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Review

A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies?

Maria Domenica Cappellini^{a,*}, John B. Porter^b, Vip Viprakasit^c, Ali T. Taher^d^a Department of Clinical Sciences and Community, University of Milan, IRCCS Ca' Granda Foundation Maggiore Policlinico Hospital, Milan, Italy^b Department of Haematology, University College London, London, UK^c Department of Pediatrics, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand^d Department of Internal Medicine, American University of Beirut Medical Centre, Beirut, Lebanon

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ABSTRACT

Beta-thalassaemia causes defective haemoglobin synthesis leading to ineffective erythropoiesis, chronic haemolytic anaemia, and subsequent clinical complications. Blood transfusion and iron chelation allow long-term disease control, and haematopoietic stem cell transplantation offers a potential cure for some patients. Nonetheless, there are still many challenges in the management of beta-thalassaemia. The main treatment option for most patients is supportive care; furthermore, the long-term efficacy and safety of current therapeutic strategies are limited and adherence is suboptimal. An increasing understanding of the underlying molecular and cellular disease mechanisms plus an awareness of limitations of current management strategies are driving research into novel therapeutic options. Here we provide an overview of the current pathophysiology, clinical manifestations, and global burden of beta-thalassaemia. We reflect on what has been achieved to date, describe the challenges associated with currently available therapy, and discuss how these issues might be addressed by novel therapeutic approaches in development.

1. Introduction

Beta-thalassaemia is a monogenic disorder leading to reduced or absent synthesis of the beta-globin subunit of adult haemoglobin [1,2]. It is characterized by ineffective erythropoiesis, chronic haemolytic anaemia, and subsequent clinical complications [2–4]. Most patients with beta-thalassaemia are born in resource-constrained countries, but modern migration patterns have altered the epidemiology of this disease [5]; patients with beta-thalassaemia are now found even in northern European countries. Affected children may present with failure to thrive, growth retardation, or other more specific signs or symptoms [6]. Lifelong disease management is required; individuals with severe disease will not survive childhood without appropriate treatment [6,7].

The survival and quality of life (QoL) of patients with beta-thalassaemia in developed countries have improved markedly in recent decades [7]. The availability of blood transfusion and iron chelation strategies for patients with severe forms of beta-thalassaemia now allow long-term disease control and improved QoL. Moreover, advances in haematopoietic stem cell transplantation (HSCT) techniques have provided a potentially curative option for some patients.

Despite important improvements in the management of beta-thalassaemia, there are still many challenges to overcome before global disease control is achievable. For example, screening and prevention programmes are inadequate in many resource-constrained countries, and access to effective treatment is far from universal [5,8,9]. The main treatment option for most patients is supportive care and, as such, many patients require regular, lifelong transfusions and iron chelation therapy. Current supportive interventions have not all been evaluated in large, long-term clinical trials, and comparative studies to inform the best method of care are limited. The convenience of administration and cost of most available therapies for this chronic disease remain challenging. HSCT is only available for a subset of patients and the procedure is not without risk. Gene therapy could offer a curative approach but the technology is still considered experimental.

Our understanding of the underlying pathophysiological mechanisms of beta-thalassaemia and its associated clinical morbidity has increased substantially in recent years [10]. This new knowledge and an increasing awareness of the limitations of current management strategies are driving research into novel therapeutic options for this patient population. Here we provide an overview of the pathophysiology, clinical manifestations, and global burden of beta-thalassaemia, and

* Corresponding author at: Fondazione Ca Granda Policlinico IRCCS, University of Milan, Via F. Sforza 35, Milan 20122, Italy.

E-mail addresses: maria.cappellini@unimi.it (M.D. Cappellini), j.porter@ucl.ac.uk (J.B. Porter), sivvp@mahidol.ac.th (V. Viprakasit), ataher@aub.edu.lb (A.T. Taher).

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reflect on what has been achieved to date in the management of affected patients. We also highlight ongoing challenges associated with currently available therapeutic options, and discuss how these issues might potentially be addressed by novel, potentially disruptive, therapeutic approaches in development.

2. Epidemiology

Robust data on the frequency and natural history of the haemoglobinopathies are scarce [8]. However, it is conservatively estimated that > 40,000 babies with beta-thalassaemia are born each year [5]. Beta-thalassaemia is a truly global disease but is most prevalent in South Asia, the Far East, the Middle East, and Mediterranean countries [1,11,12]. The global distribution of beta-thalassaemia is attributed largely to natural selection of heterozygote carriers because of protection against falciparum malaria [13]. Beta-thalassaemia is also becoming increasingly common in northern Europe, North America, and Australia as a result of migration from areas of high prevalence [14,15].

Nonetheless, a large proportion of those affected by beta-thalassaemia still live in resource-constrained countries [5]. Infant and childhood mortality rates in these areas are declining, and more patients will survive to present for diagnosis and treatment of beta-thalassaemia [8]. A combination of lower childhood mortality and population growth is expected to lead to a substantial increase in the number of affected births [8]. Therefore, despite limited available epidemiological data on haemoglobinopathies, beta-thalassaemia is expected to pose an increasingly severe global burden in future years [8].

3. Diagnosis and classification of disease

A diagnosis of beta-thalassaemia should be considered for any patient who has a hypochromic, microcytic anaemia. Diagnostic algorithms utilizing red cell indices, haemoglobin levels, and reticulocyte counts can efficiently differentiate iron deficiency anaemia and common thalassaemia traits and disorders [1,16]. The level of haemoglobin and haemoglobin electrophoresis are important tools to recognize carriers, patients with intermediate phenotype, and severely affected patients. The use of DNA genotyping to obtain a definitive diagnosis is becoming increasingly important, although not exclusively, to confirm the clinical findings and predict disease severity [17]. Once a diagnosis of clinically relevant beta-thalassaemia is made, a full blood group genotype must be obtained before starting any treatment [18]. Guidance on diagnostic patient work-up is available in recent clinical management guidelines [1,2].

Beta-thalassaemia was traditionally classified as major, intermedia, or minor based on the severity of the clinical phenotype [15]. However, over the past decade, there has been a transition to a simpler classification system based on blood transfusion requirement. Today, patients are considered to have either transfusion-dependent thalassaemia (TDT) or non-transfusion-dependent thalassaemia (NTDT) (Fig. 1) [1,2]. Patients with TDT require regular, lifelong blood transfusions for survival, starting before the age of 2 years. Those with NTDT may need transfusion therapy occasionally or for limited periods of time, especially during periods of growth and development, surgery, or pregnancy [2,19,20]. Transfusion is also offered to patients with NTDT to prevent or manage disease complications [2]. It must be remembered that classification of TDT or NTDT only represents a patient's current clinical status; patients may shift clinically between TDT or NTDT over time (Fig. 1). Transfusion requirements should be re-evaluated intermittently.

