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Innovative and Needs-led research on β-thalassemia treatment methods

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Beta-thalassemia is a well-known blood genetic disorder inherited in an autosomal recessive manner. Beta-thalassemia is found everywhere in the world as a rare, relatively rare, or common disease depending on the ethnic population. Affected individuals have chronic anemia associated with delayed growth, pale skin, weakness, fatigue, and more serious complications resulting in early death. Those with the severe form need frequent lifelong transfusions and depend on blood donations to survive. This literature mini-review highlights the healthcare needs that are not optimally met by people living with beta-thalassemia. The needs-led research can help to improve clinical outcomes through more appropriate management of the disease, increase provider satisfaction, and reduce the cost of care.

KEYWORDS

beta-thalassemia, unmet needs, needs-led research, innovative therapy, pattern of epidemiology

1 Introduction

β-thalassemia (BT) is a quantitative disorder of β -globin synthesis characterized by the absence (β^0) or reduced (β^+) synthesis of the normal β -globin chains of hemoglobin A. Even though β-thalassemia turned out to be an ancient disease (1), and the life expectancy of patients with severe thalassemia has increased alongside therapeutic progresses over time (2), it still imposes an economic burden on the communities, and healthcare systems worldwide. Moreover, the therapeutic protocols can physically and psychologically impact patients and caregivers. Patients with β-thalassemia have a lower health-related quality of life than the general population, especially for those who require lifelong regular blood cell transfusions and have more disease complications and chelation-related side effects (3). Hematopoietic stem cell transplantation from healthy donors is a cure for β-thalassemia, however it is not done very often due to the significant risk involved and its high cost (usually not covered by the medical insurance). Moreover, BT is included as a disability in the Rights of Persons with Disabilities Act, 2016 that

promotes "the access to the health services they need, when and where they need them, without suffering financial hardship" (4). More treatment options would help people with thalassemia to make the appropriate choice and develop the desired clinical improvements.

2 Information about BT in different populations

BT is found everywhere in the world being a rare, relatively rare, or common disease, depending on the ethnic population. It originates in endemic populations from malaria regions, such as the Mediterranean basin, the Middle East, South-East Africa, and Sub-Saharan Africa (5). The selective geographic distribution of BT is thought to be because of red blood cell morphological abnormalities that may play a protective role against malaria disease (6). At that point, the number of cases of BT occurring in a certain population was low, such as in North-West Europe, and high in people of Mediterranean, African, Middle Eastern, Asian Indian, Chinese, and Southeast Asian countries. The descents of people from endemic areas carry a greater risk of developing the disease. There are three main forms of BT in the population based on the degrees of phenotypic severity and blood transfusion requirement: β-thalassemia major (severe microcytic and hypochromic anaemia and clinical course requiring regular, lifelong transfusions), β-thalassemia intermedia (moderate symptoms of anaemia, occasional transfusions required) and β-thalassemia minor or β-thalassemia carrier (asymptomatic or mild symptoms of anaemia) (7).

Nowadays, BT becomes a global health problem through the migration of people from endemic areas throughout Europe, the Americas, and Australia, leading to the global distribution of the disease (8). In the last 50 years, the pattern of BT epidemiology has changed. In addition to migration, implementation of β-thalassemia prevention and screening programs, or survival rate due to current therapeutic approaches, decreased the prevalence in endemic populations (1, 8, 9). So, there is a significant variation of prevalence/incidence among different countries, but according to Orphanet, the annual incidence, at birth, of symptomatic BT is approximately one in every 100,000 individuals in the general population (10). The estimates based on the group of people with different types of BT (depending on the severity of symptoms) show that around 1.5% of the global population are carriers, approximately 60000 children are born with β-thalassemia annually, and about 63% have a longer life expectancy surviving through the age of 50 (11, 12).

Abbreviations: BT, β-thalassemia; FDA, Food and Drug Administration; EMA, European Medicines Agency; HbF, Hemoglobin F; HBB, Hemoglobin Subunit Beta; HBG, Hemoglobin Subunit Gamma; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9; HSPC, hematopoietic stem and progenitor cells; HIV-1, Human Immunodeficiency Virus-1; LVV, lentiviral vector.

