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Trends in Internal Medicine

Treatment Options for Beta-Thalassemia

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ABSTRACT

Beta-thalassemia, a hereditary blood disorder caused by point mutations affecting beta-globin synthesis, impairs erythrocyte oxygen transport. Due to its resistance, beta-thalassemia originally propagated in regions with high malaria prevalence. More recently, it has spread globally through human migration, impacting millions. Early screening of beta-thalassemia can be preconception, fetal, and infant testing and is vital for diagnosis and treatment plans. The types major, minor, and intermedia are classified based on their respective mutations and result in various symptoms ranging in severity. Regular erythrocyte transfusions replace faulty blood cells to treat thalassemia major. These transfusions cause iron overload, a severe side effect that is often treated with chelation therapy. Currently, the only cure for beta-thalassemia is hematopoietic stem cell transplantation. Hematopoietic stem cells are undifferentiated cells in the red bone marrow that proliferate and differentiate into various blood cells, including erythrocytes. Transplantation replaces deleterious hematopoietic stem cells with healthy ones that can differentiate into erythrocytes with full oxygen-carrying capacity. While an effective cure, there are many challenges pre-transplantation and post-transplantation; for example, finding suitable donors and preventing complications. Through medical advances in stem cell exploration and gene editing techniques, research continues to progress and discover potential cures. CRISPR-Cas, a prominent gene editing tool, has found success in identifying and modifying specific gene sequences. Researchers hope to apply CRISPR-Cas to the beta-globin synthesis gene and prevent beta-thalassemia. As technology advances, the practical and ethical challenges of accessibility, cost, and safety persist at the forefront of patient care.

Keywords

Beta-Thalassemia, CRISPR-Cas, Hereditary, Malaria.

Abbreviations

Beta^{+}: Reduced beta-chain synthesis, Beta^{0}: Complete absence of beta-chain synthesis, Cas: CRISPR-associated systems, CRISPR: Clustered regularly interspaced short palindromic repeats, DFO: Deferoxamine, DFP: Deferiprone, DFX: Deferasirox, DNA: Deoxyribonucleic acid, FDA: Federal Drug Administration, GVHD: Graft-versus-host disease, g/dL: Grams per deciliter, HbA: Hemoglobin A, Adult hemoglobin, HbF: Hemoglobin F, Fetal hemoglobin, HIV-1: Human immunodeficiency virus 1, HLA: Human leukocyte antigen, HSC: Hematopoietic stem cell, HSCT: Hematopoietic stem cell transplantation, iPSC: Induced pluripotent stem cell, MHC: Major histocompatibility complex, PAM: Protospacer adjacent motif, PCR: Polymerase chain reaction, RNA: Ribonucleic acid, UCB: Umbilical cord blood.

Introduction

Etiology & Epidemiology

Beta-thalassemia is a hereditary genetic blood disorder [1]. Typically, autosomal recessive, beta-thalassemia is the most common autosomal recessive disorder globally but can also manifest as a dominant mutation [1,2]. It is caused by over two hundred different point mutations on chromosome 11, particularly those in the promoter or splice site of genes coding for beta-globin [3]. These mutations result in reduced beta-chain synthesis (beta^{+}) or complete absence of beta-chain synthesis (beta^{0}), which significantly impacts the ability of erythrocytes to transport oxygen and manifests in a plethora of symptoms throughout the body [2].

Three types of beta-thalassemia exist: major, intermedia, and minor. Beta-thalassemia major and intermedia are homozygous or compound heterozygous. Homozygous indicates that the same mutation on each allele causes beta^{+} or beta^{0}, and compound heterozygous describes different mutations on each allele that together result in beta^{+} or beta^{0}. Thalassemia minor is heterozygous, with only one mutated allele. Modifier genes, mutations that affect gene expression and lead to different phenotypes of the same disease, can counteract pathology and lessen beta-thalassemia symptoms [2].

