Received: 23 June 2023 | Revised: 16 October 2023 | Accepted: 6 November 2023

DOI: 10.1002/ajh.27166

CRITICAL REVIEW

Defining curative endpoints for transfusion-dependent β-thalassemia in the era of gene therapy and gene editing

Selim Corbacioglu[1] | Haydar Frangoul[2] | Franco Locatelli[3] | William Hobbs[4] | Mark Walters[5]

[1]University of Regensburg, Regensburg, Germany

[2]Sarah Cannon Research Institute and the Children's Hospital at TriStar Centennial, Nashville, Tennessee, USA

[3]IRCCS, Ospedale Pediatrico Bambino, Gesù Rome, Catholic University of the Sacred Heart, Rome, Italy

[4]Vertex Pharmaceuticals Incorporated, Boston, Massachusetts, USA

[5]Department of Pediatrics, UCSF Benioff Children's Hospital Oakland, Oakland, California, USA

Correspondence
Selim Corbacioglu, University of Regensburg, Regensburg, Germany.
Email: selim.corbacioglu@mac.com

Funding information
Vertex Pharmaceuticals

Abstract

β-thalassemia is a monogenic disease that results in varying degrees of anemia. In the most severe form, known as transfusion-dependent β-thalassemia (TDT), the clinical hallmarks are ineffective erythropoiesis and a requirement of regular, life-long red blood cell transfusions, with the development of secondary clinical complications such as iron overload, end-organ damage, and a risk of early mortality. With the exception of allogeneic hematopoietic cell transplantation, current treatments for TDT address disease symptoms and not the underlying cause of disease. Recently, a growing number of gene addition and gene editing-based treatments for patients with TDT with the potential to provide a one-time functional cure have entered clinical trials. A key challenge in the design and evaluation of these trials is selecting endpoints to evaluate if these novel genetic therapies have a curative versus an ameliorative effect. Here, we present an overview of the pathophysiology of TDT, review emerging gene addition or gene editing therapeutic approaches for TDT currently in clinical trials, and identify a series of endpoints that can quantify therapeutic effects, including a curative outcome.

1 | INTRODUCTION

β-thalassemia is one of the most common monogenic diseases worldwide, affecting approximately 288 000 individuals globally.[1] The disease is caused by mutations in the β-globin gene (*HBB*) that result in reductions in the production of the β-chains in adult hemoglobin (HbA) leading to ineffective erythropoiesis and chronic anemia.[2–5] Currently, almost 300 mutations in the *HBB* gene have been identified that lead to ß-thalassemia (http://globin.cse.psu.edu).

Traditionally, β-thalassemia has been classified based on the severity of the disease phenotype and underlying genotype, with β^0 denoting mutations in the *HBB* gene that lead to an absence of β-globin synthesis (associated with more severe disease phenotype) and β^+ denoting mutations that cause marked reductions in β-globin synthesis (associated with milder phenotypes).[2,3,6] However, new methods of classifying β-thalassemias based on red blood cell (RBC) transfusion dependence have emerged recently. Patients who are transfusion-independent or need limited transfusion support over a specific period of time are defined as having non-transfusion-dependent β-thalassemia whereas those with the most severe forms of β-thalassemia, known as transfusion-dependent β-thalassemia (TDT), rely on regular, lifelong RBC transfusions for survival.[4,7] These classifications are now being used as eligibility criteria in clinical trials and in defining management guidelines.[8]

While regular RBC transfusions are critical for the management of patients with TDT, chronic transfusions cause transfusion-related iron overload, increasing the risk of end-organ toxicity, and treatment with iron chelation therapy (ICT)[9] that also can cause adverse reactions. Iron loading from RBC transfusions overwhelms serum

transferrin capacity, increasing the amount of unbound, reactive iron species that cause end-organ damage from reactive oxygen species and lipid peroxidation.[10] Iron chelators, such as deferoxamine, deferiprone, and deferasirox, which can lower systemic, hepatic, and myocardial iron levels are used to manage iron overload in patients with TDT.[11–13] Still, chronic RBC transfusions and extended use of ICT are also associated with increased risk of alloimmunization and infection[7,14] and some patients are unable to adhere to or are ineligible for these treatments.[15,16] The recently FDA-approved drug luspatercept, an agent able to reduce ineffective erythropoiesis, can reduce transfusion requirements in patients with TDT,[17] with an uncertain impact on long-term disease manifestations.

Beyond symptomatic management of TDT, there are few potentially curative options. Allogeneic hematopoietic stem cell transplant (HSCT) has traditionally been the only potentially curative therapy for TDT; however, its use is limited by donor availability, donor and patient age, and risks of toxicity and transplantation-related complications.[18] Several novel therapies, including gene therapy and gene editing approaches, with the promise to provide a one-time functional cure for TDT are either currently in clinical trials or have been recently approved in the United States or Europe.[19] As these therapies reach late-stage clinical trial testing, defining outcomes and endpoints capable of assessing their curative potential for TDT takes on added importance. Here, we provide an overview of the pathophysiology of TDT, assess the limitations of current treatments, review emerging therapeutic approaches in clinical trials, and identify clinical endpoints that can be used to assess potentially curative outcomes in TDT.

