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REVIEW

Emerging therapies in β-thalassemia: toward a new era in management

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ABSTRACT

Introduction: The thalassemias are among the most common inherited monogenic diseases worldwide, characterized by autosomal recessive inherited defects in the production of hemoglobin. Currently available conventional therapies have many challenges and limitations. Advances in understanding the underlying pathophysiology of β-thalassemia enabled clinicians and researchers to move toward the development of novel therapeutic modalities. These can be classified into three categories based on their efforts to address different features of the underlying pathophysiology of β-thalassemia: correction of the globin chain imbalance, addressing ineffective erythropoiesis, and improving iron overload.

Areas covered: In this review, we will provide an overview of the novel therapeutic approaches that are currently in development for β-thalassemia.

Expert opinion: A thorough understanding of the pathophysiology and overall disease burden of β-thalassemia has aided clinicians and scientists to optimize disease management approaches and construct a plan for the development of novel therapies, with ultimate goals of prolonging longevity, reducing symptom burden, improving compliance and adherence for a better quality of life.

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Thalassemia; ineffective erythropoiesis; iron overload; hepcidin; JAK2; novel therapies; gene therapy; luspatercept; genome editing; sotatercept

1. Introduction

The thalassemias are among the most common inherited monogenic diseases worldwide, characterized by autosomal recessive inherited defects in the production of hemoglobin (Hb) [1]. Most thalassemia patients are born in resource-limited areas like, the Mediterranean region, sub-Saharan Africa, the Middle East, Indian subcontinent, and East and Southeast Asia [2–4]. Because of continued migration activity, however, thalassemia is becoming increasingly common in Europe and North America, making this disease a global health burden [5–7]. The disease hallmarks include ineffective erythropoiesis, chronic hemolytic anemia, and iron overload [8]

Thalassemia patients are commonly categorized today based on their need for blood transfusion. Transfusion-dependent thalassemia (TDT) patients frequently present with severe anemia in early childhood, and require lifelong transfusion treatment to survive [9]. Non-transfusion dependent thalassemia (NTDT) patients commonly present with mild to moderate anemia in a later stage of childhood or even in adulthood, and may require occasional or short-course transfusions for the management or prevention of some disease manifestations [9].

Major advancements in transfusion programs, and iron chelation therapy administration strategies have increased the life expectancy of β-thalassemia patients and improved their overall quality of life (QoL). Currently available conventional therapies have many challenges and limitations. Advances in understanding the underlying pathophysiology of β-thalassemia enabled clinicians and researchers to move toward the development of novel therapeutic modalities. These can be classified into three major categories based on their efforts to address different features of the underlying pathophysiology of β-thalassemia: correction of the globin chain imbalance, addressing ineffective erythropoiesis, and improving iron overload. In this review, we will provide an overview of the novel therapeutic approaches that are currently in development.

2. Conventional management

Before discussing the novel therapeutic strategies in the field, it is important to mention that different conventional modalities for the management of TDT and NTDT exist. These have been and still are being used in many different parts of the world and include blood transfusion, splenectomy, iron chelation therapy, and, for a subgroup of patients, hematopoietic stem-cell transplantation. While these modalities have their benefits and limitations, they remain the mainstay of treatment and they form the basis of the currently available guidelines.

2.1. Transfusion

In β-thalassemia patients, blood transfusion acts as a regulator by providing normal erythrocytes and subduing irregular erythropoiesis [10–12]. Long-term follow-up studies conducted on different cohorts of TDT patients showed that advances

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Article highlights

- Several novel therapeutic targets in β-thalassemia have been identified that can ameliorate the genetic defect, ineffective erythropoiesis, anemia and transfusion-dependence.
- Targeting ineffective erythropoiesis through activin II receptor traps has the potential to decrease transfusion requirement in TDT and increase Hb levels in NTDT patients.
- Preliminary data of the use of JAK2 inhibitors in β-thalassemia are encouraging but further research is needed to establish effects and utility.
- Therapeutic strategies aiming at improving iron dysregulation such as mini-hepcidin and TMPRSS6 inhibitors are also showing promise.
- The use of gene therapy through globin lentiviral vectors present the most advanced approach for treating hemoglobinopathies such as β-thalassemia.
- Genome editing approaches to inhibit the BCL11A gene have also been described and are currently in pre-clinical stage.

in transfusion and iron chelation therapy have been correlated with improvements in survival [13,14]. Patients with TDT require lifelong, regular blood transfusions administered every 2–5 weeks to maintain pre-transfusion Hb levels of at least 9–10.5 g/dL. The use of blood transfusion in NTDT patients has been limited to acute signs as when patients experience acute stress or a decrease in Hb concentration, such as during infection, pregnancy, or surgery. Transfusion therapy, however, does not come without side effects of its own. Although the risk of alloimmunization and blood-borne infection remain a problem in transfused patients, secondary-iron overload is the biggest challenge with routine transfusion therapy [15,16]. Maintaining pre-transfusion Hb levels above 9 g/dL can be sometimes difficult, especially that many patients around the world have limited access to regular and safe blood transfusions. A lack of voluntary non-remunerated blood donor, poor awareness of thalassemia, a lack of national blood policies, and fragmented blood services contribute to a significant gap between the timely supply of, and demand for, safe blood. In many centers, there also exists an inadequate provision of antigen testing [17]. Policies to raise awareness and increase the use of red blood cell (RBC) antigen testing and requesting of compatible blood in transfusion centers are needed to reduce alloimmunization, which limits the effectiveness of transfusions and the potential availability of blood [17].

