A Review on Current Status of Blood Disorder: Thalassemia and its Treatment

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Article History	Abstract
           	The most prevalent hereditary monogenic disorders that claim millions of lives globally are thalassemic syndromes. A thalassemia is an inherited condition, at least one parent must carry the disease's gene. Perhaps a genetic mutation/ defective globin chain or the loss of specific important gene segments is the main cause. Thalassemic illnesses started to strain the healthcare systems of several nations worldwide. Management of thalassemia is now seen as a lifelong treatment that requires continuous monitoring. In this review, we seek to compile and analyze recent research on thalassemia diagnosis and treatment, including papers, studies, and clinical trials. We also intend to present a concise yet comprehensive study. A thalassemia is an inherited condition, at least one parent must carry the disease's gene. Perhaps a genetic mutation/ defective globin chain or the loss of specific important gene segments is the main cause. ***Keywords:** Current Status, Blood, Disordes*

1. Introduction

Hematologic diseases, often known as blood disorder diseases (BDDs), are some of the illnesses caused by haematological system disorders. BDDs can be broadly divided into three groups: platelet disease, white blood cell illness, and red blood cell disease. The most well-known BDDs are iron deficiency anaemia, leukemia, thalassemia, haemophilia, and malignant lymphoma [1]. The availability of hematopoietic tissue from patients' peripheral blood (PB) or bone marrow (BM) has historically made the study of blood illnesses a prominent priority in biomedical research, in part because of this accessibility [2]. However, recent significant advancements have increased patients' average lifespans and markedly raised their quality of life. Sickle cell disease (SCD) and thalassemia, which affect people with origins in Africa, the Mediterranean region, Southeast Asia, the Middle East, and the Far East, are the most relevant of these from a clinical perspective [3]. This article presents information on pathophysiologic features, diagnosis, and therapy of SCD and -thalassemia that has just recently been available in the literature [4].

A series of genetic blood disorders known collectively as sickle cell disease (SCD) have two characteristics: the presence of sickle-shaped erythrocytes (sickle cells) in the blood and a clinical illness (disease) brought on by the sickle cells. Literally, SCD is disease caused by sickle cells. Both homozygous sickle cell disease (HbSS) and sickle cell anaemia, as well as compound heterozygous diseases such sickle cell disease with sickle cell haemoglobin C (HbSC) and sickle cell thalassemia (HbS/thal), are included as disease entities [5].

Haemophilias

Mutations in the F8 and F9 genes, which code for the blood coagulation factors VIII and IX, respectively, result in the X-linked bleeding disorders haemophilia A and haemophilia B (HA, HB) [6]. Two significant chromosomal inversions at F8's introns 1 and 22, which have both been mimicked in iPSCs, are the cause of over half of all severe HA cases (140 kb and 600 kb, respectively) [7].

Hemoglobin disorder

The haemoglobin disorders are a group of autosomal recessive diseases that are characterized by either reduced synthesis of one or more normal globin chains (the thalassemias), the synthesis of a globin chain with an abnormal structural pattern (the haemoglobin variants), or in a few instances, both phenotypes (the reduced synthesis of a Hb variant, e.g., Hb E) [8].

Thalassemia

Inheritable haemoglobin diseases, particularly thalassemias and their interactions with haemoglobin E (HbE) and haemoglobin S (HbS), are a substantial cause of morbidity and mortality in India [9]. Previous research has showed that the prevalence of -thalassemia is 3-4% worldwide, with an estimated 8,000 to 10,000 new newborns each year with significant illness [10,11]. White blood cells (WBCs), platelets, and red blood cells (RBCs) are the three major blood cell types. The soft tissue found inside your bones, the bone marrow, is where all three cell types are created. Red blood cells transport oxygen to your body's organs and tissues [12]. Your body's defense against infections includes white blood cells. Your blood clots more easily with platelets. One or more of these blood cell types are impaired by blood cell diseases in both their creation and operation [13].

Prevalence

There is a lack of information on the prevalence of -thalassemia and other hemoglobinopathies in India's various castes and ethnic groups [14] To ascertain the prevalence of hemoglobinopathies in various caste/ethnic groups using standardized techniques, the current multicenter study was conducted in six cities across six Indian states (Maharashtra, Gujarat, West Bengal, Assam, Karnataka, and Punjab)[15] .The prevalence of the -thalassemia trait was 2.78% overall and ranged from 1.48 to 3.64% in different states, whereas it ranged from 0 to 9.3% in 59 distinct ethnic groups [16].Dibrugarh in Assam (23.9%) and Kolkata in West Bengal (3.92%) have the highest rates of HbE trait. HbE trait prevalence in six Assamese ethnic groups ranged from 41.1 to 66.7% [17].