2 | PATHOPHYSIOLOGY AND OUTCOMES OF TDT

Human hemoglobin is a tetramer composed of two α-globin or α-globin-like chains and two β-globin or β-globin-like chains. In patients with TDT, mutations in the *HBB* gene cause reduced or absent β-globin chain synthesis, which disrupts the balance between α-globin and β-globin chains that drives the formation of the HbA tetramer ($\alpha_2\beta_2$). This imbalance promotes accumulation and precipitation of α-globin chains, leading to apoptosis of erythroid progenitors along with oxidative stress and stimulation of iron loading.[2,3,20,21] Clinically, these molecular events manifest in ineffective erythropoiesis and chronic anemia.

Developmentally, HbA becomes the predominant form of hemoglobin in humans by approximately 6 months of age, after a highly regulated transition from the embryonic hemoglobins ($\zeta_2\varepsilon_2$, $\alpha_2\varepsilon_2$, and $\zeta_2\gamma_2$) to fetal hemoglobin (HbF; $\alpha_2\gamma_2$) to HbA_1 ($\alpha_2\beta_2$) takes place.[8,22] During fetal development, HbF is the predominant hemoglobin present, which persists though the initial neonatal period. The production of HbF is determined by the developmentally regulated expression of the γ-globin *HBG* genes. During the first year after birth, HbF levels decline after *HBG* genes are silenced and expression is shifted to β-globin with the production of HbA, which fails to form sufficient, stable hemoglobin tetramers due to the mutations that cause β-thalassemia.[23,24] Because of the protective effect of persistently elevated HbF which effectively can substitute for HbA, patients with β-thalassemia have no symptoms, including no anemia nor ineffective erythropoiesis at birth. It is only during the first year after birth after HbF levels decline that anemia develops. Typically, after this developmental switch in globin expression, only low levels of minor hemoglobins, such as HbA_2 ($\alpha_2\delta_2$) and HbF, are produced.[8] There are some individuals who inherit a condition known as hereditary persistence of HbF (HPFH)[24,25] wherein high levels of HbF persist beyond the neonatal period due to inherited mutations that abrogate HbF to HbA switching at birth.[22,24] In addition, children and adults with TDT who co-inherit HPFH, have reduced severity of TDT, suggesting HbF mitigates ineffective erythropoiesis and globin chain imbalance that is characteristic of TDT.[22,26] This well-known protective effect is particularly pronounced when HbF is expressed in a pancellular distribution, extending the lifespan of the mature RBCs in circulation.[27] These findings highlight the potential of HbF as a therapeutic target to treat the underlying cause of TDT.

With expanded access to healthcare in a growing number of countries and continued optimization of symptomatic therapies, the overall and complication-free survival for patients with TDT has improved during the past decade.[28] However, patients with TDT still have reduced life expectancy compared to the general population and experience significant complications that negatively impact on their quality of life.[29] Adverse complications that include bone marrow expansion and bone disease, extramedullary hematopoiesis and organomegaly, and liver, cardiovascular, and endocrine organ damage can accumulate over time.[6,7,30] Additionally, patients with TDT experience reduced social, physical, and emotional functioning, reduced health-related quality of life, and significant levels of anxiety and depression.[31–35] Due to the frequency and intensity of supportive care regimens, there is also a significant time burden experienced by patients and caregivers. For example, patients spend approximately 10 h managing their disease on days when they receive transfusions and typically 39 h managing their disease throughout the week.[31] Thus, it is important to develop meaningful clinical endpoints that capture quality-of-life (QOL) and other disease burdens in addition to more conventional adverse health effects.

3 | UNMET NEED FOR NOVEL CURATIVE THERAPIES TARGETING TDT

Allogeneic HSCT is considered the potentially curative standard, particularly in pediatric and adolescent patients (aged ≤18 years) who have human leukocyte antigen (HLA)-matched siblings.[7] More recently, excellent results have also been reported after HLA-matched unrelated donor transplantation in children.[36] While clinical trials of allogenic matched donor HSCT have shown excellent overall and disease-free survival rates in children,[37,38] allogeneic HSCT is associated with inferior disease-free survival in adults with TDT and is not universally recommended.[7,39] Thus, curative options are more limited for adults with TDT.

Unfortunately, only a minority of TDT patients have a very well-matched sibling or unrelated donor (i.e., 20%–40% according to the global region and race).[40] Alternate donor hematopoietic cell transplantation, including the use of a haploidentical-related donor has been studied, but there are increased risks of graft rejection and graft-versus-host disease that have limited the suitability of these options.[41,42]

There are important safety concerns that must be considered after allogeneic HSCT regardless of donor. Adult patients with advanced disease have an increased risk of transplant-related mortality[37,43,44] graft failure, and graft-versus-host disease, which are often due to immunologic complications of allogeneic donors and the immunosuppression required for their management.[4,18,45–47] The limited availability and risks associated with allogeneic HSCT have led to interest in developing new potentially curative therapeutic approaches for TDT.

4 | CLINICAL TRIALS OF GENE ADDITION AND GENE EDITING APPROACHES FOR TDT

In recent years, several gene addition and gene editing approaches have entered early and late-stage clinical trials which are designed to address the underlying cause of TDT and have the potential to provide a one-time, curative treatment for TDT (Table S1). These approaches rely on gene addition, gene editing, and gene silencing techniques and require the mobilization, collection, selection and modification of a patient's own hematopoietic stem cells (HSCs) with subsequent autologous transplantation.[19] Of note, the use of autologous transplantation with these gene therapy and gene editing approaches eliminates risks of alloreactive complications such as graft versus host disease and use of immunosuppressive drugs.