4. Pathophysiology

Beta-thalassaemia is a recessive disorder resulting from mutations in the beta-globin gene. Based on available data and the authors' clinical experience, > 300 beta-thalassaemia alleles have been described [21];

most are point mutations in the beta-globin gene or flanking region, but deletion of the gene or upstream regulatory elements may occur [15]. Carriers with a single beta-thalassaemia allele are usually asymptomatic; such individuals have a mild hypochromic, microcytic anaemia and elevated haemoglobin A₂ levels [15]. Homozygous or compound heterozygous mutations in the beta-globin gene or promoter impair the production of beta-globin leading to specific clinical phenotypes [15,22]. Co-inheritance of a beta-thalassaemia allele and a structural haemoglobin variant, such as haemoglobin E, results in a wide range of clinical phenotypes [2,23], varying from completely asymptomatic to severe disease.

Unpaired free alpha chains precipitate and are oxidized into methaemoglobin and insoluble hemichromes [2,24]. Free iron catalyses the formation of reactive oxygen species leading to oxidative cell damage [24]. These pathogenic mechanisms result in apoptosis of erythroblasts, leading to ineffective erythropoiesis and haemolysis of mature red cells [24]. The central role of ineffective erythropoiesis in the pathophysiology of beta-thalassaemia is shown in Fig. 2.

Although beta-thalassaemia is a monogenic disorder, the clinical manifestations of the disease are diverse [25]. Indeed, patients with the same beta-globin genotype can have very different clinical phenotypes [25]. The clinical manifestations of beta-thalassaemia are now understood to result not only from the severity of the beta-globin gene mutations, but also the coinheritance of modifying factors [25].

So-called 'secondary modifiers' of beta-thalassaemia are situations that alter the relative imbalance of alpha- and beta-globin chains [2]. Co-inheritance of alpha-thalassaemia or hereditary persistence of expression of gamma-globin act to lessen the globin chain imbalance [25]. 'Tertiary modifiers' of beta-thalassaemia are those that alter the clinical complications of disease [2]. Genetic polymorphisms may modify bilirubin metabolism, iron homeostasis, bone metabolism, cardiovascular disease, and predisposition to infection [2,25]. Malaria antibody status also seems to modify the phenotype of patients with haemoglobin E/beta-thalassaemia [26].

5. Clinical manifestations

Mature red blood cells haemolyse in the peripheral circulation, and binding of immunoglobulin and complement triggers their sequestration by the spleen [24]. Chronic haemolytic anaemia negatively affects growth and organ and vascular function, and may cause other complications such as acute cholecystitis. Chronic anaemia also leads to poor tissue oxygenation and high erythropoietin levels [24]. Proliferation of erythroid precursors in the bone marrow leads to medullary expansion, skeletal deformities, and compensatory extramedullary haematopoiesis [24,27].

In the context of ineffective erythropoiesis and chronic haemolysis, red blood cells have prothrombotic potential that can cause hypercoagulability [3,28]. Vascular manifestations such as venous thrombosis and pulmonary hypertension are now well recognized in patients with beta-thalassaemia, especially those who have undergone splenectomy or have NTDT [3].

Ineffective erythropoiesis and chronic tissue hypoxia also inhibit hepatic synthesis and secretion of hepcidin [29,30]. Low circulating levels of hepcidin promote duodenal iron uptake, release of recycled iron from the reticuloendothelial system, and hepatic iron storage [18]. Iron overload, mediated by low circulating levels of hepcidin [18,28] or regular blood transfusions [1], is a major concern. The clinical effects of iron overload include cardiomyopathy, liver fibrosis or cirrhosis, and endocrinopathies [6], and are observed more commonly among patients with TDT than NTDT.

The complex pathophysiology of beta-thalassaemia leads to wide-ranging clinical manifestations (Fig. 2). Recent data suggest an association between the degree of anaemia (haemoglobin level) and morbidity development in those with NTDT [31]. Large prospective trials are required to confirm whether outcomes for patients with NTDT may

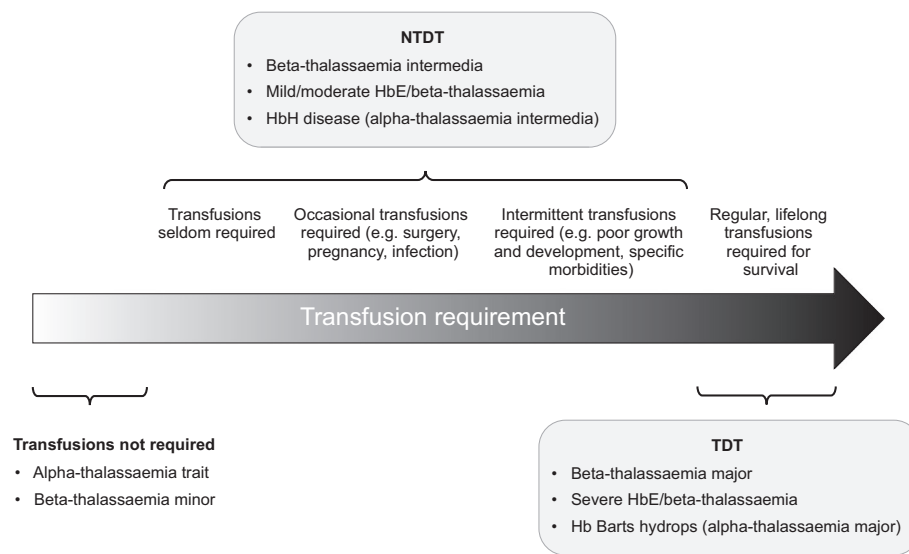


Fig. 1. Spectrum of thalassaemia syndromes based on clinical severity and transfusion requirement. HbE, haemoglobin E; HbH, haemoglobin H; NTDT, non-transfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia.

Figure adapted from Musallam et al. [28]. Published with permission; obtained from the Haematologica Journal website <http://www.haematologica.org>.

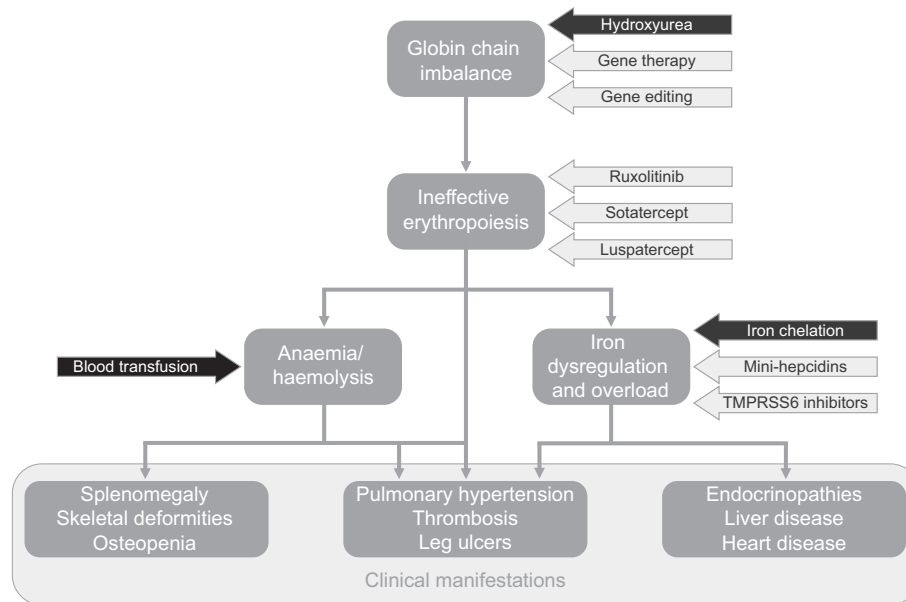


Fig. 2. Pathogenesis of beta-thalassaemia and targets for current and novel therapeutic strategies. Primary mechanism of action is shown: black arrow, current therapeutic strategy; grey arrow, novel agent.