One in 100,000 people have beta-thalassemia worldwide, and 80 to 90 million are carriers 1.5% of the world population [2,4]. The disease originated in the Mediterranean, Middle East, and Asian countries and most likely propagated due to their malaria resistance, a deadly disease common in these areas [2,5]. Today, most carriers are from the Mediterranean and Middle East, while most symptomatic individuals are from Southeast Asia, especially China and India [4]. Some areas have beta-thalassemia rates as high as 10% [3]. The recent rise in global migration has intensified the spread of beta-thalassemia beyond these geographical pockets. Beta-thalassemia now exists in almost every country worldwide, including presence in places like Northern Europe, where its historical absence can be explained by the lack of demand for malaria protection [5].

Discussion

Diagnosis

Early diagnosis of beta-thalassemia is imperative, as it provides the opportunity for more immediate treatment, increasing quality of life and possibly lightening the financial load. Carrier, prenatal, and newborn screening are all used for diagnostic purposes. Genetic testing and counseling are highly recommended for individuals from families with known carriers or a history of thalassemia or for individuals from high-risk regions. Preconception testing is the best option, extensively screening both potential parents to evaluate the child's susceptibility. Individuals who both carry the mutation are advised not to conceive. However, when only one parent is a carrier, the child has no risk of severe thalassemia, expressing thalassemia minor or no symptoms. These screening approaches have proven successful, reducing thalassemia births in the Mediterranean and Middle East. Fetal screening is also available if preconception testing is unobtainable or inconclusive [4].

Deoxyribonucleic acid (DNA) analysis identifies the order of nucleic acids and can discover carriers prior to conception or diagnosis in fetal testing [3]. Initial fetal screening utilizes high-performance liquid chromatography, a method that separates different molecules based on chemical and physical properties, to measure hemoglobin levels. If hemoglobin levels are low and thalassemia is suspected, more accurate methods, such as isoelectric focusing, polymerase chain reaction (PCR), or DNA sequencing, are used to confirm the diagnosis. Isoelectric focusing separates peptides based on charge to determine the precise amount of hemoglobin [4]. DNA sequencing focuses on locating the genetic mutation in cells from blood or amniotic fluid [3]. PCR accomplishes the same task by denaturing single strands of DNA into billions of copies and then amplifying it; this allows for gene detection based on size and charge [4]. After birth, newborn screening is used for early diagnosis, which notifies physicians to begin monitoring immediately so treatment can start promptly at the onset of symptoms. Unfortunately, current newborn screening procedures are inadequate. There is no national standard newborn screening for thalassemia in the United States, so it varies by state as of 2021, nineteen states neglected to screen for thalassemia. Additionally, existing tests only detect common thalassemia mutations, leaving many undiagnosed even after proper screening. Advocates support the inclusion of thalassemia testing in the standardized nationwide infant screening to enhance early detection and accessibility [4].

Appropriately narrowing down the type of anemia after the broader diagnosis is essential because beta-thalassemia is often misdiagnosed as iron deficiency anemia. An anemic patient without iron deficiency or improvement from iron supplements indicates non-iron-related anemia and calls for further assessment [4]. A peripheral blood smear examines erythrocyte properties and the amount of reticulocytes. Reticulocytes are cells that mature into erythrocytes, so a high number of reticulocytes indicates a constant replenishment of erythrocytes and, thus, a short erythrocyte lifespan. A complete blood count can rule out iron deficiency with low hemoglobin levels and small erythrocytes. On the other hand, high porphyrin levels indicate iron deficiency or lead poisoning; these results can rule out beta-thalassemia, which has normal porphyrin levels [3]. After identifying anemia as thalassemia, beta-thalassemia major is typically clinically diagnosed since symptoms manifest more obviously [4]. Infants with thalassemia major fail to produce any Hemoglobin A, adult hemoglobin (HbA), and produce a limited amount of hemoglobin F, fetal hemoglobin (HbF). Beta-thalassemia major is characterized by hemoglobin levels below 7 grams per deciliter (g/dL) [2]. However, this is also true of premature births, making it crucial to account for gestational age when considering possible diagnoses [4]. Infants with thalassemia intermedia produce some HbA but less than usual; they are diagnosed with hemoglobin levels between 7 and 10 g/dL [2,4]. The presence of residual HbF delays the onset of symptoms in infants, causing beta-thalassemia intermedia to maintain a low profile until nine to twelve months of age, when a mandatory hemoglobin and hematocrit test is performed [4].