Etiology

B-thalassemia is a condition where there is insufficient or no synthesis of the beta globin chains that make up the hemoglobin tetramer [18]. There are three clinical and hematological disorders that are known: beta-thalassemia carrier status, intermediate thalassemia, and major thalassemia. [19]. Two types of inherited hemoglobin tetramer abnormalities can be distinguished: those with structural abnormalities of the hemoglobin chains and those caused by a variety of molecular flaws that either lessen or stop the synthesis of one or more of the polypeptide chains that make up the hemoglobin molecule [20]. The former illnesses are referred to as "hemoglobinopathies," whilst the later are known as "thalassemia" [21].

Types of beta thalassemia

Thalassemia major (Cooley's Anemia)

The term "thalassemia major" (TM) was used to refer to homozygous (or compound heterozygous) thalassemia that required at least eight transfusions in the year before registering in the registry [22]. Patients with effective engraftment of transplanted stem cells were also excluded from this research, along with patients with haemoglobin thalassemia and non-transfused thalassemia variations [23]. Currently, thalassemia major patients are transfused from diagnosis at a pretransfusion hemoglobin level of 9.5–10 g/dl and do not develop splenomegaly or bone abnormalities as a result [24]. The prognosis for thalassemia has improved with routine blood transfusion, however the tissues are damaged by the iron buildup in the transfused red cells [25]. Additionally, it describes a severe clinical phenotype that manifests in patients who are homozygous or compound heterozygous for beta chain mutations that are more severe (such as severe B+/B+ mutations, B+/B0 mutations, or B0/B0 mutations) [26].

Thalassemia intermedia

An intermediate clinical phenotype (e.g., B+/B0, B+/B+) with diverse genetic alterations that nonetheless permit some Beta chain synthesis. [27]. There are also a few uncommon instances where beta and alpha mutations coexist. It's possible that some of these patients have thalassemia intermedia. Children with thalassemia intermedia are those that continue to grow normally until they are three years old and do not show any obvious alterations to their bones without receiving regular transfusions [28]. Some of these patients experience mild to severe hypersplenism in the years that follow, necessitating splenectomy (29) Some people may become to depend on blood transfusions as adults. 95% of the 165 Italian-origin patients in our retrospective study with thalassemia intermedia received their diagnosis after the age of two [30].43 percent had never received a blood transfusion, 30 percent had transfusions only sometimes due to illnesses, pregnancy, or surgery, and 28 percent developed transfusion dependency as adults. [31]. The age at first transfusion for this group of patients with thalassemia intermedia is shown in Figure 1.

Thalassemia minor (Beta Thalassemia carrier/trait)

A modest clinical phenotype (B+/B, B0/B) when only one healthy copy of the beta globulin gene is present [32]. The severity of the sickness caused by thalassemia minor varies, but depending on the chain formation rate, it typically manifests as moderate asymptomatic anemia (the hemoglobin is 1-2 g/dL lower than normal for people of the same age and sex. There is no specific treatment for thalassemia minor during pregnancy, however transfusions may be required if the anemia worsens [33]. There is little information available about the perinatal fate of people with thalassemia mild [34].

Symptoms and consequences

Insufficient production of -globin leads to the precipitation of free -globin chains and the subsequent oxidation of erythroid precursors in the spleen and bone marrow [35]. As a result of the inefficient erythropoiesis:

• A severe anaemia that results in heart failure, infections, and stunted growth [36]
• Bone deformation and fracture are caused by the massive bone marrow growth [37]
• Increased intestinal absorption of iron and the ensuing hemochromatosis (12- update on gene therapy) [38]

Pathophysiology

A partial or total impairment in the synthesis of the -globin chains (-thal) or -globin chains (-thal), which make up the main adult hemoglobin (HbA), a tetramer of 2, 2, causes thalassemia (thal), an autosomal recessive hereditary chronic hemolytic anemia. One or more of the several hundred mutations in the associated genes is what causes it. The unpaired globin chains are unstable; they precipitate intracellularly, causing hemolysis, early red blood cell (RBC) precursor death (via apoptosis), and a short lifespan of mature RBCs in circulation [39]. The breakdown products of hemoglobin (Hb), heme, and iron stimulate chemical reactions that produce free radicals, including reactive oxygen species (ROS), which are harmful in excess and can harm the heart, liver, endocrine system, and other important organs [40].

