



## Review

## Recent trends in treatment of thalassemia

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## ARTICLE INFO

## Keywords:

Thalassemia  
Ineffective erythropoiesis  
Iron dysregulation  
Gene therapy

## ABSTRACT

Thalassemia is a common inherited monogenic disease. It is characterized by chronic hemolysis, ineffective erythropoiesis (IE) and iron overload. Despite advances in transfusion practices and chelation therapy, still many limitations in delivering these standard therapies exist. Challenges of currently available standard care and advances in understanding the underlying pathophysiological mechanisms in thalassemia stimulated research towards development of novel therapeutic targets. Agents reducing IE as Jak 2 inhibitors and Activin II receptor traps are promising and are currently in clinical trials. Other approaches targeting iron dysregulation as mini-hepcidins, exogenous transferrin and erythroferrone inhibitors are in preclinical studies. Gene therapy, a rapidly evolving field, has exhibited remarkable progress in recent years. Studies have focused on  $\beta$  or  $\gamma$ -globin addition, over expression of endogenous  $\gamma$ -globin-activating transcription factors, silencing of  $\gamma$ -globin repressors and genome editing of  $\beta$ -globin mutations or  $\gamma$ -globin repressors. In this article we provide an overview of emerging recent trends in treatment of thalassemia targeting IE, iron dysregulation and novel curative treatments as gene therapy and gene editing.

## 1. Introduction

Thalassemia is a common inherited monogenic disease worldwide with an estimated mutation carrier rate of 1–5% among the global population [1,2]. It is characterized by chronic hemolysis, ineffective erythropoiesis (IE) and iron overload. Unfortunately, this disease is highly prevalent in resource-limited areas mostly sub-Saharan Africa, Mediterranean region, Middle East, Indian subcontinent and East and Southeast Asia [2,3]. Moreover, due to increasing and continuing modern migration, thalassemia is appearing now in many regions in Europe and North America. Thus, it is becoming a global health burden [4].

Over the last 50 years, a large body of work led to better understanding of the natural history, pathophysiology and management of the disease [5]. Major advances in transfusion practices and chelation therapy have been successful in extending life expectancy, improving quality of life, comorbidities and survival among different birth cohorts of patients with  $\beta$ -thalassemia [6,7]. Advances in allogenic hematopoietic stem cell transplantation (AHSCT) techniques have provided a potentially curative option for some patients. However, there are still many limitations to delivering these standard therapies to patients in many areas of the world, especially in low and middle-income countries. Despite improvement in blood products screening, preparation and administration practices over the last decades, availability and

access to safe and effective transfusion remains a challenge. For best results, effective chelation therapy has to be started early before irreversible iron-mediated tissue damage has occurred. AHSCT is the only established curative treatment for thalassemia patients with a disease-free survival of 80% with transplants from HLA-matched-sibling donors [8]. However, this is limited by availability of HLA-matched donor and the procedure is not without risk. Transplant related mortality and risks related to conditioning regimens, graft versus host disease, graft failure, long-term toxicities, infertility and secondary malignancies still pose considerable concerns. Amelioration of AHSCT techniques regarding the conditioning regimen, donor selection and hematopoietic stem cell source is currently under evaluation in clinical trials but is not within the scope of this review. Thus, the main therapeutic option for the majority of patients remains to be supportive care in the form of blood transfusion combined with chelation therapy. The challenge to provide blood, chelation therapy, adequate monitoring and patient support can overwhelm health care systems [9]; even in areas with relatively well-developed economies. Costs have increased with introduction of newer oral chelators and monitoring techniques for iron overload as cardiac and hepatic magnetic resonance imaging. Adherence to treatment, often suboptimal, is a further challenge that clinicians need to be aware of. Multidisciplinary health care system approach, often not institutionally available, is also needed for better outcomes. Consequently, effective treatment is often not delivered and far from

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Received 28 January 2019; Accepted 29 January 2019

Available online 04 February 2019

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universal. It was estimated that only 12% of children with transfusion dependent thalassemia (TDT) and < 40% of those transfused receive adequate chelation therapy. In United Kingdom, a lifetime treatment to 50 years of age was estimated to be about £483,454 (\$646,934) [10].