TMPRSS6, transmembrane protein serine 6.

be improved by more aggressive intervention to increase haemoglobin levels and reduce iron absorption.

6. Quality of life

The clinical manifestations of beta-thalassaemia and the demands of treatment impair the physical, emotional, social, and academic functioning of affected patients, and reduce their health-related QoL (HRQoL) [4,7,32–38]. Recent evidence suggests that better HRQoL among children with beta-thalassaemia is associated with good iron control (e.g. through improved adherence), minimization of side effects of iron overload and chelation therapy, prevention of comorbidities, and fewer hospital visits [39]. As such, early diagnosis and good clinical management are key to optimizing the overall HRQoL and survival for affected children [39–42].

Patients with beta-thalassaemia may now expect to live for 50 years or more. However, improved patient survival in recent decades has revealed previously unidentified health issues [43]. Multiple morbidities, even unrelated to thalassaemia, tend to manifest with increasing age, and these clinical sequelae negatively affect the HRQoL of those with beta-thalassaemia [4,38,43]. A holistic approach to care for patients of all ages is required [1].

7. Health economic considerations

Long-term medical care for patients with beta-thalassaemia requires specialist, multidisciplinary input and is expensive [1,44–46]. Health-care resource utilization and costs tend to increase with age [37]. The cost of treatment for beta-thalassaemia in the UK is estimated to have increased by almost one-third (32%) over the past 16 years; the cost of

Table 1
Current therapies for beta-thalassaemia.

Therapy	Advantages	Disadvantages
Blood transfusion	<ul style="list-style-type: none"> ● Suppresses ineffective erythropoiesis, thus limiting downstream pathophysiological complications ● Regular transfusion plus iron chelation therapy is associated with improved long-term survival in TDT ● Role in ameliorating certain morbidities in observational studies with NTDT 	<ul style="list-style-type: none"> ● Lifelong transfusions required every 2–5 weeks in TDT ● Risks of blood-borne infection, alloimmunization, and iron overload
Iron chelation	<ul style="list-style-type: none"> ● Long-term use improves liver and myocardial iron levels and function, and improves endocrine function in TDT ● Can reduce systemic and hepatic iron burden in NTDT ● Oral formulations now available 	<ul style="list-style-type: none"> ● Not effective for all patients ● Frequent side effects that require regular monitoring ● Demanding regimen of parenteral formulation ● Poor adherence among some patients ● High cost ● Lack of robust evidence of benefit
Hydroxyurea	<ul style="list-style-type: none"> ● May improve haematological outcomes in specific NTDT populations ● Low cost 	
Splenectomy	<ul style="list-style-type: none"> ● May improve growth, QoL, and haemoglobin concentration, thus avoiding transfusions for some patients 	<ul style="list-style-type: none"> ● Risk of sepsis ● Increasing awareness of other risks from NTDT studies, including venous thrombosis and other vascular manifestations ● May reduce ability to scavenge toxic free iron species, as evident from NTDT studies
HSCT	<ul style="list-style-type: none"> ● Potentially curative for patients with TDT ● 90% survival rate in patients; disease-free survival rates > 80% in TDT ● Improves HRQoL of children with severe disease ● Long-term cost-effectiveness 	<ul style="list-style-type: none"> ● Appropriate only for a subset of patients ○ Young age ○ Compatible sibling donor ● 5–10% risk of mortality ○ Intensive myeloablative conditioning required, graft-versus-host disease, and graft failure ● Potential impairment of fertility ● Requires access to technology at major treatment centre ● Substantial one-off cost of procedure

HRQoL, health-related quality of life; HSCT, haematopoietic stem cell transplantation; NTDT, non-transfusion-dependent thalassaemia; TDT, transfusion-dependent thalassaemia; QoL, quality of life.

treatment is now in the region of GBP 483,454 (USD 720,201) over 50 years [45].

The costs of medical care for patients with beta-thalassaemia are prohibitive for many individuals without reimbursement and for public healthcare systems around the world [47–49]. It is estimated that only 12% of children born with TDT receive adequate transfusion therapy, and < 40% of those transfused receive adequate iron chelation therapy [5]. The costs of specific therapies are discussed below.

The indirect costs of beta-thalassaemia are less well studied. Data from a large international study indicate that adults with beta-thalassaemia who are in paid employment lose a mean of 2.1 working days per month; similarly, children are reported to miss 2.8 days of school per month [36].

8. Current management of beta-thalassaemia

8.1. Current treatment options

Patients with severe beta-thalassaemia require lifelong therapy to prevent and manage the clinical consequences of disease [50]; long-term adherence to treatment is essential. Comprehensive management guidelines for both TDT and NTDT have been developed by the Thalassaemia International Federation and are widely available [1,2]. Current management strategies for TDT comprise blood transfusion, iron chelation, splenectomy (less common than in the past), and, for a subset of patients, HSCT. The benefits and limitations of current therapies for beta-thalassaemia are discussed below and summarized in Table 1.

8.1.1. Blood transfusion

Transfusion therapy for severe beta-thalassaemia provides normal red blood cells and suppresses ineffective erythropoiesis, thus limiting downstream pathophysiological complications [2]. Patients with TDT require lifelong, regular blood transfusions administered every 2–5 weeks to maintain pre-transfusion haemoglobin levels of at least 9–10.5 g/dL [1]. This transfusion schedule is usually started before the

age of 2 years and enables normal growth and physical activities, adequately suppresses bone marrow expansion in most patients, and may prevent iron absorption [1,51].

The indications for blood transfusion among patients with NTDT are less well established. Occasional transfusions should be considered for defined periods during times of anticipated acute stress or low haemoglobin levels, such as infection, surgery, or pregnancy [2]. More frequent transfusions may be beneficial for some patients, for example children with growth failure, reduced exercise tolerance, or poor QoL [2]. Controlled clinical trials to evaluate the role of sustained, regular transfusions for patients with NTDT are lacking. However, observational data on patients with NTDT suggest a potential role for transfusion in the prevention and management of thrombotic events, silent brain infarcts, pulmonary hypertension, leg ulcers, and extramedullary haematopoietic pseudotumours [2]; these findings have been attributed to suppression of ineffective erythropoiesis and subsequent pathophysiological mechanisms [18]. Therefore, it has been suggested that earlier introduction of transfusion therapy to prevent the clinical consequences of disease could benefit patients with NTDT [2,52,53]; however, others argue that increasing blood intake in NTDT may lead to more iron accumulation and, thus, complications from iron-related overload [52].