The reduced production of a-globin and subsequent buildup of mismatched a-globin precipitates are caused by the mutation in the a-globin gene on chromosome 11 [41]. These iron-containing insoluble particles cause the production of reactive oxygen species, which are harmful to the erythroid cells' cell membrane components [42]. The early apoptosis of the erythroblasts caused by oxidative stress is known as inefficient erythropoiesis [43]. (New developments in medicine) Hemolysis is produced by the macrophages of the reticuloendothelial system as a result of alterations in the membrane proteins of adult red blood cells (RBC), particularly the increased production of phosphatidylserine [44]. Ineffective erythropoiesis (new) variants of thalassemia are caused by an overactive version of transforming growth factor- (TGF-), which inhibits the final step of erythropoiesis [45]. figure 2 depicts the intricate series of occurrences in erythrocytes that lead to their rapid peripheral degradation (beta thalassemia) [46].

Diagnosis

Clinical presentation of b-thalassemia major usually occurs between 6 and 24 months of life, with severe microcytic/normocytic anemia, mild jaundice, and hepatosplenomegaly. The hematological diagnosis is based on reduced hemoglobin level (ranges between 3-4g/dl), sign and symptoms are moderate to severe. Beta-thalassemia intermedia should be suspected in subjects who present at a later age with similar but milder clinical findings than thalassemia major. The clinical spectrum of thalassemia intermedia is very wide as well as the hematological phenotype. Patients with milder forms may have moderate-to-mild anemia, and the levels of Hb. These patients are usually capable of surviving without regular blood transfusions. Even in

patients with more severe forms of thalassemia intermedia, Hb levels are >7 g/dL. sign and symptoms are mild. In thalassemia minor, Hb levels are 6-7 g/dl as shown in below table Table no.1.

Table :1 Hematologic Diagnosis

Range of sign and symptoms	Thalassemia Type	Diagnosis Treatment (HB Levels)
Mild	Thalassemia minor/beta thalassemia	6-7 g/dl
Moderate to severe	Thalassemia major	3-4 g/dl
Mild	Thalassemia intermedia	7-10 g/dl

The recognition of carriers is possible by hematological tests. Treatment for mild cases of thalassemia trait is not necessary. Treatment options for moderate to severe thalassemia include cell transplantation, chelation therapy, and blood transfusions.

Recent Advances in A Treatment of Thalassemia
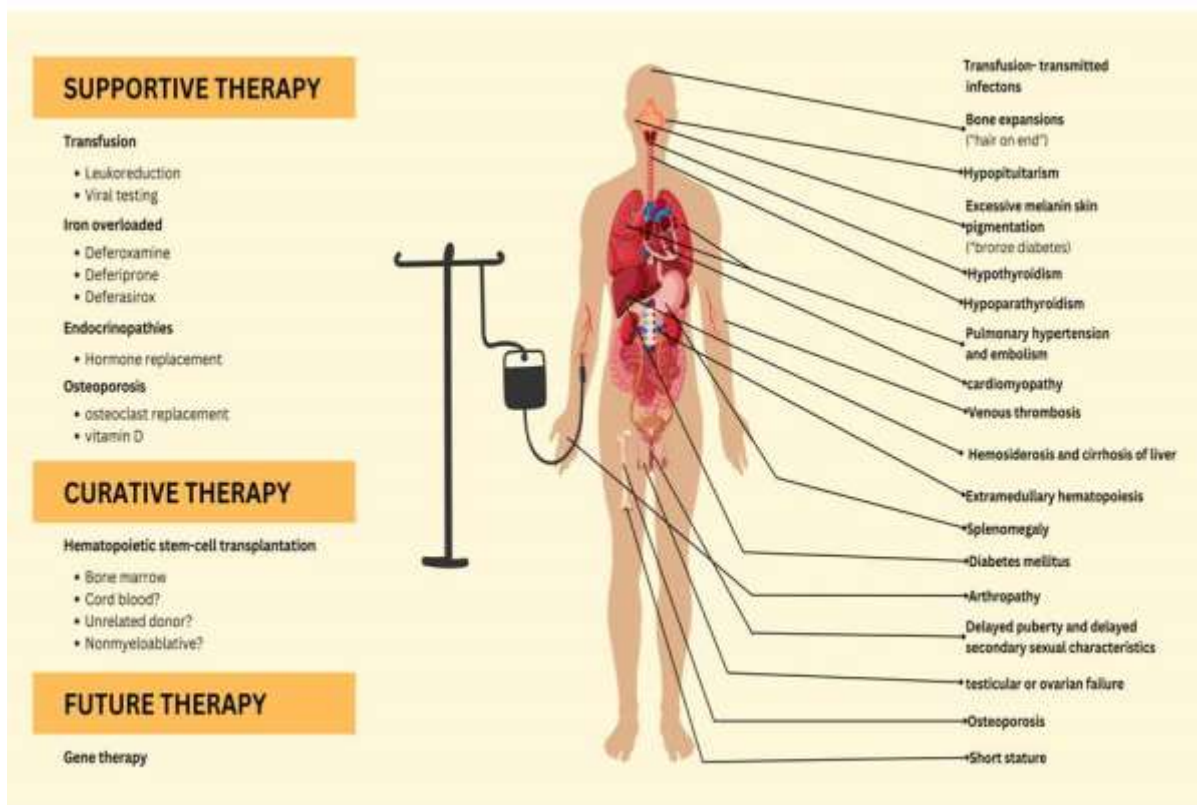
Figure 1: shows Current and future alternative therapies for beta thelasemia major

