

## LENTIVIRAL GENE ADDITION AND GENOME EDITING IN THALASSEMIA: A NEW ERA OF PRECISION MEDICINE

Razia Iqbal<sup>\*1</sup>, Shahid Mahmood<sup>\*2</sup>, Areeba Mahmood<sup>3</sup>, Sumaira Munir<sup>4</sup>, Saba Tariq<sup>5</sup>, Sundas Jabeen<sup>6</sup>, Maria Chaudhary<sup>7</sup>, Tabinda Sajjad<sup>8</sup>

<sup>\*1, \*2, 4,5, 6,7,8</sup>Department of Zoology, University of Gujrat, Gujrat 50700, Pakistan

<sup>3</sup>Aziz Bhatti Shaheed Teaching Hospital, Gujrat 50700, Pakistan

<sup>\*1</sup>razia.iqbal@uog.edu.pk, shahid.mahmood@uog.edu.pk<sup>\*2</sup>

Corresponding Author: \*

Razia Iqbal, Shahid Mahmood

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### ABSTRACT

Thalassemia is an inherited blood disorder occur due to a mutation that affect hemoglobin production in our body. It is classified as alpha or beta depending upon whether this mutation affect alpha chain or beta chain. It can be treated by inserting a functional gene or by repairing the faulty gene within the hematopoietic stem cell. Early gene transfer efforts were not successful because of limited delivery vector and insufficiently known knowledge. Recent research has developed strategies that address these challenges, including the use of lentiviral vector. Now researchers are focused on precise correction of mutant gene, modifying DNA sequences, globin gene mutation and manipulating the translocation and RNA splicing to acquire the balanced globin synthesis. In this review the place of interest is to highlight the present gene therapy approaches, including lentiviral vector delivery and comparison with emerging gene editing approaches.

**Keywords:** Gene therapy, Thalassemia, Lentiviral vector design, Globin chain, Mutation

### INTRODUCTION

Gene therapy appeared as a transformative technique to cure  $\beta$ -thalassemia which is a hemoglobin disorder occurred due to  $\beta$ -globin gene mutation it is a hereditary disorder can be transfer generation after generation. This mutation of  $\beta$ -globin causes chronic anemia and the patient need blood transfusion after regular interval of time throughout his life. Regular blood transfusion and iron chelation are the conventional methods to treat this genetic disorder. Recent therapeutic landscape has been changed because of advancement in gene addition and gene editing techniques significantly CRISPR, Cas9-based approaches. This review highlights the betterment in viral vector design and genome editing accuracy to improve the long-term efficacy with transfusion dependent  $\beta$ -thalassemia patients. (TDT) (Frangou et al., 2023 Frontiers in Hematology, 2024).

The reactivation of fetal hemoglobin production by CRISPR, Cas9 gene editing is a great medical innovation. New England Journal of Medicine, published that most of the patient treated with this technique have acquire a reliable transfusion independence, which is considered as a great milestone towards its treatment. (Frangoul et al., 2023). However patient needs to have follow-up examination to ensure independence from blood transfusion and stable hemoglobin levels. (Locatelli et al., 2024). The outcomes from patients shows that the intervention of gene therapy have play a significant role in the betterment of quality of life. Additionally, patient-reported outcomes indicate meaningful improvements in quality-of-life following gene therapy intervention (American Society of Hematology, 2025).

In the advancement of gene therapy for  $\beta$ -thalassemia lentiviral vector design pays a critical

role, significantly by the advancement of self-inactivating vector that increase the safety while safeguarding the constant  $\beta$ -globin expression. Early  $\gamma$ -retroviral vectors can sometimes induce cancerous mutations in the body. Modern lentiviral vectors remove these cancerous mutations and enhance the therapeutic effectiveness. The  $\beta$ -globin gene is combined with erythroid specific regulatory element from the locus control region (HS2, HS3, HS4) to achieve high-level expression specifically in red blood cell precursors derived from hematopoietic stem and progenitor cells. Clinical demonstration of such techniques shows remarkable results in transfusion independence and improving the safety profile (Frangoul et al., 2023; Frontiers in Hematology, 2024; ScienceDirect, 2024).