Pathology

HbA is comprised of two alpha-globin and two beta-globin polypeptide chains. In healthy adults, alpha-globin and beta-globin chains are balanced. In beta-thalassemia, the synthesis of beta-chains in hemoglobin is ineffective due to mutations in the gene that codes for beta-globin [2]. Thalassemia is classified as a type of hemolytic anemia anemias in which erythrocytes are destroyed faster than regenerated [6]. The lack of beta-globin chains results in an imbalance between beta-globin and alpha-globin chains, causing alpha-globin chains to aggregate and precipitate inside erythrocytes. Consequently, erythrocytes are unable to mature, leading to quick cell death [2]. Thalassemia major is the most severe

form of beta-thalassemia, manifesting in the first two years of life, first by fatigue and then chronic infant sickness [2,3]. The constant removal of damaged erythrocytes and production to replace them generates symptoms throughout the body. If not treated properly, which is often the case in developing countries, it can affect the liver, spleen, musculoskeletal system, hematopoiesis, and growth [2]. Splenomegaly an enlarged, over-functioning spleen working to expel the abundance of damaged erythrocytes is a common consequence of beta-thalassemia [7].

The upregulated production of erythrocytes causes the red bone marrow to expand, giving rise to skeletal deformities, osteoporosis, or extramedullary erythropoiesis [2,5]. The cortical bone grows in unnatural patterns to accommodate augmented red bone marrow, resulting in misshapen bones and skeletal deformities. Osteoporosis occurs when bone marrow travels into cortical bones to account for excess erythropoiesis, limiting the available space for minerals and decreasing bone density [6]. Extramedullary erythropoiesis, the formation of erythrocytes outside of red bone marrow, occurs because the body is trying to keep up with the hemolytic anemia. It forms asymptomatic or tumorous multipotent cell masses in other organs, typically the spleen, liver, lymph nodes, chest, and spine [2,6]. Neurological complications can occur when masses form near the spine or nerves. The combination of the various complications typically ends in early death, but life can be extended with proper treatment [2].

Thalassemia intermedia has a later onset and broader range of symptoms than thalassemia major [2]. Symptoms usually manifest between two and five years old in the more severe cases but may not occur until adulthood in milder cases. Expanded bone marrow, extramedullary erythropoiesis, enlarged spleen, and other symptoms similar to but less extreme than thalassemia major can occur. Leg ulcers and thrombosis are typically more prevalent in thalassemia intermedia than in thalassemia major. Thalassemia minor patients are generally asymptomatic or possibly moderately anemic [2]. Patients with thalassemia major require consistent blood transfusions, which combat anemic symptoms but directly create secondary iron overload [3]. The patient receives iron from the transfusion, which is constantly absorbed and cannot be excreted, generating iron buildup and various complications that affect several organ systems [8]. The liver is the main organ for iron storage, so excess iron induces liver augmentation and possible chronic liver failure [3,9].

Iron deposits occur all over the body, including in the cardiovascular, endocrine, musculoskeletal, and integumentary systems. Cardiac disease is the most common cause of death from iron overload, accounting for 71% of deaths [2]. Iron in cardiac myocytes affects rhythm, inducing arrhythmias or heart failure. Iron accumulation in endocrine organs produces endocrinopathies such as hyperthyroidism, hypothyroidism, hypogonadism, Parkinson's disease, and more [3]. Diabetes is also prevalent, occurring in more than 70% of patients with repeated blood transfusions. Arthropathy pain, stiffness, and enlargement in symmetrical joints from iron deposits occurs in 25-50% of transfusion-dependent patients [9]. Slow growth or sexual maturation can also result [2]. Usually, the first symptom of iron overload, hyperpigmentation, occurs in up to 90% of patients when melanin or iron deposits in the basal layer of the epidermis. Iron deposits can also cause atrophy in cutaneous tissue, which is evident in flat nails and hair loss [9].