3 What are the functional changes that accompany β-thalassemia?

The β-thalassemia is caused by more than 350 pathogenic variants of b-gene involved in the defective β-globin chains synthesis (13). The result is either reduced or absent synthesis of β-globin chains but the unaffected chains, such as a-globin chains, continue to be synthesized at relatively normal levels. As a result, the HbA (a_2b_2) formation does not work properly. Excess free a-chains precipitate in the cytoplasm of erythroid precursors. The molecular aggregates are toxic and highly insoluble, and form inclusions in nucleated erythroid precursors in the bone marrow. These inclusion bodies cause accelerated red blood cell destruction by apoptosis and intramedullary haemolysis leading to ineffective erythropoiesis responsible for anaemia. Along with haematological features, abnormal iron metabolism and bone abnormalities are present. Both patients with dependent and non-dependent transfusion experience an iron overload because of an inappropriate increase in intestinal iron absorption. Excess iron deposited in the heart, pancreas, liver, and other organs damages tissues and disfunctions. Symptomatic patients exhibit erythroid hyperplasia, bone marrow expansion, and extramedullary haematopoiesis. Under these conditions, the bones show marked decrease in mineral density. Skull and face bone deformities, cortical thinning, and pathological fractures of long bones are noted (14–20).

Considering all the actual burdens, there is no other option than seeking new available, accesible, and affordable therapies to improve the BT patients' quality of life. Therefore, what treatments bring hope in the patients' community? Is any new therapy that can treat Beta-thalassemia without side effects?

4 Novel therapeutic methods

4.1 Pharmacological approach

4.1.1 Luspatercept

A promising therapy recently approved by the FDA (in 2019) and EMA (in 2020) for the treatment of β-thalassemia is luspatercept (ACE-536) (21). It acts by inhibiting the Smad2/3 signaling pathway, which promotes the attenuation of ineffective erythropoiesis. It shows as well an improvement of iron balance parameters (22–25). It is currently approved only for the treatment of transfusion-dependent BT, but there is hope it could also be used for non-transfusion-dependent BT, according to ongoing clinical trials (26). However, an important disadvantage of using luspatercept today is the very high cost

of the drug, as it is estimated that the total annual amount that a patient could pay for luspatercept reaches up to $170,000 (27).

4.1.2 Hydroxyurea

Of all the drug therapies tested or used in β thalassemia, hydroxyurea certainly remains a mystery, but equally a challenge for the medical world to unravel the mysteries behind this substance. In addition to its antineoplastic effect that recommends it for the treatment of many types of cancer, it is currently approved for the SCD patients, but is also used in some cases of β thalassemia. Its importance has increased since the start of the COVID-19 pandemic as a result of the difficulties encountered by medical systems related to the adequate implementation of transfusion therapies (28). The main mechanism of action justifies its cytotoxic activity, as it blocks ribonucleoside diphosphate reductase (rNDP), an important enzyme involved in DNA synthesis. Therefore, it does not allow cells to go beyond the S phase of the cell cycle. It is also an HbF-inducer by interfering with various transcription factors (represses BCL11A and GATA1, stimulates GATA2), but also by modulating some epigenetic processes (21, 28). Recently, the results of the first placebo-controlled, double-blind, randomized clinical trial which aimed to study the effectiveness and safety of oral administration of hydroxyurea, for 6 months, in patients with TDT, were published (29). The primary outcome (a significant improvement in blood transfusion volume) was not met. However, 89% of participants who received the drug experienced increases in HbF during treatment, and 79% experienced decreases in serum sTfR (soluble transferrin receptor) levels, which is associated with improvements in hematopoiesis. All these data regarding the mentioned drugs support the idea that they can play the role of adjunctive therapies that bring an additional therapeutic benefit in β thalassemia. Therefore, more trials using therapeutic combinations should be organized.

4.2 Gene therapy

One of the researchers' priorities when treating β-thalassemia is to increase the γ chain production, which binds the α chains, leading to the production of HbF levels. This results in reduced precipitation of free α chains and mitigation of iron overload and cellular oxidative stress (30). Thus, it combates both the hemolytic syndrome and the dyserythropoiesis. Gene editing came in as a solution to increase the levels of γ-globin, by manipulating the genome of the hematopoietic stem and progenitor cells (HSPCs) from patients (31). In this regard, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) system is continuously tested.