4.1 | Gene addition

Betibeglogene autotemcel (beti-cel) is a gene addition therapy in which a self-inactivating, third-generation lentiviral vector encoding a modified β-globin gene (HbA^{T87Q}) is transduced into autologous CD34+ HSCs ex vivo to produce exogenous HbA^{T87Q}.[45] An early phase trial showed that treatment can successfully produce functional HbA composed of tetramers containing the modified HbA^{T87Q} and that 68% of patients with β^0/β^0 and non-β^0/β^0 genotypes achieved transfusion independence.[4] A more recent study reported outcomes in patients with non-β^0/β^0 genotypes, where 20 out of 22 patients (91%), including 6 out of 7 patients younger than 12 years of age, treated with beti-cel achieved transfusion independence and near-normal levels of hemoglobin.[45] The suitability of this treatment with β^0/β^0 genotypes is currently being evaluated in a phase 3 clinical trial (NCT03207009).[45] Beti-cel was approved by the United States Food and Drug Administration in August 2022 for the treatment of both adult and pediatric patients with TDT. The treatment previously received conditional approval by the European Medicines Agency in 2019 but was withdrawn in 2022 by the marketing authorization holder for commercial reasons.

Similar to beti-cel, OTL-300 is a gene addition therapy that utilizes a lentiviral vector encoding *HBB*, known as GLOBE, to transduce autologous CD34+ HSCs ex vivo.[48] In a phase 1/2 clinical trial (NCT02453477), transduced CD34+ HSCs were infused intra-bone into nine patients with differing TDT genotypes (β^0/β^0, β^+/β^+, and β^0/β^+). One-year post-infusion, six of the seven patients eligible for efficacy evaluations experienced decreased RBC transfusions and three, all children, achieved RBC transfusion independence.[48] A clinical trial is ongoing to establish the long-term safety of OTL-300 in patients with TDT (ClinicalTrial.gov number, NCT03275051).[49]

4.2 | Gene editing

Exagamglogene autotemcel (exa-cel) is a gene editing approach that utilizes CRISPR-Cas9 technology to edit a target GATA1 binding site at +58 in the erythroid specific enhancer of the *BCL11A* gene in autologous CD34+ HSPCs.[50,51] The BCL11A protein is a repressor of γ-globin; therefore, gene editing at the erythroid enhanced region of *BCL11A* will reduce expression of BCL11A specifically in erythroid cells and reactivate synthesis of HbF.[50,51] Initial data from a Phase 1/2/3 trial demonstrated that a one-time infusion of exa-cel after single agent busulfan-based myeloablation provided clinically meaningful efficacy and had a safety profile consistent with myeloablation and autologous HSCT. Exa-cel has now moved into pivotal Phase 3 trials. Efficacy and safety data from a pre-specified interim analysis were reported in 2023 for 48 pediatric and adult patients with TDT dosed with exa-cel. Transfusion independence was observed for up to 40.7 months after exa-cel infusion, and almost all patients with more than 3.5 months of follow-up (95.5%) stopped RBC transfusions. Patients had early and sustained increases in total hemoglobin and fetal hemoglobin with pancellular distribution, with durable allelic editing observed in peripheral blood and bone marrow cells. All patients engrafted neutrophils and platelets, and the safety profile was consistent with myeloablative busulfan conditioning and autologous HSCT.[50,51] Exa-cel was approved by the Medicines and Healthcare products regulatory Agency in November 2023 for the treatment of both adult and pediatric (12 through 17 years of age) patients with TDT and sickle cell disease.

BIVV003 is a gene editing therapy that leverages zinc finger nucleases to target the same +58 GATA site in the erythroid-specific enhancer of *BCL11A* in autologous HSCs, thus also aiming to reactivate endogenous production of HbF.[52] While preliminary results from a Phase 1/2 clinical trial (PRECIZN-1; ClinicalTrial.gov number, NCT03653247) suggests this therapy may be effective in adult patients with sickle cell disease,[52] an earlier trial in patients with TDT did not show clinical benefit, presumably related to inefficient editing at the intended DNA target.

5 | DEFINING A "FUNCTIONAL CURE" IN TDT

The phrase "functional cure" was first introduced in the early 2000s in the context of human immunodeficiency virus to describe attainment of

TABLE 1 Important clinical endpoints for curative genetic therapies for TDT.

Clinical efficacy	Clinical laboratory measures	Clinical safety
Transfusion independence A weighted average hemoglobin of ≥9 g/dL without RBC transfusions for ≥12 months (e.g., Locatelli et al. 2022; CLIMB-121 ClinicalTrial.gov number, NCT03655678)	Hemoglobin concentrations, edited stem cells, and ineffective erythropoiesis • Total Hb and HbF levels • F-cell expression • Persistence of edited stem cells/allelic editing frequency (gene editing) • Vector copy number (viral vector) • Bone marrow M/E ratio	Engraftment • Time to neutrophil and platelet engraftment • Graft failure
Quality of Life/Patient Reported Outcomes measures[32,34] • EQ-5D-5L • EQ-5D-Y • FACT-BMT • PedsQL	Chelation therapy/iron overload • Ferritin • Frequency of iron chelation therapies and change in iron burden • Cardiac iron concentration (cardiac T2* magnetic resonance) • Liver iron concentration End organ function • Long-term end organ function (e.g., cardiac, pulmonary, renal, neurological, endocrine, bone, and splenic function)	Treatment-related mortality and malignancies • Transplant-related mortality • Peri-transplant complications (e.g., febrile neutropenia, infections, bleeding events, veno-occlusive liver disease) • Hematologic malignancies • Emergence of replication-competent lentiviruses

Abbreviations: EQ-5D-5L, EuroQol 5 Dimensions 5 Levels; EQ-5D-Y, EuroQol 5 Dimensions Youth; FACT-BMT, Functional Assessment of Cancer Therapy-Bone Marrow Transplantation; Hb, hemoglobin; HbF, fetal hemoglobin; PedsQL, Pediatric Quality of Life Inventory; RBC, red blood cell; TDT, transfusion-dependent thalassemia.

an average life expectancy while remaining free from routine treatment.[53] Other definitions of functional cure have since been established for chronic hepatitis B virus therapy,[54,55] in oncology clinical trials[56] and associated with diabetes mellitus.[57] However, there is no established consensus definition of functional cure for TDT.