Current Standard of Care

Patients with thalassemia major undergo regular erythrocyte transfusions every two to four weeks [2]. Transfusions aim to prevent further damage by decreasing erythropoiesis and iron absorption, so patients should start them immediately at the onset of symptoms [2,8]. The amount of blood in the transfusion is a multifaceted decision based on the patient and the desired hemoglobin concentration [2]. Physicians typically aim for a hemoglobin concentration of 13 - 14 g/dL, an increase from the initial 9 to 10 g/dL, to slow damage to organ systems and allow for everyday activity [2,4].

As discussed earlier, transfusions are fundamental in treating anemic symptoms but result in iron overload, so physicians couple transfusions with a plethora of other treatments to mitigate their harmful effects. Iron overload also presents in non-transfusion-dependent thalassemias because the intestines absorb excess iron due to ineffective erythropoiesis [10]. Iron chelators are the primary countermeasure for iron overload in transfusion-dependent and non-transfusion-dependent thalassemias.

Iron chelators remove excess iron by binding to the ferric ion, thus allowing for excretion through urine and stool [2,10]. Chelators target specific organs based on patient needs [10]. Some examples of chelators are deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX) [2]. DFO is the traditional chelator, and it must be delivered intramuscularly, subcutaneously, or intravenously at least five days a week [3]. DFX is the most widely studied chelator, and it is taken orally [11]. DFO and DFX are both approved for use in children older than two; however, DFO causes growth retardation, so DFX is the only chelator approved for children over ten who are not receiving transfusions. DFP, a newer chelator, is used secondarily because it has not been studied in children younger than six. Iron chelation therapy has reduced cardiac deaths but contributed to an increase in liver complications and does not treat endocrine diseases from iron overload [10].

Current treatment for thalassemia intermedia may not entail blood transfusions, depending on the patient's circumstances. Therefore, treatment focuses on managing anemic symptoms rather than iron overload symptoms [2]. Folic acid supplements are commonly used to increase erythrocyte production, while a splenectomy may occur in more extreme cases [2]. Splenectomies minimize the number of necessary transfusions by decreasing blood consumption; however, they require immunizations to prevent infection and can increase the risk of thrombosis [3,7]. Hypercoagulation may occur after a splenectomy because destroying fewer erythrocytes results in more abnormal erythrocytes and activated platelets [7]. At-home remedies such as tea and vitamin C supplements can limit iron absorption and assist in removing iron in the gut, respectively

[3,10]. Higher levels of HbF, whether natural or induced, can relieve clinical symptoms in beta-thalassemia patients by lowering the amount of unbalanced alpha-globin chains [2,12]. An increased number of balanced globin chains curtails aggregation and erythrocyte death [2]. Hydroxyurea is the most common drug used to induce HbF, but its mechanism has yet to be entirely understood [7]. The most accepted theory proposes that its cytotoxicity causes stress erythropoiesis, increasing HbF levels. When successful, hydroxyurea works within three to six months and improves symptoms for up to a year, but it does not work for everyone [12].

Use of Stem Cells

Stem Cells

The main characteristics that define stem cells are their ability to differentiate and proliferate unlimitedly [13]. Stem cells can access all their DNA, but availability diminishes as cells differentiate [14]. There are three major classes of stem cells based on potency: totipotent, pluripotent, and multipotent [13,14]. Totipotent stem cells can differentiate to form an entire organism, including embryonic and extraembryonic structures, and are present from the zygote formation until blastocyst formation [14]. The inner cell mass of a blastocyst consists of pluripotent stem cells that can differentiate into any of the three germ layers and become any cell [13,15]. Multipotent stem cells can become multiple types of cells within the same germ layer [13].