2.2. Splenectomy

Splenectomy has conventionally been performed in β-thalassemia patients as an alternative to blood transfusion [8,18]. Studies have shown a significant improvement in the QoL and Hb concentration in splenectomized patients. However, many observational studies have showed serious adverse events in those patients. It was reported that splenectomy increases the risk of infections and sepsis, increases risk of thrombosis by 4–5 folds, as well as other vascular complications such as pulmonary hypertension, leg ulcers, and strokes [19,20]. It also decreases the ability of the body to scavenge toxic-free iron species. Therefore, indications have been made in order to confine this procedure for patients with hypersplenism that lead to cytopenia, symptomatic splenomegaly with left upper quadrant pain, early satiety, and an increased blood requirement [21,22]. Furthermore, a special consideration should be offered to the patients when needed, such as proper vaccinations, antibiotics, and thromboprophylaxis with aspirin or low molecular weight heparin for patients at high risk [21,22].

2.3. Iron chelation therapy

The goal of iron chelation therapy is to maintain a safe iron level at all times. Appropriate use of chelators and dose adjustment is mandatory to control iron overload. Three chelators are currently available: deferoxamine in subcutaneous or intravenous injection, oral deferiprone as tablet or solution form three times daily, and deferasirox used once daily in the form of dispersible tablet and more recently as film coated tablet. Serum Ferritin, liver iron concentration (LIC), and cardiac T2* are the key factors controlling the decision to start, adjust, and stop iron chelation therapy. As first line of treatment, the following is recommended: deferoxamine 30–60 mg/kg/day, administered over a span of 8–12 h a day, 5–7 days a week; or deferasirox 20–40 mg/kg/day administered once daily. For second-line treatment, and when ICT with deferasirox or deferoxamine is inadequate, deferiprone is given at a dose of 75–100 mg/kg/day divided over three doses. In TDT patients, deferoxamine led to a reduction in serum ferritin levels and liver iron concentration, improvement in cardiac T2* and improvement in cardiac dysfunction with continuous infusion [23,24]. The use of deferiprone alone or in combination with deferoxamine has been shown to improve cardiac T2* and cardiac dysfunction in TDT patients [25,26]. Moreover, when combined with either deferoxamine or deferasirox, deferiprone showed improvement in endocrine dysfunction [27,28]. As for deferasirox, its use in TDT patients was associated with a reduction in serum ferritin and liver iron concentration after up to 5 years, and cardiac T2* after up to 3 years of therapy, even in patients with severe iron overload; in addition to improvements in cardiac T2*, hepatic fibrosis, and inflammation [29–32]. Indications to intensify iron chelation therapy in TDT: include: serum ferritin levels ≥2500 ng/mL and/or LIC > 7 mg/g dry wt. and/or cardiac T2* < 20 msec. In TDT patients, the indication to stop iron chelation therapy include SF < 300 ng/mL and/or LIC <3 mg/g dry wt.

Deferoxamine was the first chelator to be studied in NTDT [33,34]. While deferoxamine was able to generate significant urinary iron excretion in NTDT patients, its demanding subcutaneous administration and association with pain inconvenience led to poor compliance and adherence. Data on the use of deferiprone in NTDT are restricted to case series and small studies only. Deferasirox, on the other hand, is the only iron chelator to receive FDA and EMA approval in NTDT, where it showed significant decrease in serum ferritin levels and liver iron concentration after up to 2 years of therapy with a starting dose of 10 mg/kg/day. Indications to intensify iron chelation therapy in NTDT include LIC after 6 months of treatment >7 mg/g dry wt. or serum ferritin levels >1500–2000 ng/mL and <15% decrease from baseline. Indications to stop/discontinue iron chelation therapy in NTDT patients include a serum ferritin <300 ng/mL and/or LIC <3 mg/g dry wt.

2.4. Hydoxyurea

Hydroxyurea is a cytotoxic antimetabolic and anti-neoplastic drug agent that increases hemoglobin F (HbF) levels. Data from early case reports have suggested that hydroxyurea may be beneficial for β-thalassemia patients [35]. Hydroxyurea has been shown to exert a 2–9 fold increase in γ-mRNA in patients with β-thalassemia [36–40]. This increase improves α- and non-α chain imbalance and leads to more effective erythropoiesis [37]. However, subsequent small studies among heterogeneous β-thalassemia populations have provided inconsistent findings, and suggested a decline in hematological response with long-term hydroxyurea treatment [35]. While there are no randomized clinical trials that recommend an evidence-based use of hydroxyurea, its use is limited to only certain group of NTDT patients [22].

2.5. Hematopoietic stem-cell transplantation

Hemopoietic stem-cell transplantation remains the only approach to cure patients with β-thalassemia today, particularly when a matched donor is available [8,18]. The majority of patients who undergo transplant from human leukocyte antigen (HLA) matched sibling donor reach 80% survival rate [41]. Moreover, nowadays with the improvement in the control of graft versus host disease and graft rejection, the use of unrelated donors or umbilical cord blood has become possible. However, the risk of mortality from the conditioning of this procedure remains 5–10% which makes it an important concern for the patient [41].

