024
Thalidomide	Hemoglobin F inducer	β-thalassaemia TDT Aged ≥ 14 years	Phase II Multicenter, randomized, double-blind, placebo-controlled trial	■ Significantly increased Hb levels and reduced transfusion burden compared to placebo in the placebo-control period	ChiCTR1800015702

Hb, hemoglobin; NTDT, non-transfusion-dependent thalassemia; QoL, quality of life; SCD, sickle cell disease; TDT, transfusion-dependent thalassemia; TGF-β, transforming growth factor-beta

tial therapeutic strategy targeting metabolic pathways in ineffective erythropoiesis. Recently, oral drugs that activate PK function have been evaluated, including mitapivat, which activates both pyruvate kinase receptors (PK-R) and pyruvate kinase M2 (PK-M2), and etavopivat, which selectively activates PK-R.[73]

- **Mitapivat**

Mitapivat is an oral PK activator that has demonstrated safety and efficacy in treating patients with thalassemia, including both equation and β-thalassemia.[74] It was previously hypothesized that β-thalassemic RBCs, which have reduced ATP concentrations, may benefit from increased PK activity induced by mitapivat, thereby improving ineffective erythropoiesis and RBC survival.[75,73] Supported by preclinical studies in mouse models of β-thalassemia, mitapivat has shown beneficial effects on ineffective erythropoiesis, chronic hemolytic anemia and iron overload.[76] These findings, together with promising outcomes in a Phase 2 proof-of-concept study, have led to Phase 3 clinical studies evaluating the efficacy and safety of mitapivat in patients with TDT (ENERGIZE-T study, NCT04770779) and NTDT (ENERGIZE study, NCT04770753), including both equation and β-thalassaemia (Table 1).[77]

Recent preliminary results from the ENERGIZE study, focusing on NTDT patients, have demonstrated that mitapivat leads to statistically significant improvements in hemoglobin levels and hemolytic markers compared with placebo.[78] Although interim results from these trials have achieved the primary endpoints suggesting the therapeutic efficacy of mitapivat in thalassemic patients, the reports are still in the preliminary stages. Final results from these studies and extended long-term observation are required to confirm the potential benefits of the treatment.

- **Etavopivat**

Etavopivat, a potent, selective, oral erythrocyte-specific PK activator that increases ATP and decreases 2,3 diphosphoglycerate, has emerged as a potential therapeutic agent for both equation and β-thalassemia. Enhancing PK activity and improving ATP efficiency in erythroid precursors have been shown to promote effective differentiation and reduce apoptosis.[79,80] A Phase 1 study conducted in patients with sickle cell disease (SCD) demonstrated that etavopivat was well-tolerated and led to increased hemoglobin levels and improved markers of hemolysis.[81] Based on the result of the Phase 1 study, a Phase 2 open-label, multicenter study (GLADIOLUS trial, NCT04987489) evaluating the efficacy and safety of etavopivat in TDT, NTDT or SCD is currently ongoing (Table 1).[82]

Inducing Fetal Hemoglobin Approaches

Several studies have previously reported that TDT patients who have coinherited HPFH exhibit reduced clinical severity, suggesting Hb F mitigates ineffective erythropoiesis and globin chain imbalance. In addition, individuals with elevated Hb F levels due to genetic modifiers, such as polymorphisms in the *XmnI*, *BCL11A*, *KLF1* or *HBS1L-MYB* genomic regions, may also correlate with a milder disease phenotype. These findings highlight the potential of HbF induction as a therapeutic target to ameliorate the disease severity of β-thalassemia.[83] Currently, several HbF-inducing agents have been investigated for their ability to reduce anemia and transfusion dependence with fewer side effects. Although numerous promising strategies have been reported, no universally effective HbF-inducing agents have been identified.[84]

- Hydroxyurea

Hydroxyurea (hydroxycarbamide) has long been recognized as an HbF inducer by interfering with various transcription factors, for example, repressing *BCL11A* and *GATA1*, upregulating *GATA2* and also modulating some epigenetic processes.[85] Studies have shown that hydroxyurea can improve ineffective erythropoiesis in β-thalassemia patients, demonstrating safety and tolerability.[82] Furthermore, the positive effects of hydroxyurea in reducing the risk of thalassemia-related complications in NTDT patients have also been reported. However, highly variable effects on hemoglobin and RBC indices

improvement have been observed. In addition, clinical data supporting the consistent efficacy of hydroxyurea for β-thalassemia remain debated.[86,85] The results of the first randomized, double-blind clinical study evaluating the efficacy and safety of hydroxyurea in TDT showed significantly increased HbF levels and reduced ineffective erythropoiesis in the hydroxyurea group. However, the significant improvement in blood transfusion burden, the primary outcome of the study, was not achieved (Table 1).[87] Recently, several genetic polymorphisms, especially *XmnI*, have been found to be associated with an increased response to HbF inducers, suggesting that genetic characterization might be useful for guiding therapeutic options in β-thalassemia patients.[88]