Any definition of functional cure must convey a realistic therapeutic goal across a patient's lifetime, considering the views and interpretations of both patients and healthcare providers.[53,55] For gene therapy and gene editing approaches in TDT, the functional cure should be applied to therapies that abrogate the need for regular RBC transfusions with patients attaining normal or near-normal total hemoglobin concentrations (i.e., 10 g/dL or greater for adult females and 12 g/dL or greater for adult males) that are sustained, and which together translate into a near-normal quality of life. In addition, controlling the ineffective erythropoiesis associated with TDT and restoring iron homeostasis represents additional therapeutic goals when treating patients with TDT.

6 | CURATIVE ENDPOINTS FOR TDT

Historically, allogenic HSCT clinical trials have reported overall and event-free survival rates defined as time from transplantation to death or recurrent TDT, based on an assessment of whether or not full donor engraftment or stable mixed donor chimerism occurred without anemia and sufficient for stopping transfusions.[38,58] A number of factors can influence the risk of death, including the degree of pre-existing end-organ damage and complications due to iron overload, which are especially relevant for older patients with TDT.[59] In addition to overall survival and event-free survival, it is also important to evaluate a comprehensive set of clinical, laboratory, and safety-related endpoints (Table 1) when the goal is to assess the potential for any gene therapy or gene editing approach to provide a functional cure in patients with TDT.

6.1 | Clinical endpoints

6.1.1 | Transfusion independence

The most important clinical endpoint is achieving durable transfusion independence. In one study, 258 children and adults with TDT who received allogenic HSCT had a 30-year survival of 82.6%, and 77.8% were transfusion-free.[60] This suggests that most patients were able to stop RBC transfusions after successful allogeneic HSCT. Since patients with TDT typically receive transfusion support as often as every 2–5 weeks,[6] a duration of 12 months or more without RBC transfusions and a weighted average total hemoglobin of ≥9 g/dL is sufficient to establish transfusion independence. Gene therapy and genetic editing studies typically involve long-term follow-up for up to 15 years after treatment, during which time ongoing evaluation of transfusion independence will be monitored.

6.1.2 | Iron chelation

Chronic iron overload in patients with TDT, which persists even after any form of curative approach, perpetuates inflammation and

continues to damage sensitive organs such as the liver. Therefore, discontinuation of iron chelation therapy and establishing a normal iron status are also important in defining a curative therapy. Following HSCT, this endpoint encompasses the removal of pre-existing iron without ongoing iron loading. When assessing the need for iron chelation therapy, it is important to consider the baseline degree of iron overload and the additional transfusion support administered through the transplant procedure. The pace and ease of pre-existing iron removal are also affected by the timing of initiation of iron removal therapy after transplant, whether the option of phlebotomy is practical, and patient adherence to iron reduction treatment in the post-transplant setting. It is expected that removal of pre-existing iron will occur slowly over time after gene therapy and gene editing approaches, potentially taking years to normalize, consistent with observations from the allogeneic HSCT experience.[61,62] As such, iron overload and the use of iron chelation therapy should also be monitored in long-term follow-up studies to best inform achievement of a functional cure.

6.1.3 | Additional clinical endpoints for assessing a curative therapy in TDT

After establishing a normal or near-normal hemoglobin concentration without transfusions, it is important to then assess the trajectory of end-organ disease. Assessments of organ function (cardiac, renal, hepatic, and endocrine) estimate a patient's physical health and whether stabilization of end-organ damage associated with TDT and long-term RBC transfusions has occurred. Organ function in TDT is influenced by tissue iron content, particularly in cardiac, hepatic, and endocrine organs where there is a predilection for iron overload to occur. The evaluation of tissue iron is separately discussed below. It is also important to note that end-organ damage may not be reversible (e.g., hypogonadism or diabetes mellitus) according to the duration or severity of iron overload, which tends to me more pronounced in adults than children. These additional organ-specific endpoints should be monitored over the course of clinical studies and long-term follow-up. Monitoring in pediatric patients with TDT might be particularly informative, where the expectation is that organ function will return to normal. Therefore, inclusion of pediatric and adolescent patients in gene therapy and gene editing trials for TDT is important for the determining long-term success of a curative therapy.