3. Novel therapies

In recent years, a better understanding of the pathogenesis and clinical impact of both TDT and NTDT has encouraged a lot of research into several promising therapeutic approaches. Figure 1 provides a summary of the pathogenesis of β-thalassemia, with its associated clinical manifestations and targets for the novel therapeutic strategies.

3.1. Correction of the a/β globin chain imbalance

3.1.1. Gene therapy

When treating β-thalassemia, the use of autologous, and genetically modified hematopoietic stem cells (HSCs) by gene therapy provides an alternative to allogeneic HSCT. Upon the isolation of hematopoietic stem and progenitor cells (HSPCs), exogenous β- globin genes are then incorporated into the host-cell genome using a lentiviral vector [42]. These genetically modified autologous HSPCs, after full or partial myeloablation, are then returned to the patient where they repopulate in the hematopoietic compartment [42,43]. For gene therapy to be successful procedure in β-thalassemia the following are requirements: high-efficiency HSC engraftment, and gene transfer, high-level expression of β/γ-globin gene and safe expression, with minimal to no risk of insertional mutagenesis. The use of gene therapy technology has proven to be effective and curative in many animal models of β-thalassemia [44–47].

Advancements in the safety profile of lentiviral vectors and the discovery of the β-globin's locus control region (LCR) have made gene therapy possible in β-thalassemia. The first clinical study on gene therapy (LG001) was conducted by Cavazzana-Calvo et al. in a young male patient with transfusion-dependent β^E/β^0-thalassemia [48]. A myeloablative conditioning regimen was

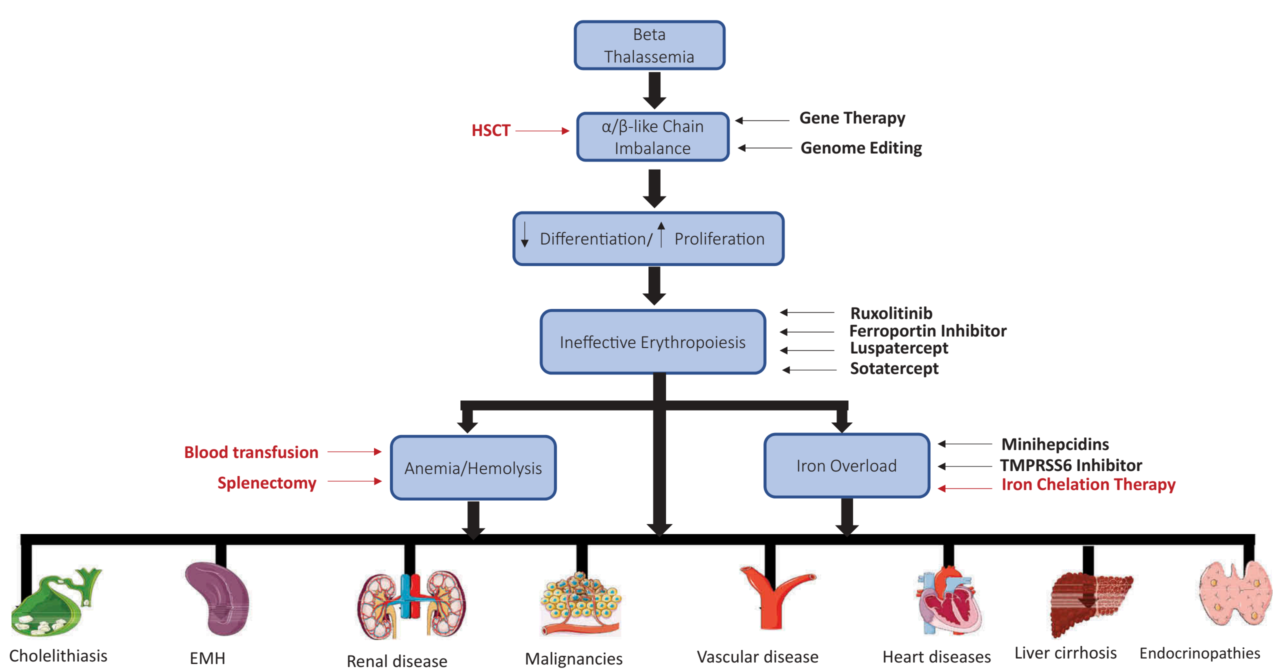

Figure 1. Pathogenesis of β-thalassemia, with its associated clinical manifestations and targets for the novel and conventional therapeutic strategies. Dark red color: Conventional therapies; Black color: Novel therapies.

used, and was followed by a bone marrow–derived HSPC, transduced with a βA-T87Q vector named HPV569 [48–50]. One year after gene therapy, the patient became transfusion independent, and exhibited an increase in Hb variants, including HbF with equal proportions of HbA containing the T87Q mutation [48].