### Molecular and genetic basis of $\beta$ -Thalassemia

$\beta$ -Thalassemia occur due to mutation in the  $\beta$ -globin gene (HBB) present on the chromosome 11p15.5 within the  $\beta$ -globin gene cluster, which contain embryonic, fetal and globin genes organized with reference to developmental expression. The HBB gene 1.6 kb have three exon and two introns, encoding 147 amino acids  $\beta$ -globin chain.  $\beta$ -globin attaches with  $\alpha$ -globin to make adult hemoglobin (HbA,  $\alpha_2\beta_2$ ). Erythroid-specific expression is regulated by promoter elements and the upstream locus control region (LCR), enabling the fetal-to-adult hemoglobin switch. (Orkin & Bauer, 2009). Any disturbance of coding sequence, splice junction, promoter regions, or regulatory element disruption of coding sequences, splice junctions, promoter regions, or regulatory elements damages transcription, RNA processing, or translation, it results in reduced ( $\beta^+$ ) or absent ( $\beta^0$ )  $\beta$ -globin synthesis (Thein, 2005).

Recent researches have described more than 200 disease causing mutations in HBB, which have significant geographical variations reflecting historical selective pressure (Thein, 2005). The majority of mutation cause defect in RNA splicing. (e.g., IVS-I-110 G>A), nonsense mutations producing premature termination codons (e.g., codon 39 C>T), frameshift insertions or deletions, and promoter mutations that decrease transcriptional efficiency. The genotype and phenotype correlation describes the quantifiable heterogeneity of  $\beta$ -thalassemia. Homozygosity or compound heterozygosity for  $\beta^0$  mutations is related with critical, transfusion-dependent  $\beta$ -

thalassemia, if the organism inherits at least one  $\beta$ -thalassemia allele he has the maximum chances to suffer from thalassemia (Cao & Galanello, 2010; Thein, 2005).

Imbalance between the production of  $\alpha$  and  $\beta$ -globin chains results in pathophysiology of  $\beta$ -thalassemia. In  $\beta$ -thalassemia, aggregation of unpaired  $\alpha$ -globin chains causes precipitation due to the formation of deficit  $\beta$ -globin chains results in oxidative damage to the cell membrane. This causes the programmed cell death of developing erythroblasts, which results in futile erythropoiesis, the actual mechanism that leads to  $\beta$ -thalassemia (Rund & Rachmilewitz, 2005). Peripheral hemolysis also worsens anemia. Abnormalities in the skeletal system occur due to the production of erythropoietin due to the prolonged hypoxia, which results in the proliferation of red blood cells. Individuals with transfusion dependent thalassemia faces the problem of iron overload. Iron hoard in liver, heart and endocrine organs which greatly increase the morbidity and mortality. Together, these molecular and cellular mechanisms explain the wide spectrum of clinical manifestations observed in  $\beta$ -thalassemia (Rund & Rachmilewitz, 2005; Cao & Galanello, 2010).

### Evolution of gene therapy for thalassemia

From the late 2000s to early 2010s the idea to cure thalassemia by gene therapy shifts from pre-clinical to clinical investigation phase. The aim of early research was to develop a mean that aid in long-term  $\beta$ -globin gene expression in HSCs.  $\gamma$ -retroviral vectors (Cavazzana-Calvo et al., 2010; Ribeil et al., 2013). Lentiviral vectors are the best to way to incorporate therapeutic  $\beta$ -globin gene in the genome of that particular organism without contaminating its DNA with previously developed mutant  $\gamma$ -retroviral vectors (Ribeil et al., 2017; LentiGlobin historical data) The first important regulatory accomplishment for gene therapy of  $\beta$ -thalassemia genes was attained by betibeglogene autotemcel (trade name: Zynteglo), which is lentiviral vector gene therapy. The first significant regulatory achievement for gene therapy of the  $\beta$ -thalassemia genes was achieved by betibeglogene autotemcel (trade name: Zynteglo), a lentiviral vector gene addition therapy. In 2019 U.S granted a conditional endorsement to the European Medicines Agency. Due to success in clinical trial of higher hemoglobin level and decrease in transfusion dependence, FDA in 2022 allowed the

gene therapy of transfusion dependent  $\beta$ -thalassemia to check the efficacy of zynteglo (Ribeil et al., 2017; *Frontiers in Hematology*, 2024)

. This achievement confirmed the autologous gene addition technique showing the progressive refinement of several years, advancement in stem cell mobilization and optimization of ex vivo manufacturing method that increases both efficacy and strength. In the late 2010s due to the development of precise genome editing there has been a great level of progress in evolution of gene therapy techniques to target the corresponding gene rather than adding the transgene. CRISPR-Cas9 gene editing has been applied to disrupt critical elements, including the erythroid enhancer of BCL11A, to reactivate fetal hemoglobin (HbF) expression to compensate for deficient  $\beta$ -globin chain synthesis. In 2023 and 2024 U.K, U.S, and European Union approved the exagamglogene autotemcel for transfusion dependent  $\beta$ -thalassemia for first CRISPR-based gene therapy (Hoy, 2024).