Chelation Therapy

The optimal body iron should minimize both the risk of adverse effects from the iron-chelating agent and the risk of complications from iron overload as shown in fig 1 in supportive therapy [47]. The lower the desired level of body iron, and in the absence of any further confounding variables, the higher the amount of iron chelator required. The primary goal of chelation therapy for P-thalassemia major is to stop or at least reduce the secondary transfusion iron overload [48].

The following qualities should be present in an ideal iron chelator: specificity and affinity for iron; a molecular weight low enough to promote good gastrointestinal absorption but high enough to prevent toxicity; adequate lipophilicity for gastrointestinal absorption and liver extraction with intracellular chelation;

sufficient hydrophilicity to prevent toxicity to the central nervous system and bone marrow; absence of iron redistribution; and scavenging at low iron concentrations; Another desirable quality might be the ability to be used as a prodrug [49].

Nanomedicine

It is a novel area of medicine that integrates nanotechnology into the advancement of conventional medicine [50]. In order to successfully deliver medications to cancer locations, nanomedicine focuses on developing a precise mix of nanomaterials (such as nanoparticles, nanovesicles, and nanocarriers) and small molecules [51]. By approving Elzonris (tagraxofusp-erzs) infusion for the treatment of blastic plasmacytoid dendritic cell neoplasm (BPDCN) in adults and children's patients (2 years or older), the FDA approved the first medication for uncommon BDDs on December 21, 2018 [52]. A cytotoxin called tagraxofusp-erzs targets cells that express CD123, the IL-3 receptor's alpha chain, which is overexpressed in BPDCN. It is made of human IL-3 and truncated diphtheria toxin (DT) [53]. The medication is more readily taken up by CD123-overexpressed cells, which starts irreversible protein synthesis and kills the cells [54].

Dosage forms for the treatment

Due to their low molecular weights, DFX and DFP are designed for oral delivery to lower iron overload while also enhancing patient compliance and quality of life as shown in figure 1 [55]. The first oral iron chelator to successfully treat thalassemia is DFP According to recent research, it is just as effective as DFO at protecting the heart from iron overload, which it has often successfully chelated at doses of 50–100 mg/kg [56]. The medication comes in liquid and tablet form, and it should be taken three times daily at a recommended dose of 75 mg/kg per day, not more than 100 mg/kg per day. DFP is quickly absorbed by the gastrointestinal tract after oral administration. Peak plasma concentration is reached in patients who are fasting after 45 to 60 minutes, and it may take longer in individuals who are fed [57]. Only peak serum concentrations were considerably impacted by ingesting meals; the entire area under the curve and the elimination half-life remained unaltered. The stated mean elimination half-life is 1.5–2.6 hours [58]. A 3:1 ratio of water-soluble DFP-iron complexes is produced and eliminated in urine. (Combination therapy vs. monotherapy) [59].

Deferiprone

-L1, also known as 1,2 dimethyl-3-hydroxypyrid-4-one, is the sole life-saving medication available to thalassaemia patients in impoverished nations because deferoxamine and deferasirox are too expensive and hard to find there [60].