Then came the TNS9 trial (ClinicalTrials.gov number NCT01639690), which is a phase 1 study treating TDT patients with autologous CD34+ hematopoietic progenitor cells transduced with TNS9, a lentiviral vector encoding the normal human ß-globin gene [51]. Three patients had β^0/β^+-thalassemia and one patient had β^0/β^0 thalassemia. Three patients received an 8 mg/kg dose of busulfan, and the fourth patient received a dose of 14 mg/kg of busulfan. The patients received 8.3 to 12.0 $CD34^+$ TNS9.3.55 cells/kg [51]. The transduction in the final $CD34^+$ cell product had a vector copy number (VCN) of 0.25. No toxicity profile greater than grade 3 was observed. All patients showed durable and stable gene marking. There was no evidence of clonal dominance. One patient experienced a significant decrease in transfusion requirements that lasted for more than 5 years.

Additional data for gene therapy in TDT patients came from the HGB-204 and HGB-205 trials (ClinicalTrials.gov numbers, NCT01745120 and NCT02151526). In these two trials, autologous CD34+ cells were obtained from 22 TDT patients and the cells were transduced ex vivo with LentiGlobin BB305 vector. Patients received a 12.8 mg/kg of myeloablative busulfan followed by 5.2 to 18.1 $CD34^+$ $BB305^+$ cells/kg [52]. The transduction VCN was 0.7 copies. Approximately 26 months after the infusion process, almost all patients with a non-β^0/β^0 genotype stopped receiving transfusions [52]. Moreover, all biologic markers of dyserythropoiesis were corrected. The median annual transfusion volume decreased by 73% in 9 patients with a β^0/β^0 genotype, and transfusions were discontinued in 3 patients [52]. Adverse events from the treatment were similar to those associated with autologous stem-cell transplantation. The HGB 212 and HGB 207 trials (ClinicalTrials.gov numbers NCT03207009 and NCT02906202) are currently ongoing phase III studies using the BB305 vector for TDT subjects with a β^0/β^0 genotype and non-β^0/β^0 genotypes.

In a more recent study by Marktel et al., three adult patients and six pediatric patients with β^0 or severe β^+ mutations were enrolled and treated in a phase 1/2 trial (ClinicalTrials.gov number NCT02453477) with an intrabone administration of HSCs transduced with the lentiviral vector GLOBE, which removes the HS4 locus control region element. A quick hematopoietic recovery with polyclonal multilineage engraftment of vector-marked cells was attained, a VCN per cell of 0.58 in erythroid precursors at 1 year, with no presence of any clonal dominance [53]. In the adult patients, transfusion requirement decreased [53]. Of the four pediatric patients, three discontinued transfusions post-gene therapy and eventually became transfusion independent [53].

A summary of all lentiviral β-thalassemia trials is provided in Table 1

3.1.2. Genome editing

Newer approaches have been in development to correct genetic mutations at the DNA level of the cell or to disrupt specific DNA sequences in the genome [43]. Known as genome editing, this approach relies on enzymes that play a role in introducing DNA breaks in specific sections of the genome. BCL11A has been shown to be a potential target for genome editing [42]. A mouse model with a specific erythroid enhancer deletion led to an impaired expression of BCL11A in erythroid precursors without affecting other hematopoietic lineages [54]. Many in vitro studies have shown that knock-out gene-editing technology could permanently produce HbF in adults with thalassemia [55–57]. Knock-down studies of BCL11A using RNA interference (RNAi) showed it to be an effective method to silence the γ-globin gene [58–60]. A new approach to induce HbF by silencing BCL11A involves the use of an erythroid-specific promoter to drive a microRNA-adapted short hairpin (shRNA) [56,61]

Other gene-editing approaches to inhibit BCL11A that have been described and are currently in pre-clinical stages include: clustered regularly interspaced palindromic repeats (CRISP) in association with Cas9 (CRISP/Cas9), transcription activator-like

Table 1. Lentiviral β- thalassemia trials.

Gene	Vector	Location	National Clinical Number (NCT)	Study Type	Condition	Start Date	Status
$\beta^{A\text{-}T87Q}$-Globin	HPV569	France	LG001	Phase 1/2	β-thalassemia or severe sickle cell disease	September 2006	Terminated
β-globin	TNS9.3.55	United States	NCT01639690	Phase 1	Beta-Thalassemia Major	July 2012	Active, not recruiting
$\beta^{A\text{-}T87Q}$-Globin	LentiGlobin BB305	United states, Australia, Thailand	NCT01745120 (HGB-204)	Phase 1/2	Beta-Thalassemia Major	December 2012	Completed
$\beta^{A\text{-}T87Q}$ Globin	LentiGlobin BB305	France	NCT02151526 (HGB-205)	Phase 1/2	Beta-Thalassemia Major; Sickle Cell Disease	May 2014	Completed
β-Globin	GLOBE	Italy	NCT02453477	Phase 1/2	Transfusion Dependent Beta-thalassemia	May 2015	Active, not recruiting
$\beta^{A\text{-}T87Q}$-Globin	LentiGlobin BB305	United States, France, Germany, Greece, Italy, United Kingdom, Thailand	NCT02906202 (HGB-207)	Phase 3	Transfusion-Dependent β-Thalassemia, who do not have a β^0/β^0 genotype	September 2016	Recruiting
$\beta^{A\text{-}T87Q}$-Globin	LentiGlobin BB305	United States, France, Germany, Greece, Italy, United Kingdom	NCT03207009 (HGB-212)	Phase 3	Transfusion Dependent Beta-thalassemia	July 2017	Recruiting
β-globin	OTL-300	Italy	NCT03275051	Phase 1/2	Transfusion Dependent Beta-thalassemia	September 2017	Enrolling by invitation

effector nucleases (TALENS), and zinc finger nucleases (ZFNs) [62–65]. Several studies using CRISP/Cas9, TALENS, and ZFNs have also shown to be effective in both patient-derived CD341 cells and induced pluripotent stem cells (iPSC) [66–69].