Approximately 100,000 patients currently receive regular transfusions for beta-thalassaemia worldwide [6]. The requirement for blood transfusion therapy for patients with beta-thalassaemia can be a huge burden in some countries [49,54]. It is estimated to cost EUR 131 to produce one unit of blood in Greece and at least USD 25 in Iran [49,55]. An estimated 33–47% of the cost of treatment for patients with severe beta-thalassaemia is attributed to blood transfusion [37,45].

Blood transfusion exposes patients to a number of risks: blood-borne infection, alloimmunization, and iron overload are among the key concerns for patients with beta-thalassaemia [2,7]. Although processes for screening, preparation, and administration of blood products have generally improved over time, challenges persist in some countries [7]. Alloimmunization occurs in 10–20% of patients with thalassaemia [1]; it is more common in patients who begin transfusion therapy later in

life (aged > 3 years), have NTDT [56–58], or have undergone splenectomy [59]. The use of extended antigen-matched donor blood reduces alloimmunization rates [1].

Blood transfusions are responsible for iron accumulation, as iron cannot be excreted physiologically; iron accumulation is already evident in children with TDT from 2 years of age. Adults with TDT receive an average of 0.3–0.6 mg of iron per kg per day and, without effective chelation, will accumulate approximately 6–12 g of iron each year [1]. Iron overload leads to complications that affect the heart, liver, and endocrine tissues [1,2]. Although iron chelation therapy is available, death due to iron overload remains an issue [2].

8.1.2. Iron chelation

As described, primary iron overload in NTDT due to increased iron absorption, and secondary transfusional iron overload in TDT, act to increase iron stores far beyond normal physiological levels unless effective chelation therapy is provided. Iron chelators interact mainly with low molecular weight ‘labile’ iron so only a small fraction of body iron is available for chelation at any time [1]. Hence treatment is most successful when chelation activity is present throughout the day [1]. Magnetic resonance imaging is useful to monitor body iron concentration and tailor iron chelation therapy to individual patient needs [1,60] but its availability varies by country.

Transfusion plus iron chelation therapy have been associated with improved long-term survival among successive birth cohorts of patients with TDT over the past 50 years [1]. Long-term use of iron chelators from early childhood improves liver and myocardial iron concentrations and can improve endocrine function [61–66]. Three iron chelators are currently approved by regulatory authorities for the treatment of iron overload among patients with beta-thalassaemia; indications and approval status vary across countries [67–72]. Evidence suggests that the short-term clinical effectiveness of all three iron chelators is similar [73], although efficacy in different organs may vary [74,75].

Deferoxamine, the first commercially available iron chelator, is administered by parenteral infusion for 8–12 hours, 5–7 nights per week [67,71,76]. The benefits of deferoxamine are well documented [63,64], but the demanding treatment regimen can lead to poor adherence [1,77]. Therefore, the introduction of two orally active iron chelators represented a great advance in the management of patients with beta-thalassaemia. Deferiprone is administered orally three times daily; deferasirox is administered orally once daily [69,70]. A combination of iron chelators is also sometimes used in clinical practice [78].

The success of currently available oral iron chelators has been somewhat limited by availability, efficacy, and safety issues [1,79]. For example, not all patients have the same access to chelators or are equally responsive to iron chelation therapy, and many patients around the world continue to live with high levels of iron in their liver and heart [80]. In addition, both deferiprone and deferasirox have been associated with some adverse events that require close and continuous monitoring [15,73]. Moreover, successful management of iron overload requires long-term adherence to treatment [81]. Satisfaction with, and adherence to, oral iron chelation therapy is greater than that observed for subcutaneous deferoxamine, but is still often suboptimal, particularly among adolescents and young adults [36,37,73,81].

Iron chelation therapy accounts for approximately half (43–55%) of the total current cost of treatment for patients with severe beta-thalassaemia [37,45]. Reported estimates of the comparative lifetime treatment costs of the three available iron chelators are variable [82,83]. However, most cost-effectiveness analyses support the use of oral iron chelators compared with deferoxamine [73,82–84]. The higher acquisition costs of oral iron chelators are generally considered to be offset by the avoidance of infusion-related equipment costs, convenience, and QoL benefits [45,73,82–84]. Regardless of the comparative cost-effectiveness of different formulations, it should be remembered that many patients in resource-constrained countries have no access to any iron chelation therapy [5,7].

8.1.3. Hydroxyurea

Hydroxyurea is a cytotoxic antimetabolic agent that increases fetal haemoglobin levels [2,85]. It is approved for use in sickle cell disease to reduce the frequency of painful crises and need for blood transfusions. Data from initial case reports suggested that hydroxyurea may also be beneficial for patients with beta-thalassaemia [85]. However, subsequent small studies among heterogeneous beta-thalassaemia populations produced inconsistent findings, and some suggested a decline in haematological response with long-term treatment [85]. No large, randomized, placebo-controlled trials have been conducted to prospectively evaluate the use of hydroxyurea among patients with beta-thalassaemia [85]. Accordingly, a recent Cochrane review found no robust evidence that hydroxyurea reduces transfusion requirement among patients with NTDT [86]. Nonetheless, treatment with hydroxyurea is supported cautiously for specific patient populations in current NTDT treatment guidelines [2]. Randomized, controlled trials with long-term follow-up are needed to assess the safety and efficacy of different doses of hydroxyurea in reducing transfusion requirement and complications of chronic anaemia [85–87].

8.1.4. Splenectomy

Splenectomy has been performed conventionally as an adjunct or alternative to transfusion therapy. Data from observational studies suggest that splenectomy may improve growth, QoL, and haemoglobin levels for some patients. However, data on serious adverse events after splenectomy are continuing to accumulate [28]. In addition to the accepted risk of sepsis, observational data on patients with NTDT suggest that splenectomy may be associated with a 4–7-fold increased risk of overt venous thrombosis and other vascular manifestations, including pulmonary hypertension [52,88]. It has also been suggested that splenectomy may reduce the body's ability to scavenge toxic free iron species [2]. Accordingly, indications for splenectomy among patients with beta-thalassaemia are becoming increasingly restrictive [1,2,89].