Blood Transfusion

The cornerstones of thalassemia treatment are blood transfusions [61] Early and frequent blood transfusions reduce the effects of severe anaemia and increase survival time [62]. However, there is a chance of problems with transfusion. Understanding the many negative effects of blood transfusions is therefore crucial for treating thalassemic individuals [63]. Blood transfusions come with risks of iron excess that can cause endocrine dysfunction and an increased chance of contracting a transfusion-transmitted illness like hepatitis [64]. Chronic anaemia is treated with blood transfusions, which also prevent bone abnormalities, promote normal growth and activity levels, and improve patients' quality of life (QoL) [65]. Transfusions deliver brand-new, healthy RBCs that treat anaemia and decrease inefficient erythropoiesis, reducing the risk of hepatosplenomegaly and bone marrow hyperplasia [66].

Pharmacokinetic study

Deferiprone 25 mg/kg was administered orally to the subjects after an overnight fast (GPO-L1- ; Government Pharmaceutical Organization, Bangkok, Thailand) [67]. Two and eight hours after medication, uniform meals were provided. 5 mL blood samples were taken through a venous catheter before to dosing as well as 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 480 minutes afterwards [68].

The samples were centrifuged, and the separated serum samples were kept at -20 ^{0}C for storage. A volume of 10 mL from each collection time was held at - 20 ^{0}C until analysis. Urine output was pooled and collected at baseline, 0-2, 2-4, 4-8, 8-12, and 12-24-hour intervals following treatment [69]. Blood transfusions were given anywhere from infrequently to three times a year. Prior to the trial, none of the patients had received a transfusion in at least three months [70]. Except for folic acid, no patient was allowed to use any prescription or over-the-counter medication for a week prior to the trial and for the duration of it (Pharmacokinetics of deferiprone) [71].
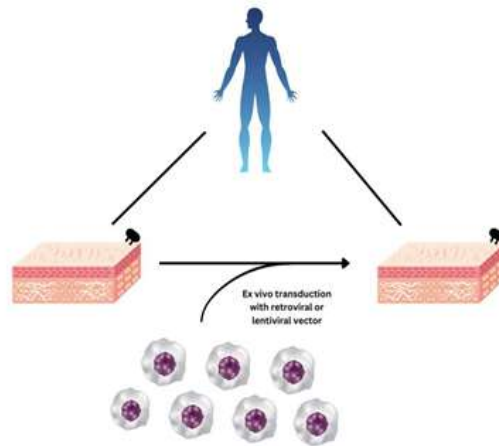
Gene Transfer

As shown in Fig 2, Bioscientists are researching an investigational gene therapy approach as a potential treatment for beta thallasemia major. In a laboratory, a corrected or functioning copy of beta globin gene is packaged into a linty viral vector. The vector contains a small number of parts from the human immunodeficiency virus or HIV. The HIV parts are used because they are effective at entering cells and delivering the functioning copy of the gene. The vector has been changed so it cannot cause or grow HIV infection.

- Now as shown in Fig 3, the blood stem cells are selected from the beta thallasemia patient from the bone marrow of peripheral blood after the successfully collection of blood stem cells,
- In the laboratory, the linty viral vector will be used for insert the functioning copy of the beta globin gene into the DNA of the collected blood stem cells this is also being explained as the gene editing to correct the beta globin mutation or disrupt the BCL11A gene to increase fetal heamoglobin.
- After the quality control procedure is successfully done. Inside the body RNA with functioning gene converts RNA to DNA.
- The corrected functioning is inserted into the DNA of the patients cell. Now gene modified stem cells will provide the proper functioning in the patient.
- Inside the body chemotherapy medicine is used to remove existing stem cells to make a room for the new ones which contain the functioning copy of the beta globin gene. When the modified blood stem cells are returned to the patient's body, they will be able to establish a home and multiply. The goal is for modified blood stem cells to become a permanent source of new blood cells with a functioning copy of the beta globin gene produce normal blood cell.
- This investigational gene therapy approach may potentially help patients produce healthy red blood cells and decrease or eliminate the need for continued blood transfusions.

Gene transfer to hematopoietic stem cells (HSCs) has shown therapeutic efficacy in recent trials for several individuals with inherited disorders, transduction incompleteness of the HSC population remains a hurdle to yield a cure for all patients with reasonably low integrated vector numbers. In previous attempts at HSC selection, massive loss of transduced HSCs, contamination with non-transduced cells, or lack of applicability to large cell populations has rendered the procedures out of reach for human applications.