Currently, there is an ongoing phase 1/2 study trial where CRISPR/Cas9 technology is being used to target an erythroid-specific enhancer of the BCL11A gene in up to 12 TDT patients (ClinicalTrials.gov number NCT03655678). This study will evaluate the safety and efficacy of autologous CRISPR-Cas9 Modified CD34+ human HSPCs using CTX001. Primary endpoints include: a) proportion of subjects achieving transfusion reduction for ≥6 months, b) proportion of subjects with engraftment, c) time to neutrophil and platelet engraftment, d) severity and frequency of adverse events (AEs), e) incidence of transplant-related mortality, and f) all-cause mortality.

Other genome editing approaches include the direct silencing of α-globin. In 2017, Mettananda et al created a targeted mutation of the MCS-R2 core element in human HSCs using CRISPR/Cas9 genome editing technology to knockdown α-globin expression [70]. They demonstrated the successful knockdown of α-globin expression *in vitro* in erythroid cells generated by genome-edited HSCs without perturbing erythroid differentiation or having detectable off-target events [70]. Another 2017 study showed that IOX1, a pan-histone demethylase inhibitor selectively downregulates α-globin expression without perturbing erythroid differentiation or general gene expression, more specifically β-like globin expression [71]. IOX1 caused a dose-dependent decrease in α-globin expression, whereas the expression of β-globin was largely unaffected. IOX1 also downregulated α- and other α-like globin genes and reduced cell expansion by about 40%. The overall data from this data showed that IOX1 did exert the desired changes in erythroid cells and could potentially develop as a novel therapy in β-thalassemia [71]. Most recently, the synergistic silencing of α-globin and induction of γ-globin by histone deacetylase inhibitor, vorinostat was shown to be a potential therapy for β-thalassemia [72]. In this study, vorinostat demonstrated a dose-dependent down-regulation of α-globin expression at low nanomolar concentrations without significant changes in erythroid cell growth or viability and with only mild changes in erythroid differentiation [72].

3.2. Addressing ineffective erythropoiesis

3.2.1. Activin II receptor traps: sotatercept (ACE-011) and luspatercept (ACE-536)

Growth differentiation factor 11 (GDF-11) has a critical role as a potential regulator in erythropoiesis [73]. GDF-11 shows a higher expression in immature erythroblasts in β-thalassemia. Abnormal production of this ligand is the principal cause of the proliferation of erythroid progenitors and the increase of oxidative stress, followed by preventing the maturation of late erythroid precursors and ineffective erythropoiesis [74]. Targeting GDF11-ActRIIB-Smad2/3–dependent signaling, by blocking the interaction of GDF-11 with activin receptors could reduce the amount of reactive oxygen species (ROS) and α-globin aggregates, thus inducing terminal erythroblast maturation [75,76]. This pathway has been inhibited through the development of agents that are capable of extracellularly binding to ligands and preventing their signaling cascades, known as Activin receptor-II binding traps. Two agents have been described, Sotatercept (ACE-011) and Luspatercept (ACE-536).

In order to determine if sotatercept can clinically benefit patients with diseases of ineffective erythropoiesis, a phase 2a, multicenter, open-label, dose-finding study was conducted in β-thalassemia [74]. This study by Cappellini et al. was conducted on 16 TDT patients and 30 NTDT patients. The median duration of treatment was 13.8 months for TDT patients and 19.6 months for NTDT patients. All patients were provided with two or more doses of sotatercept. The drug was up to be administrated at doses of 0.1, 0.2, 0.3, 0.5, 0.75, or 1.0 mg/kg, by subcutaneous injection every 3 weeks. Results showed that in TDT patients, 63% achieved ≥20% reduction of transfusion burden, sustained for ≥24 weeks; 44% achieved a reduction of ≥33%, and 13% a reduction of ≥50% [74]. In TDT patients, the mean change in Hb levels from baseline to the end of treatment was 0.7 g/dL and the active dose of sotatercept was ≥0.5 mg/kg [74]. Sixty percent of NTDT patients achieved a mean Hb increase of ≥1.0 g/dL, and 37% had a mean Hb increase of ≥1.5 g/dL, sustained for ≥12 weeks [74]. The starting active dose of the drug in NTDT patients was ≥0.3 mg/kg.

ACE-536 or Luspatercect, on the other hand, binds with high affinity with TGF-β ligands, such as GDF-11 and GDF-8. Its administration to β-thalassemia mice improved intracellular accumulation of hemichromes, oxidative stress, and splenomegaly and had a significant role in achieving terminal erythroid maturation and decreasing ineffective erythropoiesis. This was achieved by increasing the transcription of genes, inducing erythroid differentiation, and processing of unpaired α-globins [77,78]. In a phase 1 study involving healthy post-menopausal women, luspatercept was found to increase Hb levels [79]. A subsequent open-label, dose-ranging phase 2 study was conducted and showed that luspatercept increases Hb levels in NTDT patients and reduce transfusion burden in TDT patients [80]. The transfusion burden (the total number of RBC units transfused) during any 12-week interval was reduced by at least 20% from baseline in 26 of 32 patients with TDT (81%) and in 18 of 31 patients with NTDT (58%) who received luspatercept at a dose of 0.60 to 1.25 mg/kg [80].