These challenges and limitations of currently available care highlighted the needs for newer therapeutic options whether curative treatments or non-curative that would decrease the patients' transfusion requirements. Advances in understanding the underlying pathophysiological mechanisms in thalassemia stimulated research towards development of novel therapeutic options. In this article, we aim to provide an overview of emerging therapeutic options for thalassemia patients including novel therapies that target the main pathophysiological aspects of the disease; ineffective erythropoiesis (IE), iron dysregulation and curative treatments as gene therapy and gene editing.

### 1.1. Approaches targeting ineffective erythropoiesis

Ineffective erythropoiesis due to globin chain imbalance remains to be the hallmark of thalassemia. Precipitation of unpaired free alpha chain forming methemoglobin and insoluble hemichromes in erythroid cells, together with oxidative mediated cell damage result in premature cell death, apoptosis, inside (IE) and outside the bone marrow (peripheral hemolysis) [11]. IE associated with peripheral hemolysis leads to anemia, hypoxia and reactive increase of erythropoietin (EPO) production with subsequent bone marrow hyperplasia and extramedullary hematopoiesis and hepatosplenomegaly. EPO acts through its receptor (EPOR) via multiple signaling pathways mainly the JAK2/STAT5 (Janus kinase 2/signal transducer and activator of transcription5) [12]. IE induces the release of factors, such as GDF15 (growth and differentiating factor 15), TWSG1 (twisted gastrulation protein homolog 1), HIF (hypoxia-inducible factor), and ERFE (erythroferrone) which inhibit hepcidin, the major regulator of iron homeostasis. Inappropriately low hepcidin level in thalassemia promotes duodenal iron uptake, iron release from macrophages and hepatic iron storage and leads to progressive tissue iron overload. Free iron hinders erythropoiesis creating a vicious cycle between IE and increased iron absorption and release [13]. Therefore, potential benefits of reduced IE are improved anemia, reduced splenic size and extramedullary expansion, indirect increase in serum hepcidin and improved QoL [14]. Agents enhancing erythropoiesis either directly as EPO or by induction of HbF synthesis have been studied for decades. However, reported responses were largely variable and unpredictable.

#### 1.1.1. Erythropoietin

Induction of erythropoiesis using recombinant human EPO has been studied but with varying results [15,16].

#### 1.1.2. HbF induction

Thalassemia patients with persistently high levels of fetal globin typically have less severe anemia, have milder clinical syndromes, and are often transfusion independent [17]. Attempts to restore the balance between  $\alpha$  and non- $\alpha$  chains of hemoglobin have been made using agents that induce  $\gamma$  chain production such as hydroxycarbamide (HU), short-chain fatty acid derivatives, 5-azacytidine and thalidomide. However, responses were diverse, often partial, without long-term beneficial results [18–21]. Some may act via epigenetic mechanisms. This may be explained by (i) the strong correlation between epigenetic modification and the developmental pattern of globin gene expression [22,23,24] and (ii) the link found between the variation of HbF synthesis in adults and loci sensitive to epigenetic regulation [25]. However, how these compounds act is still not fully understood because they belong to several categories, including cytotoxic and hypomethylating agents (5-azacytidine, decitabine, HU), cytotoxic and histone deacetylase inhibitors, such as butyrate derivatives [26].