8.1.5. Haematopoietic stem cell transplantation

HSCT offers a potentially curative therapeutic approach for patients with beta-thalassaemia [1,90]. Almost 90% of patients with TDT who undergo HSCT at experienced centres in Europe now survive, with 2-year disease-free survival rates of over 80% [91]. HSCT improves the HRQoL of children with severe disease compared with lifelong blood transfusions, iron chelation, and management of complications [92–94]. HSCT for thalassaemia still carries an overall mortality risk of 12% within 2 years of transplantation [91]. The risks of HSCT are related largely to the intensive myeloablative conditioning regimens required, graft-versus-host disease, and graft failure. Myeloablative conditioning may also cause hypogonadism and infertility.

The best clinical outcomes of HSCT among patients with thalassaemia are reported in those aged under 14 years at transplantation [91]; this is likely to be because older patients have existing morbidity related to iron overload and other complications [15]. Young patients with TDT who have a human leukocyte antigen (HLA)-matched sibling donor should be offered HSCT at an early age [1,95]. Adults with a matched sibling donor may be offered HSCT within a clinical trial context [95].

Other techniques are required for the 70–75% of patients worldwide who do not have an existing suitably HLA-matched related donor [90]. Outcomes using matched unrelated donor HSCT are improving and may be attempted providing that the donor is selected using high-resolution molecular typing for both HLA class I and II loci, and according to stringent compatibility criteria [1,90,96]. Matched sibling donor HSCT following preimplantation genetic diagnosis and HLA typing may be feasible after appropriate ethical and legal consideration [97]. The use of HSCs from haploidentical related donors, HLA-mismatched family members, and unrelated umbilical cord blood is still considered experimental [90,96].

The economic costs of HSCT and lifetime follow-up vary globally.

Table 2
Selected therapeutic strategies under active investigation for treatment of beta-thalassaemia.

Therapeutic strategy	Mechanism of action	Key published efficacy and safety data	Most advanced stage of development
Improving globin chain imbalance			
Gene therapy	Exogenous beta- or gamma-globin genes are integrated into the genome of autologous stem cells using lentiviral vectors	Interim Phase1/2 study data suggest increased levels of haemoglobin A and reduced transfusion requirements [108]	Phase 3 (NCT02906202) and long-term follow-up study (NCT02633943)
Gene editing	Designer nucleases are used to genetically modify the endogenous DNA of stem cells	In vitro and early in vivo data suggest that BCL11A knock-out genome-editing and/or promoter-targeted gamma-globin gene-induction technology could allow permanent production of fetal haemoglobin in adults with thalassaemia [109–111]	Preclinical [109–111]
Improving ineffective erythropoiesis			
Ruxolitinib	Inhibits JAK2, the intracellular signal transducer of erythropoietin	Data from a single-arm, multicentre Phase 2a study among adults (n = 30) with TDT and splenomegaly (NCT02049450) indicate that ruxolitinib, administered orally at a starting dose of 10 mg twice daily, reduced spleen volume but had little effect on pre-transfusional haemoglobin levels or transfusion requirement; the most commonly reported adverse events associated with ruxolitinib were respiratory tract infection (8/30), nausea (6/30), upper abdominal pain (5/30), anaemia (5/30), diarrhoea (5/30), and weight increase (5/30) [122]	Phase 2a [122]
Sotatercept	Activin type IIA receptor fusion protein that binds to activin A and other TGF-beta superfamily ligands to target late-stage erythropoiesis	Data from a multicentre, open-label Phase 2a study among adults with TDT or NTDT indicate that sotatercept at 0.1, 0.3, 0.5, 0.75, or 1.0 mg/kg administered once every 3 weeks (NCT01571635) increased haemoglobin levels and reduced transfusion burden; sotatercept was shown to have a reasonable safety profile [129]	Phase 2a [129]
Luspatercept	Activin type IIB receptor fusion protein that binds to select TGF-beta superfamily ligands, such as GDF11 and activin B to target late-stage erythropoiesis	Data from a multicentre, open-label Phase 2 study among adults with beta-thalassaemia indicate that luspatercept administered subcutaneously every 3 weeks at doses of 0.2–1.25 mg/kg (NCT01749540 and NCT02268409) reduced transfusion requirements and liver iron concentration among patients with TDT [135], and increased haemoglobin levels, reduced liver iron concentration, and improved the QoL of those with NTDT; luspatercept was generally well tolerated [136]	Phase 3 (NCT02604433)
Improving iron dysregulation			
Mini-hepcidins	Short peptides that mimic the activity of endogenous hepcidin	Mouse model data suggest significant improvements in ineffective erythropoiesis, anaemia, and iron overload [140]	Preclinical
TMPRSS6 inhibitors	Gene-editing or small-interfering RNA techniques inhibit TMPRSS6, and thereby stimulate endogenous hepcidin production	Data from mice and other preclinical models of beta-thalassaemia suggest improvements in anaemia, reduction of ineffective erythropoiesis, splenomegaly, and iron overload [141–144].	Preclinical

GDF11, growth differentiation factor 11; JAK2, Janus kinase 2; NTDT, non-transfusion-dependent thalassaemia; QoL, quality of life; RNA, ribonucleic acid; TDT, transfusion-dependent thalassaemia; TGF, transforming growth factor; TMPRSS6, transmembrane protein serine 6.

For example, the estimated total lifetime cost for a patient with TDT who undergoes successful HSCT in Taiwan is USD 110,588 [44]. The estimated costs of HSCT for thalassaemia are USD 12,915 in Mexico, in the range of USD 10,000 in Islamabad and Jaipur, and up to THB 735,839 in Thailand in the first year [98–100]. In Austria, the median cost of HSCT in children is EUR 136,382 (USD 175,815) [101]. The estimated costs associated with HSCT are lower than those of lifelong blood transfusion and iron chelation and, as such, HSCT is considered a cost-effective treatment option for children with TDT [1,45,77,98]. Nonetheless, a considered clinical judgement is required to assess the suitability of HSCT for individual patients with beta-thalassaemia [15].

9. Novel strategies for treatment of beta-thalassaemia

A better understanding of the pathogenesis and clinical effects of beta-thalassaemia in recent years has stimulated research into a number of promising therapeutic approaches (Fig. 2). Key data on novel strategies under investigation for the treatment of beta-thalassaemia are summarized in Table 2.

9.1. Improving globin chain imbalance

Gene therapy technology has potential to correct the underlying

alpha-/beta-globin chain imbalance in beta-thalassaemia.

9.1.1. Gene therapy

Gene therapy using autologous stem cells could offer an alternative curative approach to HSCT, which is limited to patients with an appropriately matched donor [102]. Haematopoietic stem and progenitor cells (HSPCs) are isolated and exogenous beta- or gamma-globin genes, which are integrated into the host cell genome using a lentiviral vector [102]. After full or partial myeloablation, the genetically modified autologous HSPCs are returned to the patient where the modified cells repopulate the haematopoietic compartment [22,102].

Gene therapy technology has proved curative in several animal models of beta-thalassaemia [103–106]. A successful outcome was also reported after the use of additive globin techniques in one adult patient with severe beta-thalassaemia [107]. Several clinical trials of the efficacy and safety of gene therapy for patients with TDT are in progress, including NCT03207009, NCT02906202, and NCT02633943. Interim data from a Phase 1/2 study of autologous haematopoietic stem cells transduced ex vivo with a lentiviral vector among patients with TDT (NCT01745120) suggest increased levels of haemoglobin A and reduced transfusion requirements; final study data are awaited [108].