6.1.4 | Patient-reported outcomes on HRQoL

The effect of curative therapy on quality-of-life also should be assessed. Patient-reported outcomes can measure the emotional, social, and physical functioning of patients with TDT after gene therapy. There are established tools to measure health status and quality of life in adults with TDT across these domains. Patient-reported outcome tools utilized in HSCT studies include the EuroQol Quality of Life Scale (EQ-5D-5L) and the FACT-BMT while the EQ-5D-Y and PedsQL Core are tools for measuring general well-being and overall health status in children and their caregivers. These assessment tools have defined minimal clinically important differences to evaluate potential clinical benefit and effectiveness of therapy. The EQ-5D-5L assesses the health status of adults whereas the EQ-5D-Y assesses the health status of children (≥12 and <18 years of age) in a standardized way. The FACT-BMT is commonly used in adult subjects undergoing HSCT. The PedsQL Core is a brief, standardized, generic instrument that systematically assesses patients' and parents' perceptions of health-related quality of life in pediatric subjects with chronic health conditions.[63]

6.2 | Laboratory and radiologic endpoints

Laboratory measures including total hemoglobin and Hb electrophoresis, assessing the number of genetically targeted cells, markers of ineffective erythropoiesis, and assessments of iron overload (Table 1) are typically assessed after gene therapy. Improvements in total hemoglobin (including normalization) above RBC transfusion thresholds (generally ≥9 g/dL) support the clinical endpoint measure of transfusion independence, although ideally a normal or near normal hemoglobin would be achieved. For gene editing approaches that reactivate HbF, the capacity to achieve HbF concentrations that result in total hemoglobin concentrations ≥9 g/dL is a clinically relevant measurement because total hemoglobin and transfusion independence are directly related to the magnitude of HbF induction and because HbF elevation is clinically associated with reduced or absent disease manifestation, including improvement in ineffective erythropoiesis.[64,65] Measurements of HbF concentration and F-cell expression are thus important endpoints for establishing the effectiveness of gene editing therapies[50] (Table 1).

Laboratory biomarkers that directly measure the proportion of genetically targeted cells should include the level and persistence of allelic editing at the intended locus (gene editing approaches) or vector copy number (viral vector approaches). These measurements in peripheral blood and bone marrow over time will prove essential for demonstrating the long-term treatment efficacy of curative therapy. The persistence of gene-edited alleles and the impact of gene insertion using lentiviral vectors should be also assessed in long-term follow-up studies to evaluate the durability of the treatment effect.

Ineffective erythropoiesis is a hallmark of TDT, which is marked by the expansion of erythroid precursors in the bone marrow and their death prior to the production of mature erythroid cells.[66] Ineffective erythropoiesis leads to comorbidities such as bone marrow expansion, concomitant bone deformities, splenomegaly, and further contributes to iron overload even in the absence of RBC transfusion. Assessment of ineffective erythropoiesis, including measure of the myeloid to erythrocytes (M:E) cell ratio in bone marrow, can be used to determine changes in TDT disease trajectory (Table 1). Patients with TDT generally have an M:E ratio of <0.1 due to ineffective erythropoiesis.[3,67] Increases in the M:E ratio toward 1 or greater than 1 in

the setting of transfusion independence indicate improvement in ineffective erythropoiesis. In patients with TDT, iron overload in the liver and heart can be predictive of risk for end-organ damage associated with disease progression and mortality. Therefore, evaluation of tissue iron content in these organs using non- invasive imaging approaches such as with T2* magnetic resonance imaging should be considered as endpoints in assessing the impact of treatment on tissue iron concentrations. Improvements in liver and cardiac iron content are important correlations for the risk of end-organ damage and to assess the potential of curative therapy. In addition, laboratory measures of iron overload, including serum ferritin, should also be included (Table 1).

6.3 | Safety evaluations

Assessments of safety in clinical trials will be critical in the context of novel gene therapies to determine the balance between treatment benefit versus risk. Patients should be monitored for engraftment of both neutrophils and platelets. Adverse events and serious adverse events should be monitored and attributed to cause whenever possible. Additionally, it will be important to consider the long-term potential side effects of novel therapies, including secondary malignancies (Table 1). For gene addition therapies, it will be necessary to conduct integration site analysis and to monitor for the emergence of replication-competent lentiviruses as well as insertional oncogenesis, while for gene editing therapies, evaluation of off-target editing sites is warranted if there are identified off-target sites. Notably, the exa-cel gene editing approaches currently in pivotal trials have no identified off-target sites using highly sensitive measures.[50]

7 | CONCLUSIONS

Conventional treatment for patients with TDT includes regular, lifelong RBC transfusions and ICT, both of which are associated with substantial negative impacts on long-term physical health and HRQOL. Allogeneic HSCT is a potentially curative option but unfortunately continues to be limited by patient and donor eligibility. Several novel therapies, including approaches that utilize gene therapy or gene editing approaches, with the potential to provide new curative options for patients with TDT are now in clinical trials.

These new gene addition and gene editing approaches offer a great clinical advantage in that the use of autologous transplantation eliminates the need for a fully matched human leukocyte antigen donor, and thus eliminates the risk of graft versus host disease and the need for immune suppression. Thus, gene addition therapy and gene editing are valuable potentially curative options for those patients who are eligible for allogeneic HSCT but either do not have an HLA-matched related donor or prefer an autologous gene therapy option. Many of these emerging therapies for TDT will require myeloablative conditioning regimens prior to infusion of gene-modified stem cells; these conditioning regimens may include the use of myeloablative regimen such as busulfan. The conditioning regimen, which typically includes well-established agents with known safety profiles, will be associated with well-described and manageable risks that should be monitored. Novel conditioning therapies, that are more specific in targeting bone marrow progenitor cells, and therefore reduce toxic effects in other tissues, are currently in development.[68] Characterizing the safety profile of these novel conditioning agents will be critical to assessing their ability to expand the availability of novel therapies to a broader set of patients as well as inform the overall benefit: risk of the overall treatment.