#### 1.1.3. Jak2 inhibitors

The process of erythropoiesis is tightly regulated by an array of events that include cytokine signaling and cell-cell interactions, mainly in the context of erythroblastic islands, a specialized niche for the maturation of erythroid progenitors [27]. EPO, the master regulator of erythropoiesis, signaling through EPOR, controls virtually all stages of erythroid differentiation. Activation of Jak2 ultimately leads to activation of the signal transducer and activator of transcription Stat5 a and b and parallel signaling pathways [28]. Studies using both mouse models of  $\beta$ -thalassemia and specimens from thalassemic patients showed that, together with apoptosis, a significant number of erythroid progenitors undergo increased proliferation and decreased differentiation in the spleen [29]; erythroid proliferation outpaces differentiation in thalassemia. Jak2 is a member of the Jak tyrosine kinase family. Its association with EPO is unique because Jak2 is the only kinase that is associated with EPOR and therefore the only signal transducer of EPO [14]. Theoretically, Jak2 inhibitors may decrease IE by improving the balance between proliferation and differentiation. In mouse models of thalassemia, Jak2 inhibition was shown to improve IE and decrease splenic size but at the cost of decreased overall erythropoietic activity that was not improved by blood transfusion [29,30]. Ruxolitinib, a JAK2 inhibitor, is approved for the treatment of patients with polycythemia vera and myelofibrosis [31,32]. A single-arm, multicenter, 30-week Phase 2a study was undertaken to evaluate the efficacy and safety of ruxolitinib among 30 adults with transfusion dependent thalassemia (TDT) and splenomegaly (NCT02049450) [33]. Treatment for 30 weeks at a starting dose of 10 mg twice daily, was associated with a significant reduction in splenic volume (26% at week 30) but did not decrease overall transfusion requirement (12 decreased, 7 increased and 8 showed no change). Commonly reported adverse events were upper respiratory tract infection (8/30), nausea (6/30), upper abdominal pain (5/30), anemia (5/30), diarrhoea (5/30), and weight increase (5/30) [33].

#### 1.1.4. Activin II receptor traps: Sotatercept and Luspatercept

Growth differentiation factor (GDF-11) is upregulated in spleen and erythroid cells of thalassemic animals and inhibits murine erythroid maturation. Inactivation of GDF-11 and blocking its interaction with activin receptors interfering with downstream signaling cascades decreases oxidative damage and ameliorates IE [34,35]. In mice models, decreased levels of GDF11 promoted terminal erythropoiesis by inducing apoptosis through the Fas-FasL pathway and reducing reactive oxygen species and  $\alpha$ -globin aggregates. Activin receptor-II ligand traps are agents that bind to ligands extracellularly, and act to prevent signaling at intended receptors. They were originally developed to prevent osteoclast-dependent bone resorption by inhibiting activin-dependent signaling; thus, improving bone mineral density in postmenopausal women. Unexpectedly, a significant increase in Hb values was shown [36].

Two agents, Sotatercept (ACE-011) and Luspatercept (ACE-536), were developed for treatment of conditions associated with IE. Sotatercept is a recombinant human homodimeric activin type IIA receptor fusion protein comprising the extracellular domain of the human activin type IIA receptor fused to the Fc domain of human immunoglobulin G1 (IgG1) with 2 disulfide-linked chains, dimerized through the Fc region [37]. Luspatercept is another recombinant fusion protein but for human activin type IIB receptor. Luspatercept binds with high affinity to select TGF-beta superfamily ligands, such as GDF11 and GDF8; unlike sotatercept, it binds only minimally to activin A [34,37]. The murine analogue of Luspatercept (RAP-536) corrected complications of IE, splenomegaly and bone pathology in mouse model of NTDT [38]. Both agents exert their effects on late stage erythropoiesis to increase mature red cells independent of erythropoiesis stimulating agents and EPO, which act on earlier stages of erythropoiesis [34,37].

In a phase 2a, open-label, dose-finding study of sotatercept (ACE-

011) in adult patients with beta-thalassemia, dose-dependent increments in Hb (> 1 g/dl) with improvements of red cell morphology were shown [39]. Preliminary data suggested reduction in transfusion burden, good tolerability and favorable safety profile. Grade 3-bone pain in one TDT patient and grade 2-phlebitis in a NTDT patient were reported as adverse events [39].