Nonetheless, the last decade has witnessed the development of treatments based on lentiviral vectors, which rely on the gene transfer of healthy HBB genes (32, 33).

4.2.1 Genome editing-based treatments

Researchers turned their attention to the genetic manipulation of the BCL11A gene enhancer in red blood cells. BCL11A is considered to be the most important regulator of HBG expression. By deleting its enhancer through CRISPR/Cas9, the transcription of BCL11A is suppressed, which allows the resumption of HBG activity and HbF synthesis (34, 35).

A phase 1/2/3 study (CLIMB-111), supported by Vertex and CRISPR Therapeutics, developed a cell therapy product called CTX001 using CRISPR/Cas9 mechanisms in CD34+ HSPCs (NCT03655678). Researchers deleted the BCL11A gene enhancer, thus increasing the levels of γ-globin and restoring the production of fetal hemoglobin, when transplanted in the patients' blood (36). This study offers promising preliminary data: remarkable and sustained increases in total hemoglobin (new values being between 8.9 and 16.9 g/dL) and HbF (between 67.3% and 99.6%) for all 15 participants at 4-26 months after infusion. This progress allowed patients to be categorized as transfusion-independent (37).

Analogous to CLIMB-111, a recently initiated Phase 1/2 study (NCT04211480) also uses the CRISPR/Cas9 editing tool in HSPCs, targeting the BCL11A enhancer loci. It is dedicated exclusively to pediatric patients aged between 5 and 15 years and the partial results are encouraging as well (38).

Gene editing techniques can be used to modulate the α chain level, too. There are 2 ways to apply these tools in order to prevent the excess of free α chains. The first of them damages directly the α gene. Using CRISPR/Cas9 technology, its deletion can be induced [640], genetically "simulating" the appearance of α thalassemia. The main reason why this "association" (α and β thalassemia) is favoured is represented by the clinical and molecular observations made during the last decades that show the patients with co-inheritance of α and β thalassemia manifest a milder form of the disease (39), precisely because of an α/β ratio less unbalanced and with lower levels of cellular oxidative stress compared to β thalassemia patients. As a consequence of these findings, an *in vitro* study applying the theory was conducted and revealed remarkable results due to a significant reduction in the precipitation of free α chains (40).

The second way to control the α gene expression through gene editing techniques involves affecting gene enhancers. It is considered that gene activity is mediated by 4 enhancers (MCS-R1 to R4), but among them MCS-R2 is the key element as researches show (39, 41, 42). According to an *in vitro* study, the deletion of MCS-R2 with CRISPR/Cas9 led to significant reductions in the α chains' levels: 60% in the case of monoallelic mutation and 90% if the deletion targeted both alleles of the enhancer (41, 42). These results demonstrate the huge potential that gene therapy techniques have in the context of treating β thalassemia by modifying HBA activity and suggest the need for further investigations to study the concrete efficacy of these techniques.

4.2.2 Gene transfer-based treatments

August 2022 saw the first FDA approval of a genetic treatment method for transfusion-dependent β-thalassemia, Zynteglo (betibeglogene autotemcel) (43). Such treatment represents an innovation for patients suffering from BT, as it is a one-time therapy with an efficacy rate of over 86%, including pediatric patients. It uses the patient's own CD34+ bone marrow cells, modifying them genetically, *via* the lentiviral vector BB305, in order to encode the β-globin (βA-T87Q) gene, thus increasing the production of β-globin (44). Moreover, Zynteglo proves itself as a more favorable treatment method than the standard of care, from an economical perspective, as compared to lifelong treatment (45). BB305 is a lentiviral vector constructed after the structure of Human Immunodeficiency Virus-1 (HIV-1), but lacking in HIV-1 protein-coding genes, which are replaced by the β-globin ones. It does, however, maintain the structures needed in the processes of viral genome packaging and cellular transductions (46). The principle behind this treatment has been under investigation for the last 12 years, thus it is expected that more treatment options of this kind will emerge, such as the DEST LVV and others (47–49).

5 Conclusion

Considering the current needs of the patients, the new therapies should increase the quality of life by reducing the transfusion burden, disease complications and chelation-related side effects. Moreover, the outcomes of these newly-approved treatments should encourage the patients' community to support further research.

Author contributions

These authors contributed equally to this work. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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