Overall, sustained RBC transfusion independence and maintenance of normal or near-normal levels of hemoglobin are likely the most critical endpoints in determining if a gene therapy treatment provides a functional cure to patients with TDT. Elimination of ineffective erythropoiesis and cessation of iron chelation therapy are supportive endpoints. Finally, patient health-related quality of life is also of key importance in determining the overall impact of curative genetic therapy. We propose that for any of these novel treatments to be considered a functional cure for TDT patients should be able to attain RBC transfusion independence that is durable, with normal or near-normal hemoglobin concentrations, improved measure of quality of life, effective erythropoiesis, and reduced iron overload with an acceptable safety profile that includes engraftment of neutrophils and platelets and absence of clonal hematopoiesis or insertional oncogenesis.

ACKNOWLEDGMENTS

The authors would like to thank Nathan Blow, PhD, of Vertex Pharmaceuticals Incorporated, who may own stock or stock options in the company, and Emma Guy, MScR, and Nicholas Strange of Complete HealthVizion, IPG Health Medical Communications, Inc., Chicago, IL, USA, for providing medical writing and editing support under the guidance of the authors. Open Access funding enabled and organized by Projekt DEAL.

FUNDING INFORMATION

Medical writing assistance was funded by Vertex Pharmaceuticals Incorporated, in compliance with Good Publication Practice guidelines.

CONFLICT OF INTEREST STATEMENT

Selim Corbacioglu has no conflicts of interest to disclose. Haydar Frangoul has served as a consultant for Editas Medicine, Rocket Pharmaceutical, and Vertex Pharmaceuticals Incorporated; on a speaker's bureau for Jazz Pharmaceuticals; on a data safety monitoring board for Rocket Pharmaceutical; and on a steering committee for Vertex Pharmaceuticals Incorporated. Franco Locatelli has received research support from Bellicum; served on a speaker's bureau for Amgen, Bellicum, bluebird bio, Gilead, Jazz Pharmaceuticals, Medac, Miltenyi, Neovii, Novartis, and SOBI; and served on an advisory board for Amgen, Bellicum, Neovii, Novartis, Sanofi, and Vertex Pharmaceuticals Incorporated. William Hobbs is an employee of Vertex Pharmaceuticals Incorporated and holds stock and/or stock options at the company. Mark Walters has served as a consultant for AllCells, Inc.,

BioChip, Inc., Ensoma, Inc., and Editas Medicine; served on an advisory board for Vertex Pharmaceuticals Incorporated; and holds stock/stock options in Ensoma, Inc.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable—no new data are generated, or the article describes entirely theoretical research.

ORCID

Selim Corbacioglu https://orcid.org/0000-0003-1070-8486
Franco Locatelli https://orcid.org/0000-0002-7976-3654
Mark Walters https://orcid.org/0000-0002-6515-4559