A phase 3, randomized, double-blind, placebo-controlled study also conducted by Cappellini et al. in order to evaluate the efficacy and safety of luspatercept in adult TDT patients receiving regular RBC transfusions [81]. The BELIEVE trial (ClinicalTrials.gov number NCT02604433) included 336 patients, 18 years of age or older, requiring regular transfusion of 6 to 20 RBC units in the 24 weeks before randomization. Patients were randomly assigned in a 2:1 ratio to receive either luspatercept or placebo subcutaneously every 21 days for at least 48 weeks [81]. The starting dose level was 1.0 mg/kg and titrated up to 1.25 mg/kg. The primary endpoint was a reduction of ≥33% in transfusion burden during weeks 13 through 24 and a reduction of at least 2 red-cell units over this 12-week interval. A reduction of ≥33% reduction in RBC transfusion burden was detected in 44 of 224 patients receiving luspatercept, at weeks 37–48, compared to 4 of 112 patients receiving placebo.

Seventeen and 23 patients of 224 receiving luspatercept achieved a reduction of ≥50% in RBC transfusion burden at weeks 13–24 and 37–48, respectively, compared with 2 and 1 of 112 patients receiving placebo. In conclusion, the percentage of TDT patients who had a reduction in transfusion burden was significantly greater in the luspatercept group than in the placebo group. Luspatercept was well tolerated in this population and no deaths reported. Reported adverse events that were more common with luspatercept than placebo included transient bone pain, arthralgia, dizziness, hypertension, and hyperuricemia [81]. On 8 November 2019, the Food and Drug Administration (FDA) approved luspatercept for treatment of anemia in adult β-thalassemia patients with who require regular RBC transfusions.

Along with the same concept, a phase 2, double-blind, randomized, placebo-controlled, multicenter study is currently ongoing and aims at determining the efficacy and safety of luspatercept (ACE-536) versus placebo in adults NTDT (BEYOND trial) (ClinicalTrials.gov number NCT03342404). The primary endpoint is to achieve an increase from baseline ≥1.0 g/dl in mean of Hb values after 13 weeks (between 12 and 24 weeks), in the absence of transfusions.

Table 2 compares the two current ongoing clinical trials on luspatercept.

3.2.2. JAK2 inhibitor

Preclinical studies by Libani et al. showed that Janus kinase 2 (JAK2) inhibition not only improved ineffective erythropoiesis but also reversed splenomegaly in an NTDT mouse model [82]. More recent data from a study conducted by Casu et al. on TDT and NTDT mouse models also showed that JAK2 inhibitors are able to decrease splenomegaly [83]. These findings have suggested that the use of ruxolitinib, a JAK1/JAK2 inhibitor, could benefit β-thalassemia patients. A single-arm, multicenter, 30-week Phase 2a study by Taher et al evaluated the efficacy and safety of ruxolitinib in 30 TDT patients (TRUTH Study) [84]. A decrease in spleen size from baseline was observed in ruxolitinib-treated patients. There was also a mean change in spleen volume from baseline to week 12 (n = 26) and week 30 (n = 25) [84]. At week 30, one patient who initially had a 15% decrease in spleen volume at week 12 demonstrated an increase in spleen volume [84]. There was however no clinically significant improvements in pre-transfusion Hb, thus no related reduction in transfusion needs. Moreover, although hepcidin levels increased in the ruxolitinib treatment group, no significant changes in iron parameters were observed over time [84]. For all the above-mentioned reasons, the study did not proceed into a Phase III.

Table 2. Current ongoing clinical trials using luspatercept.

	BELIEVE	BEYOND
Molecule	Luspatercept	Luspatercept
Study Protocol	ACE-536-B-THAL-001	ACE-536-B-THAL-002
Study Type	Phase 3	Phase 2
National clinical number (NCT)	NCT02604433	NCT03342404
Condition	TDT	NTDT
Location	Lebanon, Greece, Italy, Thailand, UK, USA	Lebanon, Canada, France, Greece, Israel, Italy, Malaysia, Taiwan, Thailand, Tunisia, Turkey, UK, USA
Primary objective	Determine the proportion of subjects treated with Luspatercept plus best supportive care (BSC) versus placebo plus BSC who achieve an erythroid response defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs/time) with a reduction of at least 2 units from week 13 to week 24	Evaluate the effect of Luspatercept on anemia as measured by mean Hemoglobin concentration in the absence of transfusion over 12 week interval from week 13 to week 24 compared to baseline
Age	Above 18	Above 18
Start Date	May 2016	February 2018
Status	Active, not recruiting	Active, not recruiting

3.2.3. Ferroportin inhibitor

A more recent approach to target ineffective erythropoiesis involves the use of ferroportin inhibitors. Ferroportin is the only known exporter of iron which releases it into the plasma. It is constitutively expressed on the cell surface and is a target of hepcidin. It binds directly to the receptor triggering the latter's internalization and degradation. This, in turn, will lead to an increase in ferritin levels. In theory, decreasing levels of ferroportin will result in an increase in cytoplasmic iron in target cells (e.g. hepatocytes, macrophages, and enterocytes). This increase in iron will trigger a negative feedback loop which would supposedly suppress the absorption of iron by these cells. This decrease in absorption coupled with the continued utilization of plasma iron mainly for erythropoiesis and Hb production will culminate in restoration of the system to a steady state. Control of the ferroportin concentration can be mediated either by ligand-induced internalization and degradation through affecting synthesis or directly inhibiting ferroportin receptors.