Another way to define stem cells is by origin. Embryonic stem cells are pluripotent stem cells found in the inner cell mass of embryos, but most embryonic cells used in research are from *in vitro* fertilization rather than *in vivo* fertilization [13,14]. Somatic stem cells are derived from nonreproductive cells [13]. Some examples of somatic stem cells are mesenchymal cells in bone marrow that differentiate into bone, cartilage, and fat cells and hematopoietic stem cells (HSC) that differentiate into all blood cells [14].

Scientists have discovered that somatic cells can be reverted to pluripotent stem cells by transferring a nucleus into an enucleated oocyte these are known as nuclear transfer stem cells [14,15]. However, many ethical issues persist with harvesting embryonic stem cells and nuclear transfer stem cells. Induced pluripotent stem cells (iPSC), discovered in 2006, are made using retroviruses to revert almost any somatic cells to pluripotent stem cells [13,16]. The timeline for iPSC reversion is about three weeks of culturing and four weeks of programming [15]. IPSCs improve upon embryonic stem cells because they are autologous and avoid rejection; however, they also can be challenging to make, less efficient, and more variable, so their safety warrants further evaluation [15-17].

Hematopoietic Stem Cell Transplantation

Despite many improvements in managing transfusion patients, their quality of life remains much lower than cured patients [8]. Currently, the only cure for thalassemia is a hematopoietic stem cell transplantation (HSCT), also known as a bone marrow transplant [2]. HSCTs replace mutated genes with healthy genes, allowing bone marrow cells to undergo normal hematopoiesis and produce healthy erythrocytes [5,14]. They address the issue of iron overload and are highly successful when patients do not have compounding risk factors [2]. HSCTs can be autologous, using cells from the patient, or allogeneic, using cells from a donor [14]. Allogeneic HSCT is the only option for thalassemia since an autologous HSCT requires healthy cells [5]. One downfall of allogeneic HSCT is the need for a suitable donor match. A good match constitutes a donor with the same human leukocyte antigens (HLA), cell markers encoded by the major histocompatibility complex (MHC), which typically depends significantly on ethnicity [5,17]. Matching ensures that the donor's immune cells recognize and do not attack the host cells [17]. Related donors are used when possible; they are typically siblings, who are a good match approximately 25-30% of the time [2,8]. More than 60% of patients do not have related matched donors, and finding an unrelated matched donor can be difficult [5]. Patients have the same cure rate with matched related and unrelated donors 80-87% [8]. The difference occurs in the graft-versus-host disease (GVHD) rate, which is higher in unrelated donors [8].

When a matched donor cannot be found, patients can use partially mismatched donors whose antigens do not entirely match the recipient. Partially mismatched donors can be related or unrelated, but related donors are preferable because of lower costs and an enhanced ability to find and contact them. More complications can arise with mismatched than matched allogeneic donors because their HLAs differ; for example, the graft failure rate for mismatched unrelated donors is about 10%, much higher than matched related and matched unrelated donors. Partially mismatched related donors include umbilical cord blood (UCB) and haploidentical donors [18]. UCB is the best option when no matched donors are available, but its minimal use can be attributed to small amounts of umbilical blood and cost [17,18]. Haploidentical donors are individuals whose HLAs half-match with the recipient [5]. Mothers are the best option because maternal and fetal blood overlap during pregnancy, but other family members, such as siblings, are also good donors [18]. UCB and haploidentical donors can be unrelated but are primarily related due to increased compatibility and access.