A multicenter, open-label, dose-ranging Phase 2 study of Luspatercept in 31 NTDT and 32 TDT adults (NCT01749540) at doses of 0.2–1.25 mg/kg administered subcutaneously every 3 weeks showed similar findings [40]. In NTDT, Luspatercept increased Hb > 1 g/dl over baseline in 71% of patients and improved QoL score in patients showing > 1 g/dl increase. In TDT, a  $\geq 20\%$  reduction of transfusion requirement was shown in 78% of patients and a  $\geq 30\%$  reduction in 69% of patients after a median of 14 months duration. In both TDT and NTDT patients, a parallel reduction of liver iron concentration was observed. Luspatercept was generally well tolerated with majority of reported adverse events being bone pain (38%), headache (28%), myalgia (22%) and arthralgia (19%). A 5-year extension phase (NCT02268409) of this study is ongoing [40]. Consequently, randomized double-blind phase 3 studies have begun to evaluate the efficacy and safety of Luspatercept (ACE-536) versus placebo in adults who require regular transfusions for TDT (NCT02604433) and in NTDT (NCT03342404). Efficacy will be determined by at least a 33% improvement in the number of transfused red blood cell units from baseline.

#### 1.1.5. Forkhead-box-class-O3

Forkhead-box-O3 (Foxo3) is a critical transcription factor that protects the cell from oxidative stress by upregulating antioxidant enzymes during early stages of erythropoiesis [41]. Foxo3 is phosphorylated by proteins of the EPOR-p13K/AKT/mTOR signaling pathway and is translocated out of the nucleus where it remains inactivated. In  $\beta$ -thalassemia intermedia (TI) mice, Foxo3 has been found to be down-regulated due to persistent activation of EPOR-p13K/AKT/mTOR pathway. Inactivation of Foxo3 leads to oxidative damage in late erythroblasts and plays a significant role in the process of IE [42]. Thus, activation of Foxo3, as a potential HbF inducer, could be beneficial in improving anemia in  $\beta$ -thalassemia. However, its role in hemoglobinopathies remains to be elucidated. The use of rapamycin, an mTOR inhibitor, has remarkably improved erythroid cell maturation,  $\beta$ -globin production and anemia through Foxo3 activation in  $\beta$ -TI mice model [42]. In cultured erythroblasts from  $\beta$ -TI patients, rapamycin increased  $\gamma$ -globin mRNA expression and HbF production [43]. Similar findings were reported with the use of another Foxo3 activating agent, resveratrol (3,5,4'-trihydroxy-*trans*-stilbene), a non-flavonoid polyphenol that up-regulates antioxidant enzymes in  $\beta$ -TI mice [44]. Metformin, an approved drug for diabetes type 2, is a Foxo3 inducer. Its use as an HbF inducer is being investigated in an ongoing phase 1 clinical trial in patients with sickle cell anemia and NTDT (NCT02981329) [45]. These agents are in preclinical studies, and need further evaluation.

#### 1.2. Approaches targeting iron dysregulation

Iron is a key element for erythropoiesis, and its distribution is controlled by hepcidin, a hormone synthesized in the liver [46]. IE and chronic hypoxia inhibit hepatic production and secretion of hepcidin. Inappropriately low hepcidin level in thalassemia increases duodenal iron uptake, iron release from macrophages and hepatic iron storage and leads to iron overload in parenchymal tissues [13]. Restricting iron delivery to thalassemic normoblasts decreases heme synthesis, heme-chrome formation and consequent oxidative stress and apoptosis thus the efficiency of erythropoiesis is improved. In principle, plasma hepcidin can be increased either by administration of exogenous hepcidin (s) or by upregulating hepcidin synthesis. Studies in thalassemic mice models showed that moderate overexpression of hepcidin improved IE, increased hemoglobin, reversed splenomegaly, and limited iron

overload [47,48]. These observations led to the development of hepcidin agonists, which are now under study. Administration of mini-hepcidin, short peptides that mimic the activity of endogenous hepcidin, significantly improved IE, anemia, and iron overload in a mouse model [48]. Another possible approach is to increase hepatic synthesis of hepcidin by suppressing TMPRSS6, a transmembrane serine protease (matriptase-2) that normally suppresses hepcidin synthesis by deactivating hemojuvelin. Deletion of the TMPRSS6 gene in mouse model increased hepcidin expression, improved anemia and reduced IE, splenomegaly, and iron loading [49]. Approaches using antisense oligonucleotides or small interfering RNAs (siRNA) decreasing TMPRSS6 were shown to ameliorate IE, improve anemia and iron overload in mice and preclinical studies [50,51]. Increased hepcidin synthesis was also achieved by the use of exogenous transferrin through the down-regulation of TFR1, which led to an increase of erythroid precursors enucleation and amelioration of terminal erythroid differentiation and maturation in  $\beta$ -thalassemia mice [52,53].