- **Thalidomide**

Thalidomide is known as an immunomodulatory and antiangiogenic agent. Recently, it has emerged as a potential therapeutic strategy for β-thalassemia, capable of increasing HbF production, potentially through its effects on transcription factors such as GATA1 and KLF1, which are involved in β-globin gene regulation. Consequently, the increased HbF production ameliorates globin chain imbalance and reduces ineffective erythropoiesis.[89,90] Although some case reports have shown the positive outcomes with thalidomide use in β-thalassemia patients, only one multicenter randomized study has reported clinical benefits, including increased hemoglobin concentration and reduced RBC transfusion requirements (Table 1).[89,91-93] Additionally, a recent study evaluating combination therapy with thalidomide and hydroxyurea in TDT patients has shown that almost half of the patients can maintain Hb ≥ 9 g/dL with transfusion independence.[94] A recent meta-analysis on the efficacy of thalidomide in β-thalassemia patients has demonstrated significant benefits, particularly in TDT, by reducing transfusion requirements and increasing hemoglobin levels. Together with previous clinical studies, the findings support the role of thalidomide as a potential therapeutic option for β-thalassemia.[95] Studies have also demonstrated that *HBG2* and *HBS1L-MYB* polymorphisms are associated with thalidomide response in Chinese patients and may serve as predictors of treatment response.[96] Although clinical trials have shown promising results, including improved hemoglobin and reduced transfusion, thalidomide use is limited by its possible short-term and long-term adverse effects, such as peripheral neuropathy, thromboembolic events and secondary malignancies. Moreover, thalidomide is contraindicated in pregnant women, and effective contraception must be considered due to its severe teratogenic effects. Thus, thalidomide may be considered as an alternative pharmacological therapy in selected cases where the benefits outweigh the risks. Additionally, it should be noted that most clinical studies have been conducted in adults, and available information for pediatric patients remains limited.[97]

- **Other hemoglobin F inducers**

Sirolimus, also known as rapamycin, has been shown to induce γ-globin gene expression and increase HbF production in erythroid precursor cells from β-thalassemia patients. More recently, additional mechanisms inhibiting mammalian targets of rapamycin (mTOR) by sirolimus have been found to activate *ULK1* and autophagy, leading to a reduction in free α-globin accumulation. Ultimately, these related studies suggest a potential therapeutic strategy using sirolimus that may improve the clinical status of β-thalassemia.[98-100] Currently, ongoing Phase 2 pilot clinical trials, NCT03877809 and NCT04247750, are being conducted to evaluate the biochemical parameters and clinical outcomes of sirolimus-based therapy in TDT.[101]

Previously, some short-chain fatty acids, such as butyrates, a class of histone deacetylase inhibitors, have been demonstrated to increase γ-globin gene expression and HbF levels in erythroid cells from β-thalassemia patients. However, individual responses to butyrates have been inconsistent and insufficient.[102,103] Other HbF inducers, including DNA hypomethylating agents such as 5-azacytidine and decitabine, have also been reported to improve hemoglobin levels; however, long-term safety

remains a concern, and their roles in the management of β-thalassemia are not well established.[37,104]

MicroRNA-Based Approaches

Micro ribonucleic acids (RNAs), or miRNAs, are small, non-coding RNAs that play a critical role in the post-transcriptional regulation of gene expression to maintain optimal levels of targeted proteins. Currently, several miRNAs have been demonstrated to be involved in β-thalassemia.[105] The role of miRNAs in Hb F regulation in β-thalassemia has been recently investigated, demonstrating that miR-210-3p, miR-92a-3p, miR-30a and miR-486-3p are positive regulators of γ-globin gene expression, primarily through the suppression of *BCL11A* messenger RNAs (mRNAs). The expression pattern of these miRNA expression should be further studied to assess their potentials in predicting responses to Hb F inducers.[106-109] Dysregulation of several miRNAs has been shown to contribute to ineffective erythropoiesis in β-thalassemia by targeting proteins involved in erythroid maturation. For example, miR-15a and miR-16-1 can suppress the anti-apoptotic protein Bcl-2, leading to increased apoptosis of erythroid precursors, while miR-144 inhibits the erythroid transcription factor, GATA1, resulting in impaired erythroid differentiation.[110,111] Overexpression of miR-27a targets the erythroid transcription factor KLF8, causing impaired differentiation of erythroblast cells. Additionally, the miR-99a/let-7e/miR-125a cluster has been shown to be upregulated, leading to increased reactive oxygen species and death of thalassemic erythroblasts.[112,113] Although miRNA-based therapies have not been specifically studied in clinical trials for β-thalassemia, several miRNA-based therapies, including miRNA mimics or anti-miRNAs, are ongoing in the preclinical development.[114] Investigating these dysregulated miRNAs may potentially lead to novel therapeutic approaches that ameliorate the clinical severity of β-thalassemia. However, a better understanding of the numerous targets and interactions of miRNAs is essential for optimizing the use of miRNA-based therapies. Further research is required to develop novel therapeutic strategies for the management of β-thalassemia.

Conclusion

β-thalassemia is among the best-understood disorders at the molecular level. Understanding the pathophysiology and molecular basis of the disease has led to the development of emerging novel therapeutic approaches, including both curative and pharmacological therapies. Given the substantial number of available novel therapeutic approaches, along with improvements in conventional therapies, β-thalassemia patients have the potential to achieve significantly better outcomes and quality of life. The efficacy and safety of these novel treatments are currently being evaluated by several clinical trials, and ongoing research is essential for the continued development of innovative therapeutic approaches. Long-term outcomes and comparative studies will be crucial in the future to determine the optimal and personalized use of these treatments. Additionally, accessibility to these therapies remains a significant challenge in resource-limited countries where β-thalassemia is more prevalent.

Author contribution

All authors contributed to manuscript drafting or critical review and final approval for submission.

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