Partially mismatched unrelated donors fall into two categories: permissive and nonpermissive. Permissive mismatches, the category unrelated haploidentical donors fall into, involve antigens encoded by similar MHCs and belong to the same immunogenicity group. Despite differing donor and recipient HLAs, permissive mismatches are effective because T cells often fail to recognize the difference. Nonpermissive mismatches display antigens outside the immunogenicity group, resulting in less favorable outcomes [18]. Deciding to proceed with nonpermissive mismatches can be dangerous and depends on many factors affecting patient outcomes. Before transplantation, the patient prepares with procedures and medication. Myeloablative conditioning regimens destroy faulty bone marrow to make for better transplantation [19]. Busulfan, an extensively researched myeloablative conditioning drug, is commonly used in non-malignant transplants. Cyclophosphamide is an immunosuppressant administered to the graft to reduce the risk of GVHD before and after transplantation [5].

GVHD occurs when a graft's immune cells, particularly T cells, attack recipient HLAs. A suppressed recipient immune system can increase the risk of GVHD because the recipient cannot defend against the transplant attack [17]. When grafts proliferate, complications with GVHD can affect many organs, including the skin, gastrointestinal tract, and liver [5]. Acute GVHD occurs within one hundred days and affects the skin first, causing rash due to epidermal apoptosis [17]. Blisters, ulcers, liver disease, diarrhea, and sometimes vomiting or anorexia are also common. Chronic GVHD is characterized by disease beginning after one hundred days. Inflamed donor lymphocytes damage host tissue, causing T-cells to proliferate and cytokines to increase [17].

GVHD has been studied in hopes of decreasing graft complications. Acute treatment involves steroids and blocking cytokines to manage inflammation. Extracorporeal photopheresis, inducing apoptosis of leukocytes via ultraviolet radiation, is also utilized. Chronic disease progression is less understood because it displays various symptoms, but current treatment employs immunosuppressive agents to manage it. Prevention is the best treatment for GVHD, which entails removing donor T cells *ex vivo* to avoid attack or implementing T cell antibodies *in vivo* to respond to the attack [17]. Along with GVHD, graft rejection and graft failure are the other primary complications of transplantation [8]. Graft rejection is the opposite of GVHD, occurring when the host immune system recognizes the transplant as foreign and attacks it. Mixed hematopoietic chimerism, the presence of two genetically distinct cells in one organism, causes rejection [5]. Graft failure occurs when the HSCT fails to engraft properly and produce functioning cells [8]. While preparation has preemptively counteracted some complications since the 1990s, HSCT remains a complex and dangerous procedure [5]. Additional issues include the limited number of donor HSCs and no efficient way to collect them [14]. Physicians work with patients to weigh the risk of HSCT with the risk of chronic transfusions and decide which option is better suited [8]. Children are better candidates for HSCT since their disease is less advanced. They have lower risk and higher cure rates because adults have accumulated complications from prior treatments. Portal fibrosis, hepatomegaly, and chelation irregularity are all examples of complications that can lessen the chances of survival that children likely have yet to experience [5].

Potential Stem Cell Treatments

One option for parents who are both carriers of beta-thalassemia is *in vitro* fertilization coupled with preimplantation genetic diagnosis. First, several oocytes are retrieved from the mother and fertilized in a lab. Then, genetic testing is performed on each embryo to screen for beta-thalassemia. The couple selects an embryo free from beta-thalassemia, eradicating the possibility of disease and the obligation of transfusions or HSCT later in life [4]. The chosen embryo is implanted into the uterus and carried to term.

Autologous HSCTs are common in cancer treatment but are not currently applicable in beta-thalassemia patients. Cancer is caused by an acquired gene mutation not present in all cells, so patients have healthy bone marrow outside affected areas. Beta-thalassemia is hereditary, so all cells in the body contain deleterious mutations, resulting in no healthy bone marrow. Similarly, iPSCs are effective in treating many diseases, excluding beta-thalassemia. Treatment of thalassemia necessitates correcting the mutation, which inducing cells back to pluripotency does not accomplish. Gene therapy, or gene editing, can add a DNA coding strand for a functioning beta-globin chain into the host genome of iPSCs or HSCs. This technology rectifies deleterious mutations, allowing for the use of autologous HSCTs or iPSCs. Autologous cells improve patient outcomes by abolishing the need for a donor match and the risk of GVHD, graft rejection, and graft failures [8].