### **Lentiviral design and engineering**

The ability of lentiviral vectors to transduce the therapeutic gene into long-lived hematopoietic stem and progenitor cells (HSPCs) make it a standard for gene therapy strategies focusing  $\beta$ -thalassemia. Third-generation self-inactivating (SIN) lentiviral vectors have remarkable ability to transduce gene HPSC with high efficiency because they greatly reduce the risk of inserting mutagenesis by removing majority of viral genes and modifying the long terminal repeats to decrease the activity of viral promoters. These vectors use  $\beta$ -globin locus control region and erythroid-specific promoter to gain high level of  $\beta$ -globin transgene expression, which is critical for the therapeutic efficacy of gene therapy. (Jang et al., 2020; Rattananon et al., 2021).

The cautious addition of intronic sequences and optimization of transcriptional orientation are the main engineering strategies to maximize the expression and maintain vector sustainability during packaging and reverse transcription. This technique has great advantages and potential but still there are difficulties exist in it, particularly with respect to high titers and efficient transfer of gene to human HSPCs. The complex nature of regulatory elements, including the addition of whole LCR sequence, is important for high levels of  $\beta$ -globin expression. Addition of these

regulatory elements can cause my problems and affect the overall efficiency. Recent researches have solved these problems by developing novel designs. For example the many novel vectors including G2B vectors have been prepared by the optimization of LCR element and promoter region. These vectors increase the  $\beta$ -globin expression four-five times as compared to old clinical vectors without compromising on safety (Gao et al., 2025).

These advances illustrate how iterative engineering of vector architecture can substantially improve both therapeutic output and production efficiency. Overall, the evolution of lentiviral design from SIN backbone and erythroid promoters to refined regulatory elements and high titers represents an advancement in the underlying technology, which on now being understood in the present gene therapy success for  $\beta$ -thalassemia and future potential improvements (Gao et al., 2025).

### **Mechanism of lentiviral gene transfer in CD34<sup>+</sup> hematopoietic stem cells for $\beta$ -thalassemia**

Lentiviral vectors are the preferred vectors for  $\beta$ -thalassemia therapy because they stably integrate the therapeutic genes into both dividing and non-dividing HSCs. In contrast to the inefficacy of  $\gamma$ -retroviral vectors, lentiviral vectors infect quiescent CD34<sup>+</sup> cells. Once the viral genome is released into the host cell nucleus, the viral RNA is reverse-transcribed into double-stranded DNA. The viral genome is then actively transported into the nucleus and integrates into the host chromosomal DNA. This leads to the stable inheritance of the therapeutic  $\beta$ -globin gene in all the progeny cells derived from the infected HSCs. Additionally, the use of self-inactivating (SIN) vectors is beneficial because they minimize promoter activity in the viral long terminal repeats and the risk of insertional mutagenesis (Cartier et al., 2009; Nalini, 2015).

The process of CD34<sup>+</sup> HSC collection starts with the mobilization of stem cells and progenitor cells from the bone marrow to the peripheral blood using granulocyte colony-stimulating factor (G-CSF), sometimes supplemented with plerixafor. After mobilization, the cells are collected from the peripheral blood using a process called leukapheresis. CD34<sup>+</sup> cells are obtained from the collected cells using immunomagnetic separation. Before transducing the cells, the cells are cultured for a short period with cytokine-supported medium supplemented with stem cell factor, Flt3 ligand,

and thrombopoietin to support the stemness of the cells and increase the viral susceptibility. Lentiviral transduction occurs *ex vivo* at a controlled multiplicity of infection to ensure the best vector copy number. However, the culture duration must be tightly controlled to avoid the loss of engraftment potential (Aiuti et al., 2013; Biffi et al., 2013).

Before the reinfusion of gene-corrected cells, patients undergo conditioning therapy, which most commonly involves busulfan-based myeloablation. Conditioning therapy decreases the levels of endogenous HSCs. This provides an opportunity for the engraftment of corrected autologous HSCs. Conditioning therapy has a direct impact on the levels of gene-corrected cells that engraft and expand. Myeloablative conditioning regimens enhance the efficacy of therapy but also lead to toxicities and potential adverse effects. Thus, pharmacokinetic monitoring is critical to ensure safety and efficacy (Lucarelli et al., 1994; Thompson et al., 2018).