Manolova et al defined the profile and mode of action of the compound VIT-2763, a small oral molecule that acts as a ferroportin inhibitor [85]. Experiments in $Hbb^{th3/+}$ mice showed that VIT-2763 improved anemia and ameliorated erythropoiesis by remarkably reducing the aggregation of α-globin in RBC [85]. The drug also decreased oxidative damage by reducing the number of mitochondria-containing RBC and subsequently lowering levels of ROS. VIT-2763 also improved tissue oxygenation in $Hbb^{th3/+}$ mice, thus inhibiting the hypoxic vicious cycle behind the ineffective erythropoiesis in β-thalassemia and lowering erythropoietin (EPO) production. The drug also prevented iron overload in liver. Furthermore, after 3 weeks of dosed VIT-2763, an important amelioration of myelopoiesis was noted in spleen of $Hbb^{th3/+}$ mice [85]. A phase II study looking at the efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of VIT-2763 will be soon initiated in patients with NTDT.

3.3. Improving iron overload

While iron chelators are still being used around the world for the management of iron overload, their success has been somewhat limited by either their availability, efficacy, or safety issues. Some iron chelators have also been associated with adverse events that require close and continuous monitoring. Despite advancements in iron chelation therapy, adherence and compliance with treatment also continue to be a problem especially in adolescents and young adults. For all the above-mentioned reasons current ongoing clinical trials are trying to address and correct iron dysregulation with novel approaches, specifically by targeting hepcidin. These include minihepcidins and transmembrane protease serine 6 (TMPRSS6) inhibitors.

3.3.1. Minihepcidins

Hepcidin limits both iron utilization and absorption. Preclinical studies have proposed that minihepcidicns could restrict iron absorption and thereby be utilized to address iron overload, with favorable effects on ineffective erythropoiesis. Casu et al showed that minihepcidin ameliorated ineffective erythropoiesis, anemia, splenomegaly, and iron overload in young $Hbb^{th3/+}$ mice [86]. In old $Hbb^{th3/+}$ mice, a combined administration of minihepcidin with the iron chelator deferiprone also improved ineffective erythropoiesis, and anemia and reversed splenomegaly [86].

Many ongoing studies are currently assessing the efficacy and safety of minihepcidin. The first is a phase 2 multicenter, randomized, open-label, parallel-group study with LJPC-401 for the treatment of myocardial iron overload in adult TDT patients (ClinicalTrials.gov number NCT03381833). The primary objective of the study is to evaluate the effect of LJPC-401 on iron levels in adult TDT patients that suffer from myocardial iron overload. Recently, however, this trial was prematurely terminated, as an interim analysis on the safety and efficacy data showed an absence of efficacy with the protocol treatment regimen, thus indicating an unfavorable risk-benefit profile for patients.

Another phase 2 study of PTG-300 in NTDT and TDT subjects with chronic anemia (TRANSCEND trial) is also currently ongoing (ClinicalTrials.gov number NCT03802201). This is a study with dose escalation by subject cohort and with the potential for individual titration (dose increase or decrease) within each cohort. The study aims to look at the safety profile of PTG-300 in order to obtain preliminary evidence of efficacy of PTG-300 for the treatment of chronic anemia in β-thalassemia and to evaluate the appropriate dosing regimen for PTG-300 in the target population. Primary outcome measures of this study include: a) NTDT subjects who achieve an increase in Hb without transfusion and b) TDT subjects who achieve a decrease in RBC units required over an 8-week period. This study was recently stopped, however, due to efficacy issues of the drug.

3.3.2. TMPRSS6

Another approach is to increase hepatic synthesis of hepcidin by suppressing TMPRSS6. TMPRSS6 regulates hepcidin production by cleaving hemojuvelin (HJV), a key modulator of hepcidin expression, from the hepatocyte surface. The TMPRSS6 gene encodes the protein Matriptase-2 that belongs to the family of type II transmembrane serine protease (TTSP). TMPRSS6/Matriptase II downregulates the bone morphogenetic protein (BMP) signaling by cleaving HJV and releasing the soluble form [87].

In 2012 study by Nai et al, deletion of the TMPRSS6 gene improved anemia, ineffective erythropoiesis, and splenomegaly in $Hbb^{th3/+}$ mice [88]. Approaches using second-generation antisense oligonucleotides (ASOs) targeting mouse TMPRSS6 have also been described. Guo et al demonstrated that targeting TMPRSS6 by antisense technology can modulate HAMP expression in a dose-dependent manner, demonstrating that TMPRSS6-ASO treatment has the potential to prevent iron overload [89]. They showed that hemochromatosis affected mice ($Hfe^{-/-}$) treated with ASO had a significant decrease in serum iron, and liver iron accumulation [89]. A decrease in the formation of insoluble membrane-bound globins, ROS, and apoptosis was also shown in TMPRSS6-ASO treated $Hbb^{th3/+}$ mice. These animals also exhibited a significant amelioration of ineffective erythropoiesis, lower erythropoietin levels, decrease in splenomegaly, and an increase in Hb levels [89].