REFERENCES

1. Betts M, Flight PA, Paramore LC, Tian L, Milenkovic D, Sheth S. Systematic literature review of the burden of disease and treatment for transfusion-dependent beta-thalassemia. *Clin Ther*. 2020;42(2):322-337 e322.
2. Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis*. 2010;5:11.
3. Origa R. Beta-thalassemia. *Genet Med*. 2017;19(6):609-619.
4. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene therapy in patients with transfusion-dependent β-thalassemia. *N Engl J Med*. 2018;378(16):1479-1493.
5. Jaing TH, Chang TY, Chen SH, Lin CW, Wen YC, Chiu CC. Molecular genetics of beta-thalassemia: a narrative review. *Medicine (Baltimore)*. 2021;100(45):e27522.
6. Cappellini MD, Farmakis D, Porter J, Taher A. Guidelines for the Management of Transfusion Dependent Thalassemia (TDT). 2021. 2021 https://thalassaemia.org.cy/wp-content/uploads/2021/06/GUIDELINE-4th-SINGLE-PAGE.pdf
7. Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassaemia treatment: how will we manage this old disease with new therapies? *Blood Rev*. 2018;32(4):300-311.
8. Taher AT, Musallam KM, Cappellini MD. Beta-thalassemias. *N Engl J Med*. 2021;384(8):727-743.
9. Hamilton JL, Kizhakkedathu JN. Polymeric nanocarriers for the treatment of systemic iron overload. *Mol Cell Ther*. 2015;3:3.
10. Brittenham GM. Iron-chelating therapy for transfusional iron overload. *N Engl J Med*. 2011;364(2):146-156.
11. Pennell DJ, Berdoukas V, Karagiorga M, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. *Blood*. 2006;107(9):3738-3744.
12. Tanner MA, Galanello R, Dessi C, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation*. 2007;115(14):1876-1884.
13. Cappellini MD, Bejaoui M, Agaoglu L, et al. Iron chelation with deferasirox in adult and pediatric patients with thalassemia major: efficacy and safety during 5 years' follow-up. *Blood*. 2011;118(4):884-893.
14. Taher AT, Cappellini MD. Luspatercept for beta-thalassemia: beyond red blood cell transfusions. *Expert Opin Biol Ther*. 2021;21(11):1363-1371.
15. Shah FT, Sayani F, Trompeter S, Drasar E, Piga A. Challenges of blood transfusions in beta-thalassemia. *Blood Rev*. 2019;37:100588.
16. Shenoy S, Walters MC, Ngwube A, et al. Unrelated donor transplantation in children with thalassemia using reduced-intensity conditioning: the URTH trial. *Biol Blood Marrow Transplant*. 2018;24(6):1216-1222.
17. Cappellini MD, Viprakasit V, Taher AT, et al. A phase 3 trial of luspatercept in patients with transfusion-dependent β-thalassemia. *N Engl J Med*. 2020;382(13):1219-1231.
18. Cappellini MD, Motta I. New therapeutic targets in transfusion-dependent and -independent thalassemia. *Hematology Am Soc Hematol Educ Program*. 2017;2017(1):278-283.
19. Makis A, Voskaridou E, Papassotiriou I, Hatzimichael E. Novel therapeutic advances in beta-thalassemia. *Biology (Basel)*. 2021;10(6):546.
20. Taher AT, Cappellini MD. How I manage medical complications of beta-thalassemia in adults. *Blood*. 2018;132(17):1781-1791.
21. Meral A, Tuncel P, Surmen-Gur E, Ozbek R, Ozturk E, Gunay U. Lipid peroxidation and antioxidant status in beta-thalassemia. *Pediatr Hematol Oncol*. 2000;17(8):687-693.
22. Steinberg MH. Fetal hemoglobin in sickle cell anemia. *Blood*. 2020;136:2392-2400.
23. Steinberg MH, Sebastiani P. Genetic modifiers of sickle cell disease. *Am J Hematol*. 2012;87(8):795-803.
24. Thein SL, Menzel S. Discovering the genetics underlying foetal haemoglobin production in adults. *Br J Haematol*. 2009;145(4):455-467.
25. Steinberg MH. Treating sickle cell anemia: a new era dawns. *Am J Hematol*. 2020;95(4):338-342.
26. Uda M, Galanello R, Sanna S, et al. Genome-wide association study shows *BCL11A* associated with persistent fetal hemoglobin and amelioration of the phenotype of β-thalassemia. *Proc Natl Acad Sci U S A*. 2008;105(5):1620-1625.
27. Musallam KM, Sankaran VG, Cappellini MD, Duca L, Nathan DG, Taher AT. Fetal hemoglobin levels and morbidity in untransfused patients with beta-thalassemia intermedia. *Blood*. 2012;119(2):364-367.
28. Forni GL, Gianesin B, Musallam KM, et al. Overall and complication-free survival in a large cohort of patients with beta-thalassemia major followed over 50 years. *Am J Hematol*. 2023;98(3):381-387.
29. Farmakis D, Giakoumis A, Angastiniotis M, Eleftheriou A. The changing epidemiology of the ageing thalassaemia populations: a position statement of the Thalassaemia International Federation. *Eur J Haematol*. 2020;105(1):16-23.
30. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. *Lancet*. 2018;391(10116):155-167.
31. Paramore C, Levine L, Bagshaw E, Ouyang C, Kudlac A, Larkin M. Patient- and caregiver-reported burden of transfusion-dependent beta-thalassemia measured using a digital application. *Patient*. 2021;14(2):197-208.
32. Shah F, Telfer P, Velangi M, et al. Routine management, healthcare resource use and patient and carer-reported outcomes of patients with transfusion-dependent beta-thalassaemia in the United Kingdom: a mixed methods observational study. *EJHaem*. 2021;2(4):738-749.
33. Sobota A, Yamashita R, Xu Y, et al. Quality of life in thalassemia: a comparison of SF-36 results from the thalassemia longitudinal cohort to reported literature and the US norms. *Am J Hematol*. 2011;86(1):92-95.
34. Tedone F, Lamendola P, Lopatriello S, Cafiero D, Piovani D, Forni GL. Quality of life and burden of disease in Italian patients with transfusion-dependent beta-thalassemia. *J Clin Med*. 2021;11(1):15.
35. Yengil E, Acipayam C, Kokacya MH, Kurhan F, Oktay G, Ozer C. Anxiety, depression and quality of life in patients with beta thalassemia major and their caregivers. *Int J Clin Exp Med*. 2014;7(8):2165-2172.
36. Li C, Mathews V, Kim S, et al. Related and unrelated donor transplantation for beta-thalassemia major: results of an international survey. *Blood Adv*. 2019;3(17):2562-2570.
37. Angelucci E, Pilo F, Coates TD. Transplantation in thalassemia: revisiting the Pesaro risk factors 25 years later. *Am J Hematol*. 2017;92(5):411-413.
38. Baronciani D, Angelucci E, Potschger U, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000–2010. *Bone Marrow Transplant*. 2016;51(4):536-541.
39. Kanate AS, Majhail NS, Savani BN, et al. Indications for hematopoietic cell transplantation and immune effector cell therapy: guidelines from the American Society for Transplantation and Cellular Therapy. *Biol Blood Marrow Transplant*. 2020;26(7):1247-1256.
40. Galgano L, Hutt D. HSCT: how does it work? In: Kenyon M, Babic A, eds. *The European Blood and Marrow Transplantation Textbook for Nurses: Under the Auspices of EBMT*. Springer; 2018:23-36.
41. Strocchio L, Locatelli F. Hematopoietic stem cell transplantation in thalassemia. *Hematol Oncol Clin North Am*. 2018;32(2):317-328.