The clinical benefit of gene therapy technologies must outweigh the risks of myeloablative conditioning regimens. Efforts are ongoing to

improve the ability of viral vectors to express curative haemoglobin levels with fewer gene integrations per cell [22]. Such improvements are expected to reduce the required intensity of myeloablation and limit the risk of potential oncogenic integration [22]. Ongoing Phase 3 studies (NCT03207009 and NCT02906202) are investigating the potential role of gene therapy for patients aged 12–50 years; further studies will be required to assess its efficacy and safety in younger children.

9.1.2. Gene editing

Gene-editing technology can be used to genetically modify the endogenous DNA of haematopoietic stem cells [22]. Precise corrections to the genome can be made using designer nucleases. The cause of beta-thalassaemia in many patients is a single point mutation, which lends itself to correction using gene-editing strategies [102]. A key challenge of gene editing is to identify molecular targets that would benefit a wide population, among the numerous mutations associated with beta-thalassaemia.

One potential target for gene editing is the transcription factor BCL11A, which suppresses expression of gamma-globin [102]. Deletion of a specific erythroid enhancer in a mouse model impaired expression of BCL11A in erythroid precursors without affecting other haematopoietic lineages [109]. These data, and those from other in vitro studies [110–112], suggest that knock-out gene-editing technology could allow permanent production of fetal haemoglobin in adults with thalassaemia. Clinical trial data are awaited.

Gene therapies hold great promise for improving the lives of patients with beta-thalassaemia [102]. However, these techniques currently require sophisticated and expensive resources [102]. These limitations put gene therapies beyond the reach of many patients with beta-thalassaemia who live in remote and resource-constrained parts of the world [102].

9.2. Improving ineffective erythropoiesis

Ineffective erythropoiesis is the key pathophysiological mechanism underlying the chronic anaemia and complex multi-morbidity profile of patients with beta-thalassaemia. A number of agents that target ineffective erythropoiesis are currently under investigation for the treatment of anaemia due to beta-thalassaemia. Of these, the Janus kinase 2 (JAK2) inhibitor, ruxolitinib, and the activin receptor-II ligand traps, sotatercept (ACE-011) and luspatercept (ACE-536), are at the most advanced stages of development (Fig. 3).

9.2.1. JAK2 inhibitors

Erythropoiesis is regulated by a complex network of extracellular and intracellular factors [113]. Recent work has elucidated the roles of JAK2 and the transforming growth factor (TGF)-beta superfamily in the control of erythropoiesis. Binding of erythropoietin to its cell membrane receptor activates the cytoplasmic JAK2, which in turn activates multiple signal transduction pathways to increase proliferation, differentiation, and survival of erythroid progenitors [114]. JAK2 is the only intracellular signal transducer of erythropoietin and is, therefore, a potential target to treat conditions caused by disordered and ineffective erythropoiesis [114]. A JAK2 inhibitor, ruxolitinib, is approved for the treatment of patients with polycythaemia vera and myelofibrosis [115,116]. Experience in myeloproliferative disorders suggests that JAK2 inhibitors are clinically effective at reducing splenomegaly but are associated with side effects, including thrombocytopenia and anaemia [22], as well as acute relapse of disease symptoms [117]; these potential side effects could limit the clinical application of JAK2 inhibitors in NTDT. The perceived cost-effectiveness of treatment for myeloproliferative disorders has also been an issue in some countries [118,119].

JAK2 inhibition has been shown to improve ineffective erythropoiesis and reverse splenomegaly in a mouse model of NTDT [120]. Recent data from mouse models of NTDT and TDT also support the

ability of JAK2 inhibitors to reduce splenomegaly [121]. However, this positive effect on spleen size was associated with suppression of endogenous erythropoiesis that was not improved by blood transfusion [121].

Similar findings were recently reported from a single-arm, multi-centre, 30-week Phase 2a study to evaluate the efficacy and safety of ruxolitinib among adults ($n = 30$) with TDT and splenomegaly (NCT02049450) [122]. Ruxolitinib, administered orally at a starting dose of 10 mg twice daily, was associated with a noticeable reduction in mean spleen volume (26.4% reduction from baseline at week 30; $n = 25$) [122]. A slight reduction in transfused volume of red blood cells (5.9%; 95% CI: -14.7% , 2.8%) during the study was reported but no clinically relevant improvement in pre-transfusional haemoglobin levels was shown [122]. The most commonly reported adverse events were upper respiratory tract infection (8/30), nausea (6/30), upper abdominal pain (5/30), anaemia (5/30), diarrhoea (5/30), and weight increase (5/30) [122]. Given the limited benefit of ruxolitinib on pre-transfusional haemoglobin levels and transfusion requirements, no further studies in TDT are planned.

9.2.2. Activin receptor-II ligand traps

Activin receptor-II ligand traps bind to ligands, and act to prevent signalling at intended receptors [123]. The molecules were originally developed to inhibit activin-associated bone resorption among postmenopausal women [124,125]. Phase 1 data on sotatercept among healthy volunteers showed increased bone mineral density and an unexpected, clinically significant increase in haemoglobin level [124,125]. Two receptor fusion proteins, sotatercept and luspatercept, have been developed for the treatment of conditions caused by ineffective erythropoiesis, including beta-thalassaemia [126]. These recombinant proteins bind to select TGF-beta superfamily ligands that regulate late-stage erythropoiesis [126–128]. Thus, the mechanisms of action of sotatercept and luspatercept are distinct from erythropoiesis-stimulating agents and erythropoietin, which act on earlier stages of erythropoiesis.

9.2.2.1. Sotatercept. Sotatercept is a first-in-class recombinant activin type IIA receptor fusion protein [129] comprising the extracellular domain of the human activin type IIA receptor fused to the Fc domain of human immunoglobulin G1 (IgG1) [130]. Sotatercept binds with high affinity to activin A and other proteins in the TGF-beta superfamily [130,131]. Preclinical data indicate that sotatercept blocks the interaction of growth differentiation factor 11 (GDF11) with activin receptors and interferes with downstream signalling cascades [130,131]. Thus, sotatercept acts on late-stage erythropoiesis to increase mature red blood cells [130,131].

In a Phase 1b study of healthy volunteers, sotatercept led to dose-dependent increases in haemoglobin, haematocrit, and red blood cell counts that persisted for up to 4 months after treatment [125]. A multicentre, open-label, dose-finding Phase 2a study of sotatercept among adults with TDT or NTDT (NCT01571635) is now complete. Patients received sotatercept at doses of 0.1, 0.3, 0.5, 0.75, or 1.0 mg/kg administered subcutaneously once every 3 weeks [129]. Preliminary data indicate that sotatercept is associated with increased haemoglobin levels, a reduced transfusion burden, and a favourable safety profile [129]. Full final study data are awaited.