Another study by Schmidt et al. described a different approach to downregulate TMPRSS6 with small interfering RNAs (siRNA) [90]. They showed that lipid nanoparticles-TMPRSS6 siRNA treatment of both $Hfe^{-/-}$ and $Hbb^{th3/+}$ mice induced hepcidin and diminished tissue iron levels and serum iron levels [90]. Furthermore, lipid nanoparticles-TMPRSS6 siRNA treatment of $Hbb^{th3/+}$ mice led to significant improvements in anemia by altering RBC survival and ineffective erythropoiesis [90].

A phase 2a study using subcutaneous administration of IONIS TMPRSS6-LRx every 4 weeks will soon be initiated in NTDT patients ≥18 years of age. (ClinicalTrials.gov number NCT04059406). Primary outcome measures of the study will include percentage of participants with a ≥ 1.0 g/dL increase in plasma Hb from Baseline at Week 27.

4. Conclusion

A new era of novel therapies is emerging to improve outcomes in thalassemia care. Whether in pre-clinical or clinical development, several novel therapeutic approaches have been developed in β-thalassemia to ameliorate the α-β genetic defect, ineffective erythropoiesis, and iron dysregulation. Once the efficacy and safety of all these novel therapies are established, long-term, head-to-head, and comparison trials would inform decisions on the optimal management of TDT and NTDT patients. Moreover, the optimal use of these novel therapies on their own or in combination with other agents, such as iron chelators also warrants future clinical studies.

5. Expert opinion

For TDT patients, transfusion and iron chelation therapy have long been the standard treatment modality. Hematopoietic stem-cell transplantation is also another established approach, yet it is only available for few patients. Despite this, we still see disease burden due to lifelong transfusion requirement and in turn complications due to iron overload. For TDT patients, novel therapies aim at reducing transfusion burden, making β-thalassemia less of a health burden, with an overall less health expenditure. While the ultimate aim is complete transfusion independence, most novel therapies, including gene therapy, have partially and not fully decreased transfusion requirement. The clear clinical impact of this on the long term is yet to be determined. In the short term, studies must look at the impact of this reduction in transfusion requirement on the QoL of these patients. Yet in the context of the clinical trials themselves, this will be difficult to assess. However, we believe that when these novel therapies are used in the context of the clinic in real-life, it will be easier to monitor QoL parameters and look at ways to make everything more convenient for the patient.

In NTDT patients, the use of these emerging therapies is mainly to raise Hb concentrations. Yet their impact on this patient population will not be truly assessed except on the long term. On the short term, we mainly want to find out why is it important to increase Hb and manage the anemia itself. This can be assessed through improvements in QoL parameters and patient-reported outcomes. Another point to consider is that these novel therapies address different aspects of the complex pathophysiology of β-thalassemia. For example, if we address ineffective erythropoiesis on its own using luspatercept, we expect that there should automatically be a decrease in iron absorption. When we target iron dysregulation using minihepcidins, we decrease the damaging effect on the bone marrow and thus improve anemia. Whether these novel agents will be used as monotherapy or combination is yet to be determined. Moreover, novel therapies that target iron dysregulation are leading to less iron absorption and therefore less iron loading in the organs of NTDT patients. Thus, iron chelation strategies could be perhaps changed or completely abandoned in the future or even be used in combination therapy with these novel therapies. Further treatment protocol studies are therefore needed to investigate the role of monotherapy vs. combination therapy in this cohort of patients to target their pathophysiology in multiple ways and ensure that they will not develop long-term complications.

In terms of which β-thalassemia patients will benefit from which novel therapy, we believe that those living in resource-poor areas with limited access to regular and safe blood transfusions are likely to be the ones to benefit from novel therapies targeting ineffective erythropoiesis such as luspatercept as this agent will increase their Hb levels, reduce their need for blood transfusions and improve their overall quality of life. Gene therapy can also be of benefit to this subset of β-thalassemia patients to correct ineffective erythropoiesis and hemolytic anemia, thus preventing the need for blood transfusion. We believe that TDT patients with a non-β0/β0 genotype, aged >12 and <65, with no significant iron overload and organ damage and who have good compliance to treatment and are eligible for allogeneic transplant but have no HLA-identical family donor are most likely to be the ones that would benefit from gene therapy approaches. However, as these techniques require complex and expensive resources, only a limited sub-population of β-thalassemia patients will be able to benefit from them. On another note, iron chelation therapy has many disadvantages including poor compliance and adherence among some patients, frequent side effects that require regular monitoring and a demanding regimen of parenteral formulation. Therefore, novel therapies targeting iron dysregulation such as minihepcidins and TMPRSS6 inhibitors could be of benefit to this particular subset of β-thalassemia patients. Patients with NTDT, in particular, are more likely to benefit from these agents that control hepcidin expression than TDT patients, because transfusional iron overload is not mediated by low hepcidin levels.

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