Erythroferrone (ERFE), a recently discovered erythroid suppressor of hepcidin, is produced in erythroblasts in response to EPO through the JAK2/STAT signaling pathway. ERFE has been found highly expressed in murine models with  $\beta$  thalassemia intermedia, whereas ERFE-deficiency resulted in increased hepcidin expression, significant reduction in iron overload and slight amelioration of erythropoietic indices [54]. This indicates that ERFE inhibition may be a future target with therapeutic potential in diseases with iron overload and IE as thalassemia.

Agents targeting hepcidin expression are more likely to be beneficial to patients with NTDT than those with TDT because transfusional iron overload is not mediated by low hepcidin levels. However, mini-hepcidins and TMPRSS6 inhibitors are to be evaluated for use in patients with TDT because improvement in erythropoiesis could potentially reduce transfusion requirements [55].

All discussed novel agents, in preclinical or clinical development studies, merit further evaluation for efficacy and safety and are shown in Table 1.

#### 1.3. Gene therapy and editing

Despite improved outcomes, AHST still carries a substantial risk of mortality and severe adverse events [56,57]. In absence of fully matched sibling donor, alternative approaches as AHST from HLA-matched unrelated or haploidentical donors or minimally mismatched cord blood products may be used. However, these options carry a lower benefit/risk ratio [58]. Gene manipulation with the aim to treat  $\beta$ -thalassemia has been extensively investigated in several experimental systems. In principle, in vitro studies have focused on  $\beta$  or  $\gamma$ -globin addition, overexpression of endogenous  $\gamma$ -globin-activating transcription factors, silencing of  $\gamma$ -globin repressors, such as BCL11A (B-cell lymphoma/leukemia 11A) and genome editing of  $\beta$ -globin mutations or  $\gamma$ -globin repressors [59].

##### 1.3.1. Gene therapy

The concept of treating beta-thalassemia patients by inserting the  $\beta$ -globin gene into bone marrow cells emerged as early as 1978 at the University of California at Los Angeles (UCLA) [60] but these attempts were completely unsuccessful then and received an avalanche of criticism [61]. For patients who do not have HLA-matched donor, ex-vivo gene therapy using autologous HSC and lentiviral vectors (LVs) to replace key elements of the  $\beta$ -globin gene is becoming a clinical reality and a hopeful potential curative option. In principle, gene therapy typically involves isolation of HSC, ex-vivo correction of the abnormal gene, myeloablative-conditioning regimen and reinfusion of sufficient genetically modified HSC to repopulate the hematopoietic compartments [62]. This approach has the obvious advantages of lack of concerns about histocompatibility-related complications or the need for including immunosuppressive agents in the conditioning regimen. In addition, making use of a single product applicable to all  $\beta$ -thalassemia