Gene therapy was first successful in 2010 when an 18-year-old male with thalassemia major could not identify an HLA matc. The gene insertion was carried out with a lentiviral vector a retrovirus based on Human Immunodeficiency Virus-1 (HIV-1) that can carry much larger DNA strands than other vectors. One year after the transplant, the patient stopped transfusions, his hemoglobin levels were normal, and his reticulocyte and erythrocyte numbers decreased [8]. The inserted gene was found in granulocytes, monocytes, and erythroblasts but not in B or T lymphocytes.

Clustered regularly interspaced short palindromic repeats (CRISPR) CRISPR-associated systems (Cas) is a modern technology used to target specific genes. Several preclinical and clinical trials have proved it is easy, cheap, accurate, and efficient. Clinical researchers hope to use CRISPR-Cas to treat monogenic diseases like inherited blood, lung, and neurological disorders, then extend its use to eliminate genetic diseases such as hereditary diseases, viral infections, cancer, autoimmune diseases, and more [20].

The two mandatory components for CRISPR-Cas are the guide ribonucleic acid (RNA) and the Cas endonuclease. Cas proteins utilize RNA insertion and deletion and DNA or RNA base editing to modify genetic code. The base editing component is newer and can only be done within fifteen nucleotides of the protospacer adjacent motif (PAM), a sequence after the target DNA that directs Cas nuclease activity. Cas9 is the most widely used nuclease in CRISPR-Cas and the first to make clinical trials. In 2018, the first Cas9 *ex vivo* clinical trial was approved; in 2019, the first *in vivo* trial was approved. *Ex vivo* trials isolated cells, edited them, and inserted them back into the patient, while *in vivo* trials delivered CRISPR-Cas to the target cells in the patient. One trial extracted HSCs from peripheral blood, proliferated them *ex vivo*, and used Cas9 to activate HbF. Nine months after the treatment, hemoglobin levels remained normal [20]. From there, the research took off.

Cas9 operates using an RNA guide to find the PAM, which limits activity to the target site. Cas9 is not the only nuclease used with CRISPR. There are two classes and six types of Cas proteins. Class 1 uses multi-Cas protein complexes: Cas3 targets DNA, Cas10 targets RNA and DNA, and Din10, whose function is still relatively unknown. Class two consists of single Cas proteins: Cas9 and Cas12-Cas14 target DNA, and Cas13 targets RNA [20].

In addition to the CRISPR-Cas function, delivering the altered genetic material to the cells is also imperative. Transportation can occur through viral vectors, nonviral vectors, or physical delivery. Viral vectors are incorporated into the genome and cause permanent mutations. Lentivirus, an HIV-1-dependent retrovirus discussed earlier, is an example of a viral vector. Each viral vector has different benefits and drawbacks. Adeno-associated viruses have low immunogenicity but cannot carry large molecules; adenoviruses can carry large molecules but are immunogenic; lentiviruses carry large molecules and are not immunogenic but can rearrange. Nonviral vectors are not added to the genome and include nanoparticles, cationic lipids, and exosomes. Solid lipid nanoparticles are the most widely used but can harm tissues, so other nanoparticles are being studied. They are challenging to make and may be difficult to implement clinically. Cationic lipids have high delivery success rates but may be toxic. Exosomes are the opposite they are biocompatible with low immunogenicity but inefficient. For physical delivery, membrane deformation is the best method for various cells, but it is expensive and can intensify tumor risk. Electroporation is suitable for *in vitro* but can damage tissue *in vivo*. Microinjection has excellent accuracy, and hydrodynamic delivery is cheap, but both can harm tissues [18].