9.2.2.2. Luspatercept. Luspatercept is a novel recombinant protein comprising the modified extracellular domain of the human activin type IIB receptor linked to the Fc region of human IgG1 [126,128]. Luspatercept binds with high affinity to select TGF-beta superfamily ligands, such as GDF11 and GDF8; unlike sotatercept, luspatercept binds only minimally to activin A [128,130,132]. Preclinical studies indicate that luspatercept inhibits aberrant Smad2/3 signalling to improve late-stage erythropoiesis [128,133]. The murine analogue of luspatercept (RAP-536) corrected the complications of ineffective

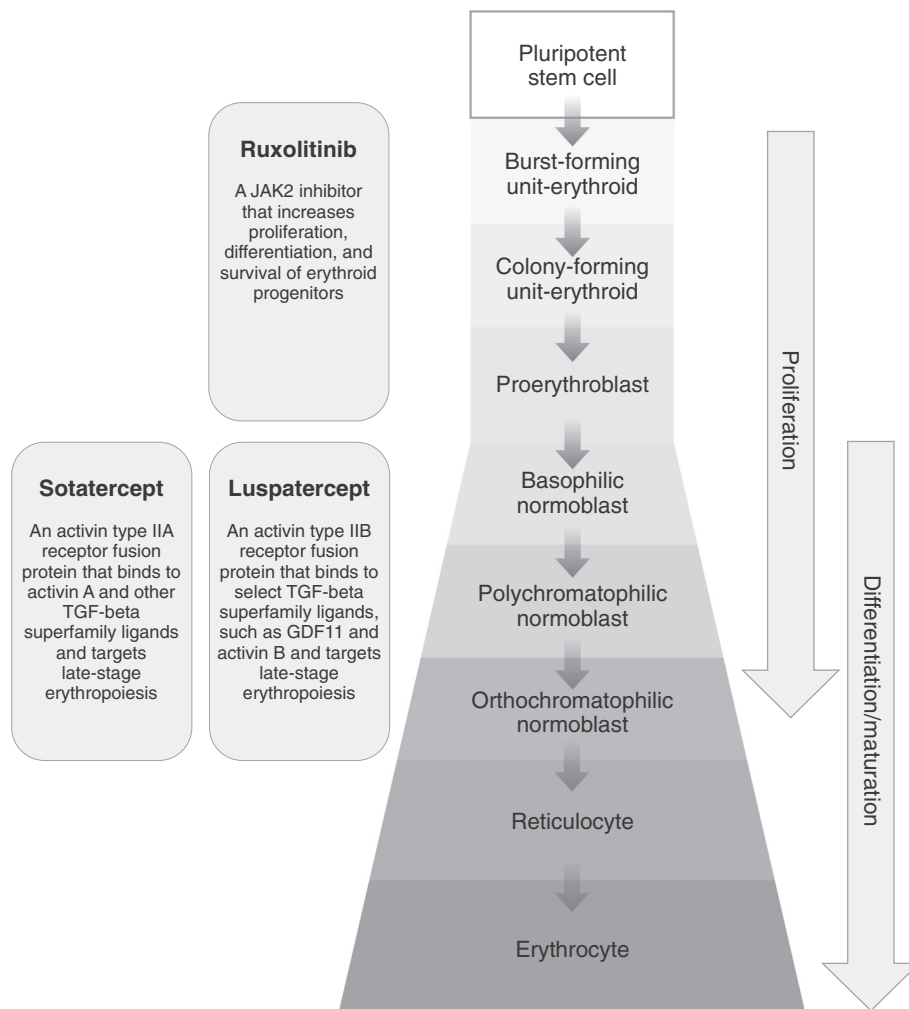


Fig. 3. Novel agents that primarily target ineffective erythropoiesis. GDF11, growth differentiation factor 11, JAK2, Janus kinase 2; TGF, transforming growth factor.

erythropoiesis, including iron overload, splenomegaly, and bone pathology in a mouse model of beta-thalassaemia [134].

In a Phase 1 study of healthy volunteers, a dose-dependent increase in haemoglobin level was observed 1 week after initiation of luspatercept, and maintained for several weeks after cessation of therapy; treatment was well tolerated [126]. A multicentre, open-label, dose-ranging Phase 2 study of luspatercept in adults with beta-thalassaemia is now complete (NCT01749540) and a 2-year extension is in progress (NCT02268409) [135]. Patients received luspatercept at doses of 0.2–1.25 mg/kg administered subcutaneously every 3 weeks [135]. Available data indicate that luspatercept was generally well tolerated and had a favourable safety profile [136]. Luspatercept reduced transfusion requirements and liver iron concentration among patients with TDT, and increased haemoglobin levels, reduced liver iron concentration, and improved the QoL among those with NTDT [136].

Following these positive outcomes, a double-blind, randomized, placebo-controlled, multicentre Phase 3 study (BELIEVE) has begun to evaluate the efficacy and safety of luspatercept among adults who require regular transfusions for beta-thalassaemia (NCT02604433). Luspatercept at a starting dose of 1.0 mg/kg [137] or placebo will be administered subcutaneously every 3 weeks. Demonstration of efficacy will require at least a 33% improvement in the number of transfused red blood cell units from baseline.

9.3. Improving iron dysregulation

9.3.1. Manipulation of hepcidin levels

Ineffective erythropoiesis and chronic tissue hypoxia inhibit the hepatic synthesis and secretion of hepcidin. Low circulating levels of hepcidin promote duodenal iron uptake, release of recycled iron from the reticuloendothelial system, and hepatic iron storage resulting in iron overload [18,29,30].

Moderate overexpression of hepcidin in a mouse model of beta-thalassaemia improved ineffective erythropoiesis, increased haemoglobin levels, reversed splenomegaly, and limited iron overload [138]. It may, therefore, be possible to prevent primary iron overload in beta-thalassaemia, and perhaps reduce the existing iron burden, by manipulating circulating levels of hepcidin.

Encouraging preclinical data on mini-hepcidins and transmembrane protein serine 6 (TMPRSS6) inhibitors have also been reported recently. Mini-hepcidins are short peptides that mimic the activity of endogenous hepcidin [139]. Administration of mini-hepcidin significantly improved ineffective erythropoiesis, anaemia, and iron overload in a mouse model [140]. TMPRSS6 is a transmembrane serine protease that reduces production of hepcidin [22]. Thus, endogenous hepcidin production can be stimulated by reducing expression of TMPRSS6. Data from mouse models suggest that deletion of the TMPRSS6 gene improves anaemia and reduces ineffective erythropoiesis, splenomegaly, and iron loading [141]. Use of antisense oligonucleotides or small interfering RNAs that target TMPRSS6 has been shown to improve

anaemia and iron overload in mice and other preclinical models of beta-thalassaemia [142–144].