**Table 1**  
Novel therapeutic agents in development targeting erythropoiesis or iron regulation in thalassemia.

Therapeutic agent	Mechanism of action	Key outcomes	Stage
Ruxolitinib (INC424)	Inhibition of JAK2, the key intracellular signal transducer of EPO	Significant reduction in splenic volume but no decrease in overall transfusion requirement	Phase 2 (NCT02049450) [33]
Sotatercept (ACE-011)	Ligand trap TGF- $\beta$ superfamily, Activin type IIA receptor fusion protein, targets late erythropoiesis	Dose-dependent increments in Hb, reduction in transfusion burden	Phase 2 (NCT01571635) [39]
Luspatercept (ACE-536)	Ligand trap TGF- $\beta$ superfamily, Activin type IIB receptor fusion protein, targets late erythropoiesis	Increased transfusion requirements and liver iron concentration among patients with TDT, and increased Hb levels, reduced liver iron concentration, and improved the QoL in NTDT	Phase 2 (NCT01749540), (NCT02268409) [40] Phase 3 (NCT02604433), (NCT03342404)
Rapamycin	mTOR inhibitor, Antioxidant effect Foxo3 activation	Increased $\gamma$ -globin mRNA expression and HbF production in cultured erythroblasts from $\beta$ -TI patients	Preclinical [42,43]
Resveratrol	Antioxidant effect, Foxo3 activation	Amelioration of erythrocyte survival, increased Hb levels and reduced reticulocyte count in murine $\beta$ -TI model	Preclinical [44]
Metformin	Foxo3 activation, HbF induction	Ongoing	Phase 1 (NCT02981329) [45]
Minipeptides	Short peptides that mimic the activity of endogenous hepcidin	Significantly improved IE, anemia, and iron overload in a mouse model	Preclinical [48]
TMPRSS6 inhibition	Gene-editing or small-interfering RNA techniques inhibit TMPRSS6, increased hepcidin expression	Improved anemia and reduced IE, splenomegaly, and iron loading in mice and preclinical studies	Preclinical [49–51]
Exogenous transferrin	TfR1 down-regulation Improvement of erythroid terminal differentiation and hepcidin production	Increase of erythroid precursors enucleation and amelioration of terminal erythroid differentiation and maturation in $\beta$ -thalassemia mice	Preclinical [52,53]
ERFE-deficiency	Hepcidin up-regulation	Increased hepcidin expression, significant reduction in iron overload and slight amelioration of erythropoietic indices	Preclinical [54]

JAK2: Janus kinase 2; EPO: erythropoietin; TGF: transforming-growth factor; Hb: hemoglobin; TDT: transfusion-dependent thalassemia; NTDT: non-transfusion-dependent thalassemia; HbF: fetal hemoglobin; TI: thalassemia Intermedia; IE: ineffective erythropoiesis; TMPRSS6: transmembrane serine protease 6; ERFE: erythroferone.

**Table 2**  
Gene therapy and editing in thalassemia.

Therapeutic agent	Mechanism of action	Key outcomes	Stage
LentiGlobin BB305 vector	$\beta$ -globin gene addition, Ex vivo autologous CD34+ stem cell transduction	Interim data reported from two-phase 1–2 showed increased levels of HbA and reduced transfusion requirements	Phase 1–2 (NCT01745120), (NCT02151526) [70] Phase 3 (NCT02906202) and long term follow-up study (NCT02633943)
TNS9.3.55 Lentiviral Vector	$\beta$ -globin gene addition, Ex vivo autologous CD34+ stem cell transduced with TNS9.3.55	Stable engraftment without clonal dominance [82]	Phase 1 (NCT01639690), Ongoing
TIGET-BTHAL GLOBE Lentiviral Vector	$\beta$ -globin gene addition, Ex vivo autologous CD341 stem cell transduction	Ongoing	Phase 1 (NCT02453477)
ZFN-driven BCL11A enhancer ablation	HbF induction, Designer nuclease used to knock-down BCL11A by gene-editing	Increased HbF production in CD34+ cells from patients with $\beta$ -thalassemia	Preclinical [76]
CRISPR-Cas9-mediated BCL11A enhancer inactivation	HbF induction, Designer nuclease used to knock-down BCL11A by gene-editing	Increased HbF production in human adult-stage erythroid cell line	Preclinical [77]
CTX001	HbF induction by disruption of BCL11A gene	Outcomes include change in HbF; change in annual RBC ex vivo; increases in $\gamma$ -globin mRNA to 39% (as a ratio of $\gamma/\alpha$ ) in 1 $\beta$ -thalassemia patient sample	Phase 1 beginning in Europe [78,79]

ZFN: zinc fingered nucleases; CRISPR- Cas-9: clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases.