Before the widespread clinical use of CRISPR-Cas, issues such as ethics and safety need to be addressed and standardized [20]. One risk of gene therapy is genotoxicity the possibility of mutations from DNA damage [8]. Nonspecific outcomes, which cleave DNA at incorrect sites and can go as far as rearranging chromosomal segments and disrupting healthy DNA, are too prevalent. Researchers are working to enhance nuclease specificity and improve the RNA guide to minimize off-target effects and mitigate genotoxicity. Inhibiting CRISPR-Cas could provide another solution to decrease off-site effects. Right now, the complex is constantly active, which can lead to unwanted functions, especially *in vivo*. Additionally, Cas or delivery methods *in vivo* can trigger an immune response, causing the treatment to fail [20]. Improving efficiency and accuracy while reducing immunogenicity will enhance safety and is vital to proper care.

CRISPR-Cas treatment is expensive, costing up to $3.5 million per treatment. Private insurance often delays or denies coverage due to the high cost, and CRISPR-Cas treatment would cost Medicaid billions if added. Currently, drugs and treatments are created for commercialization and profit, causing for-profit pharmacies and medical providers to deny service; the small number of patients benefiting would not be worth their financial investment [1].

Model solutions for combating these high costs incorporate the government, public funding, and nonprofit organizations. Some nonprofits work on nonexclusive licensing, helping people looking to develop patented technology for lower-income groups. Another proposed solution suggests mandates stating that companies provide a certain proportion of treatments to low-income individuals. The Canadian government provided $15 million to infrastructure providing care and returned their investment in six months. The Federal Drug Administration (FDA) complicates potential solutions in the United States complex. If the FDA approved the general CRISPR-Cas technology instead of approving each application, it would substantially increase financial access [1].

Conclusion

Before stem cell technology, beta-thalassemia patients were destined for a life of transfusions, severe side effects, and early death. Due to the discovery and clinical use of HSCT, many patients can live healthy lives free from constant treatment. While curative when performed, HSCT still has many barriers, for instance, finding a suitable donor and ensuring a successful transplant.

Autologous HSCTs have been highly productive in treating other diseases, such as cancer, while avoiding the need to search for a matching donor. Unfortunately, they have not been an option for beta-thalassemia patients because, unlike cancer patients, the disease is hereditary and not localized. With its ability to edit HSCs or iPSCs from a patient, CRISPR-Cas technology offers a solution by repairing the mutated gene and allowing for autologous HSCTs. CRISPR-Cas and other gene therapies eliminate the need for a donor, reducing time, cost, and risk of a failed transplant or GVHD.

With clinical advancements on the horizon, questions related to accessibility and ethics remain. Cost, the key driver for patient access to any new medical procedure, is reflected on the patient, often restricting treatment options. For conditions like beta-thalassemia, in which a cure can remedy severe symptoms and improve quality of life, it is integral that accessibility expands. As CRISPR-Cas becomes more commonplace in the clinical sphere and throughout biotechnology, increasing usage and understanding will lend to further implementation. Once deemed safe, this technology must be available to patients. While this is a multifaceted issue, improvements in private and federal insurance company policies to expand coverage and reduce costs are an excellent place to start.

Aside from accessibility, ethical issues surround gene editing technology, including questions about gene editing and embryos. Successful CRISPR-Cas technology can eliminate mutations in embryos before they can manifest; however, many groups argue against its usage for ethical reasons. It is essential to account for potential ramifications when making decisions about the direction of medicine, as they often prove difficult to reverse in the future.

After focusing on technologically advanced, controversial, and expensive treatments, reflecting on readily available and practical solutions is critical. Preconception screening, a highly successful and cost-effective method to combat the prevalence of beta-thalassemia, is underutilized. The responsibility for screening falls on the government, the public, and individuals. Adding thalassemia testing to the existing newborn testing panel would increase accurate diagnoses and allow for proactive treatment. Additionally, it is crucial that individuals and couples take responsibility for their child's health potentially high-risk

couples should obtain proper testing before deciding to conceive. For individuals who may be unaware of their risk, public health initiatives should educate on identifying high-risk traits and the importance of screening. Emphasizing parent responsibility and expanding public knowledge are viable, inexpensive avenues to combat beta-thalassemia.

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