42. Anurathapan U, Pakakasama S, Songdej D, et al. Haploidentical hematopoietic stem cell transplantation in thalassemia. *Hemoglobin*. 2022;46(1):2-6.
43. Gluckman E, Cappelli B, Bernaudin F, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. *Blood*. 2017;129(11):1548-1556.
44. Kenyon M, Murray J, Quinn B, Greenfield D, Trigoso E. Late effects and long-term follow-up. In: Kenyon M, Babic A, eds. *The European Blood and Marrow Transplantation Textbook for Nurses: Under the Auspices of EBMT*. Springer; 2018:271-299.
45. Locatelli F, Thompson AA, Kwiatkowski JL, et al. Betibeglogene autotemcel gene therapy for non-β(0)/β(0) genotype β-thalassemia. *N Engl J Med*. 2022;386(5):415-427.
46. Mulas O, Mola B, Caocci G, La Nasa G. Conditioning regimens in patients with beta-thalassemia who underwent hematopoietic stem cell transplantation: a scoping review. *J Clin Med*. 2022;11(4):907.
47. Wingard JR, Hsu J, Hiemenz JW. Hematopoietic stem cell transplantation: an overview of infection risks and epidemiology. *Infect Dis Clin North Am*. 2010;24(2):257-272.
48. Marktel S, Scaramuzza S, Cicalese MP, et al. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent beta-thalassemia. *Nat Med*. 2019;25(2):234-241.
49. ClinicalTrials.gov. Long-term Follow-up of Subjects Treated With OTL-300 for Transfusion Dependent Beta-thalassemia Study (TIGET-BTHAL). 2022. https://clinicaltrials.gov/ct2/show/NCT03275051. Accessed February 17, 2023
50. Frangoul H, Altshuler D, Cappellini MD, et al. CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. *N Engl J Med*. 2021;384(3):252-260.
51. Locatelli F, Lang, P, Corbacioglu, S et al. Transfusion independence and elimination of vaso-occulsive crises after exagamglogene autotemcel in transfusion-independent beta thalassemia and severe sickle cell disease. In: *European Hematology Association Annual Meeting*, 2023.
52. Alavi A, Krishnamurti L, Abedi M, et al. Preliminary safety and efficacy results from Precizn-1: an ongoing phase 1/2 study on zinc finger nuclease-modified autologous CD34+HSPCs for sickle cell disease (SCD). *Blood*. 2021;138(1):2930.
53. Dube K, Willenberg L, Dee L, et al. Re-examining the HIV 'functional cure' oxymoron: time for precise terminology? *J Virus Erad*. 2020;6(4):100017.
54. Choi HSJ, Tonthat A, Janssen HLA, Terrault NA. Aiming for functional cure with established and novel therapies for chronic hepatitis B. *Hepatol Commun*. 2022;6(5):935-949.
55. Cornberg M, Lok AS, Terrault NA, Zoulim F, Faculty E-AHTEC. Guidance for design and endpoints of clinical trials in chronic hepatitis B—report from the 2019 EASL-AASLD HBV treatment endpoints conference (double dagger). *J Hepatol*. 2020;72(3):539-557.
56. Terpos ERM, Ntanasis-Stathopoulos I, Kanellias N, et al. Functional cure, defined as PFS of more than 7 years, is achieved in 9% of myeloma patients in the era of conventional chemotherapy and of first-generation novel anti-myeloma agents; a single-center experience over 20-year period. *Blood*. 2018;132(1):1968.
57. Bourgeois S, Sawatani T, Van Mulders A, et al. Towards a functional cure for diabetes using stem cell-derived Beta cells: are we there yet? *Cell*. 2021;10(1):191.
58. Baronciani D, Boumendil A, Dalissier A, et al. Hematopoietic cell transplantation in thalassemia and sickle cell disease: report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry: 2000–2017. *Blood*. 2018;132(1):168.
59. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of beta-thalassemia. *Eur J Haematol*. 2020;105(6):692-703.
60. Caocci G, Orofino MG, Vacca A, et al. Long-term survival of beta thalassemia major patients treated with hematopoietic stem cell transplantation compared with survival with conventional treatment. *Am J Hematol*. 2017;92(12):1303-1310.
61. Yesilipek MA, Karasu G, Kaya Z, et al. A phase II, multicenter, single-arm study to evaluate the safety and efficacy of deferasirox after hematopoietic stem cell transplantation in children with beta-thalassemia major. *Biol Blood Marrow Transplant*. 2018;24(3):613-618.
62. Inati A, El Hachem Y, Tawk S, Kassm SA, Abbas HA. Pediatric immune thrombocytopenia in Lebanon: treatment and predictors of outcome. *Pediatr Blood Cancer*. 2016;63(2):375-376.
63. Varni JW, Zebracki K, Hwang M, Mulcahey MJ, Vogel LC. Pain, pain interference, social and school/work functioning in youth with spinal cord injury: a mediation analysis. *J Spinal Cord Med*. 2022;1–7:1-7.
64. El Hoss S, Cochet S, Godard A, et al. Fetal hemoglobin rescues ineffective erythropoiesis in sickle cell disease. *Haematologica*. 2021;106(10):2707-2719.
65. Steinberg MH. Targeting fetal hemoglobin expression to treat beta hemoglobinopathies. *Expert Opin Ther Targets*. 2022;26(4):347-359.
66. Ribeil JA, Arlet JB, Dussiot M, Moura IC, Courtois G, Hermine O. Ineffective erythropoiesis in beta-thalassemia. *ScientificWorldJournal*. 2013;2013:394295.
67. Origa R. Beta-thalassemia. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews (R)*. University of Washington; 1993.
68. Haworth KG, Kiem HP. Next-generation conditioning for bone marrow transplantation: paving the way for CAR-T cell-based conditioning. *Mol Ther*. 2018;26(5):1167-1168.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Corbacioglu S, Frangoul H, Locatelli F, Hobbs W, Walters M. Defining curative endpoints for transfusion-dependent β-thalassemia in the era of gene therapy and gene editing. *Am J Hematol*. 2023;1-8. doi:10.1002/ajh.27166