patients irrespective of the individual mutation is an additional benefit. However, this strategy shares with AHSCT the transplant procedure related risks and toxicity of myeloablative agents [63]. To be effective, achieving a satisfactory combination of high levels of sustained  $\beta$ -globin expression and stable propagation of complex sequences is essential. This was made possible by the characterization, isolation, size reduction, and blending of the  $\beta$ -globin locus regulatory elements and the advent of LVs [64]. LVs, derived from human immunodeficiency virus type 1 (HIV1), were able to transduce cells arrested at the G1-S boundary of the cell cycle [65,66] and to transfer much longer sequences than gamma-retroviral vectors ( $\gamma$ -RVs) used in earlier trials [67,68]. Limitations of  $\gamma$ -RVs included instability, low vector titers and inability to transduce non-dividing cells [69]. Self-inactivating LVs are currently used in several clinical trials for  $\beta$ -thalassemia (Table 2).

Reported clinical outcomes: Interim combined data have been reported from two-phase 1–2 studies using mobilized autologous CD34 +

cells from 22 patients (aged 12–35 years) with TDT and transduced ex vivo with LentiGlobin BB305 vector, which encodes adult Hb (HbA) with a T87Q amino acid substitution (HbAT87Q) (ClinicalTrials.gov numbers, NCT01745120 and NCT02151526) [70]. At a median of 26 months (range, 15 to 42) after infusion, all but one of the 13 patients who had a non- $\beta^0/\beta^0$  genotype stopped receiving transfusions; HbA<sup>T87Q</sup> levels ranged from 3.4 to 10.0 g/dl, and total hemoglobin levels ranged from 8.2 to 13.7 g/dl. Biologic markers of dyserythropoiesis were corrected in evaluated patients with hemoglobin levels near normal ranges. In 9 patients with a  $\beta^0/\beta^0$  genotype or two copies of the IVS1-110 mutation, the median annualized transfusion volume was decreased by 73%, and red-cell transfusions were discontinued in 3 patients. Treatment-related adverse events were typical of those associated with autologous stem-cell transplantation. No clonal dominance related to vector integration was observed [70].

### 1.3.2. Gene editing

Because of increasing knowledge of the molecular mechanisms controlling the  $\gamma$ -globin gene expression, modulation of  $\gamma$ -globin gene regulators aiming to promote  $\gamma$  chain synthesis has been used as another approach. This has been achieved in human hematopoietic cells by editing genes that suppress  $\gamma$  globin synthesis, such as BCL11A transcription factor, a strong silencer of the  $\gamma$ -globin gene, without affecting other hematopoietic lineages [71,72,73,74]. Editing techniques included the use of several nucleases such as engineered zinc fingered nucleases (ZFN), transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats linked to Cas9 nucleases (CRISPR-Cas9) [75]. ZFN-driven BCL11A enhancer ablation was shown to increase HbF production in CD34+ cells from patients with  $\beta$ -thalassemia, which could be the source for autologous transplantation in these patients [76]. Similar effect has been achieved with the CRISPR/Cas9-mediated BCL11A enhancer inactivation in a human adult-stage erythroid cell line [77]. Another approach is a gene-edited cellular product, named CTX001, that uses CRISPR/Cas9 technology to target an erythroid-specific enhancer of BCL11A gene and has been approved for clinical trials in several countries [78]. The safety and specificity of CRISPR technique to gene editing is debated [79].

In general, gene therapy, the treatment of the future, is a rapidly evolving field that has exhibited remarkable progress in recent years. Despite excellent reassuring outcomes, acceptable safety profile and great promise it holds to  $\beta$ -thalassemia patients, its overall long-term risks remain unclear; in particular, those related to the risk of insertional mutagenesis and increased oncogenic potential [80,81,79]. Moreover, the need for sophisticated and expensive resources for these approaches makes them beyond reach of many patients in remote and resource-constrained countries [62].

Conclusion: A new era of novel therapeutics is evolving for better thalassemia care. Advances in understanding the underlying pathophysiological mechanisms stimulated research towards development of novel therapeutic agents. Several therapeutic approaches, in pre-clinical and clinical development, have been developed designed to ameliorate the genetic defect, IE or iron dysregulation. Optimal use of these novel therapeutic options as monotherapies, sequentially or in combinations warrants future clinical studies.

### Funding

This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### Declaration of interest

None.

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