Novel approaches aiming to combine allo-HCT with HSC gene therapy/editing involve technical and financial difficulties. All currently existing gene therapy/editing approaches target CD34$^+$ cells, which are a heterogenous mix mostly containing short-term progenitor cells and <0.1% HSCs with long-term engraftment potential. The inability to purify and specifically target multipotent HSCs limits the targeting efficiency, increases the costs for modifying reagents, and poses the risk of potential gene therapy off-target effects transduction of hematopoietic cells with retroviral vectors is an efficient way to manipulate gene expression during development of the immune system.

Retroviral vectors are easy to manipulate in the laboratory and provide stable, long-term gene expression in the infected cells and their progeny because they stably integrate into the genome. Their major limitation is that they can only efficiently infect and integrate in cycling cells. While no patients receiving deferiprone or deferoxamine passed away during the trial period, three patients receiving deferoxamine did so as a result of an irreversible worsening of their heart disease. The results of this investigation indicate that subcutaneous deferoxamine does not have the same cardio-protective impact as long-term therapy with deferiprone against the toxicity of iron overload. Formal prospective studies are warranted to confirm this effect.

Fig 2: Shows a general view of a gene-therapy approach for β-thalassemia
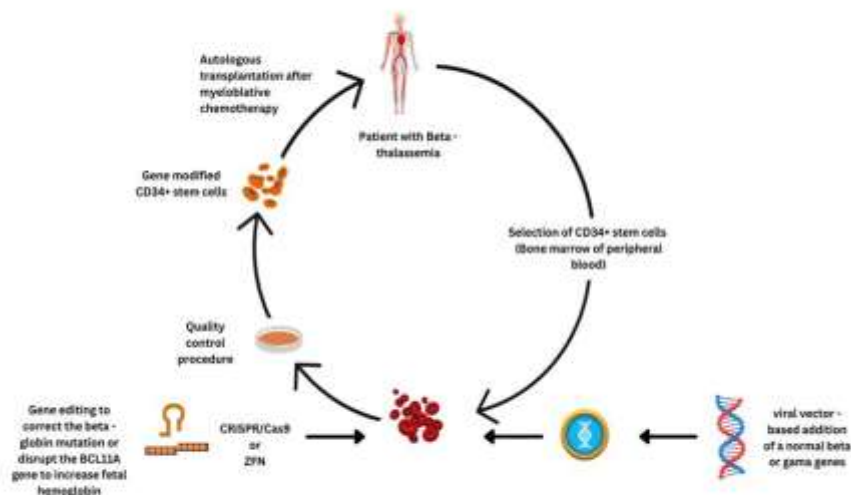
Fig 2: general strategy for ex vivo gene transfer to HSCs

The approach entails: • Isolation of autologous bone marrow CD34+ cells (containing HSCs) • Culture of cells and in vitro transduction with viral vector • Infusion of transduced cells to the subject.

Conclusion

Thalassemias are genetically derived blood disorders in which there is a reduced rate of synthesis of one or more of the globin polypeptide chains. Genetic and cellular targets are potential approaches in management of disease. Disease management in current approaches include gene therapy, prenatal diagnosis, transfusion therapy, bone marrow transplantation (BMT). Gene therapy is one of the most promising approaches for the future treatment of β-thalassemia patients and comprises several, at times complementary, strategies deliveries of transgenes in stem cell-based gene therapy are effective in the therapeutic management. Gene transfer using onco-retroviral vectors and lentiviral vectors are beneficial. The clinically most advanced approach, that of substituting nonfunctional endogenous β-globin genes with a normal β-globin gene carried by lentiviral vectors, leads to de novo production of HbA.

Lentiviral vectors have an advantage over onco-retroviral vector due to integration of larger element and minimal sequence rearrangement. Induced pluripotent stem cells, splice-switching and stop codon read-through are other genetic approaches which are showing advantages over the current therapy. Gene therapy with autologous CD34+ cells transduced with the BB305 vector reduced or eliminated the need for long-term

red-cell transfusions in many patients with severe β-thalassemia without serious adverse events related to the drug product. Further refinements in the methodology for gene transfer is likely to bring more definite success for the treatment of b-thalassemia. as in vitro evidence indicates, by additional treatment with inducers of endogenous HbF, which is firmly established as clinically beneficial. This approach can be enhanced in the further studies and the recent advances in the treatment of beta thalassemia.

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