Cellular delivery of iron for erythropoiesis depends on binding of transferrin-bound iron to transferrin receptor 1 (TfR1) [145]. Induction of TfR1 haploinsufficiency or administration of exogenous apo-transferrin increases hepcidin expression and reverses ineffective erythropoiesis in mouse models of beta-thalassaemia [145–147].

Patients with NTDT are more likely to benefit from agents that manipulate hepcidin expression than are those with TDT, because transfusional iron overload is not mediated by low hepcidin levels. However, mini-hepcidins and TMPRSS6 inhibitors still merit evaluation for use in patients with TDT because improvement in erythropoiesis could potentially reduce transfusion requirements. The magnitude of their treatment effect in clinical trials will help to determine their potential use as an alternate, sequential, or concomitant therapy to existing iron chelators in different patient subsets.

9.3.2. Manipulation of ferroportin and HIF2alpha

Ferroportin exports iron from enterocytes to the circulation and plays an important role in iron homeostasis [148]. Intestinal hypoxia-inducible factor-2alpha (HIF2alpha) is the key regulator of ferroportin expression in response to changes in systemic iron requirements [148]. In mouse models, HIF2alpha is activated early in the pathogenesis of beta-thalassaemia and contributes to the accumulation of iron [149]. Furthermore, disruption of HIF2alpha signalling corrects iron overload in mouse models of beta-thalassaemia [149].

9.4. Reflections on novel therapies

Novel therapeutic modalities are being evaluated for patients with TDT or NTDT. The ultimate aim for those with TDT is to transform the natural disease course and ultimately offer independence from transfusion and iron chelation therapy. Potential therapies must enable patients to maintain adequate haemoglobin levels, otherwise patients with TDT could simply transition to a state of uncontrolled NTDT with its substantial associated morbidity. To achieve this, therapies to reduce the transfusion burden in TDT must keep ineffective erythropoiesis and haemolytic activity within acceptable levels. If novel therapies cannot offer transfusion-independence, then the value of reduced transfusion requirements must be carefully evaluated; patient satisfaction, QoL, iron balance and chelation requirement, and cost-effectiveness should all be considered. If transfusion-independence is achieved, pre-existing iron overload or other complications must still be actively managed, especially among adult patients. Consideration of early introduction of novel therapies is warranted, provided that adequate growth and development are maintained.

Amelioration of ineffective erythropoiesis and iron dysregulation are the key targets for patients with NTDT. Novel agents that target either ineffective erythropoiesis (luspatercept) or iron dysregulation (mini-hepcidins and TMPRSS6 inhibitors) have clinical or proof-of-concept evidence, respectively, of amelioration of both disease mechanisms. However, demonstration of clinical benefit in drug development programmes is a challenge because morbidities resulting from ineffective erythropoiesis and/or iron dysregulation develop over many years. Thus, initial proof of benefit may be limited to simply showing short- to mid-term improvement in anaemia, iron overload, symptoms, and/or general wellbeing. Once the efficacy and safety of novel therapies are established, long-term, head-to-head, and comparison or combination trials would inform decisions on the optimum management of patients with TDT or NTDT. In particular, because monotherapy may not be enough to achieve the target of a transfusion-free life for patients with TDT, future clinical trials are needed to evaluate combination therapy. This approach is valid, as these novel therapies have different mechanisms of action, and may act synergistically when used in combination.

10. Conclusions

The lifelong management of patients with beta-thalassaemia represents a huge global burden, particularly in resource-constrained countries. Despite marked advances in the management of beta-thalassaemia in past decades, more work is needed to achieve effective disease control for all patients. Early initiation of effective, tolerable, and convenient therapy is needed to maximize long-term adherence to treatment, and thus limit the development of complications in adulthood. An increasing knowledge of the pathogenesis of beta-thalassaemia in recent years has facilitated the development of a number of promising therapeutic strategies. Efforts to optimize HSCT techniques will continue in the future, as will the search for a cure for beta-thalassaemia through genetic modification approaches. Various novel drugs to ameliorate ineffective erythropoiesis and improve iron regulation are also in clinical development. It is hoped that these new approaches will reduce the symptom burden and multi-morbidity profile of patients with beta-thalassaemia and so improve their long-term clinical outcomes and QoL.

Practice points

- Beta-thalassaemia is a genetic disorder of haemoglobin synthesis characterized by deficient or absent synthesis of the beta-globin subunit of adult haemoglobin. The disorder has a complex pathophysiology and affects multiple organ systems.
- Beta-thalassaemia is a global disease, but is most prevalent in South Asia, the Far East, the Middle East, and Mediterranean countries; the disorder is becoming increasingly common in Europe and North America as a result of migration.
- Patients with severe beta-thalassaemia or transfusion-dependent thalassaemia (TDT) require regular red blood cell transfusions. Iron overload damages major organs, including the heart and liver; lifelong daily iron chelation therapy is effective, but often inadequate, in preventing iron toxicity due to either varying degrees of efficacy among patients or poor adherence.
- Allogeneic haematopoietic stem cell transplantation (HSCT) is the only available potentially curative therapy for thalassaemia, but is limited by difficulty in finding suitably matched donors and safety concerns.
- A number of new therapeutic strategies are in clinical development, including genetic modification approaches and novel agents designed to ameliorate ineffective erythropoiesis and improve iron regulation.

Research agenda

- What is the true incidence and prevalence of beta-thalassaemia worldwide?
- What is the clinical and economic burden of beta-thalassaemia worldwide?
- What are appropriate therapeutic goals for patients with beta-thalassaemia? How can goals be prioritized? Are goals different for newly diagnosed patients versus those with chronic disease?
- How should patients switching between TDT and non-transfusion-dependent thalassaemia (NTDT) be best managed and maintained?
- What is the optimal allogeneic HSCT regimen?
- Would novel therapies result in the emergence of new clinical forms in terms of transfusion requirement and persisting active disease?
- How valuable is a reduction versus complete amelioration of transfusion burden for patients with TDT?
- Will a combination of these novel therapies increase their clinical impact and enhance efficacy?
- When new therapeutic options become available, will patients be best managed with monotherapy or sequential or combined treatments?

- What will be the cost implications of such comprehensive management approaches?

Conflict of interest statement

MDC is an advisory board member for Novartis Pharmaceuticals, Sanofi-Genzyme, and Celgene Corporation. JBP has received research funding from Novartis Pharmaceuticals, and served on advisory boards at Agios Pharmaceuticals, Bluebird Bio, Celgene Corporation, and Novartis Pharmaceuticals. VV received research grant support and lecture fees from Novartis Pharmaceuticals, Sanofi-Genzyme, Sebia, and Roche Diagnostics, and research grant support from Celgene Corporation, Shire, Sideris Pharmaceuticals, and the Faculty of Medicine, Siriraj Hospital, Thailand. ATT has received honoraria from Novartis Pharmaceuticals, and research funding from Novartis Pharmaceuticals and Celgene Corporation.

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