Once engrafted, the genetically modified CD34<sup>+</sup> HSCs proliferate in the bone marrow and differentiate into erythroid progenitor cells that express the therapeutic  $\beta$ -globin gene. The lentiviral vectors used in  $\beta$ -thalassemia gene therapy vectors have been engineered to include erythroid-specific promoters and  $\beta$ -globin locus control region (LCR) sequences, which enable high-level, cell type-specific expression of the therapeutic gene in erythroid cells while avoiding off-target expression in other cell types. Clinical studies of lentiviral  $\beta$ -globin gene addition approaches have shown that patients with transfusion-dependent  $\beta$ -thalassemia have sustained production of hemoglobin, reduced requirement for blood transfusions, and in some cases (Cavazzana-Calvo et al., 2010; Riebel et al., 2017)

#### **Long-term expression and genomic stability**

Long-term expression studies in murine and humanized systems have consistently shown sustained transgene expression without evidence of progressive loss. Integration site studies showed a largely polyclonal pattern of lentiviral insertions. In contrast to previous  $\gamma$ -retroviral vector systems associated with insertional oncogenicity, self-inactivating (SIN) lentiviral vector systems minimize enhancer-driven activation of adjacent proto-oncogenes. Detailed insertional site mapping

in preclinical systems did not show evidence of dominant clone formation associated with vector-driven transformation. In addition, long-term studies showed no evidence of hematologic malignancies, consistent with the improved safety profile of contemporary lentiviral vector systems (Montini et al., 2009; Nalini, 2015).

#### **Vector copy number optimization**

Vector copy number per diploid genome was identified as a key factor in therapeutic effectiveness and safety. In preclinical mouse models, a direct relationship between VCN and hemoglobin levels was established. Nevertheless, theoretical risks of insertional mutagenesis were a concern due to high copy numbers. It was established that a VCN of approximately 1 to 3 copies of the genome is sufficient to express therapeutic levels of  $\beta$ -globin to reverse anemia, while minimizing the risk of genotoxicity. Various strategies were employed to optimize the effectiveness of these vectors, including the use of controlled MOI, cytokine restimulation, and the use of a transduction enhancer. These strategies were effective in ensuring that therapeutic VCNs were achieved without compromising the engraftment capacity of stem cells capacity (Miccio et al., 2008; Thompson et al., 2018).

The introduction of betibeglogene autotemcel lentiviral gene therapy marked a major step forward in the management of transfusion-dependent  $\beta$ -thalassemia (TDT), a disease previously treated with lifelong red blood cell transfusions and iron chelation therapy. Initial humanization was demonstrated in Phase 1/2 studies such as HGB-204 (Northstar) and HGB-205, which established seminal "proof of concept" on safety and therapeutic activity following the *ex vivo* transduction of autologous CD34<sup>+</sup> hematopoietic stem and progenitor cells (HSPCs) using a BB305 viral vector encoding a modified  $\beta$ -globin gene ( $\beta^A$ -T87Q) and busulfan-based myeloablative conditioning (NCBI Bookshelf, 2025).

#### **Clinical outcomes of lentiviral gene therapy in transfusion-dependent $\beta$ -thalassemia**

These results were also supported by the small cohort Phase 1/2 HGB-205 trial. In the HGB-205 trial, the four subjects were successfully engrafted, and three subjects were also found to be transfusion-independent for a long duration. The

subjects were found to be maintaining a weighted average total hemoglobin level of 11.4 g/dL. This shows the ability of beti-cel to increase the hemoglobin levels close to normal without the need for any transfusion. The safety profile was consistent with the profile expected for a gene therapy product with myeloablative conditioning and no product-related severe adverse events. These results were further supported by the Phase 3 HGB212 trial, which assessed the safety and efficacy of beti-cel in a patient population with severe genotypes, including  $\beta^0/\beta^0$ . In this study, 16 out of 18 patients (89%) retained independence from transfusions at a median follow-up of nearly 48 months, with total hemoglobin levels remaining above traditional disease thresholds. Transfusion independence was sustained to the last follow-up, and no deaths or severe gene therapy-related adverse events reported. These results substantiated the potential for beti-cel to provide a near curative approach for patients with severe  $\beta$ -thalassemia, without the risks associated with allogeneic hematopoietic stem cell transplant (Miccio et al., 2008; Thompson et al., 2018).

#### Safety consideration in gene therapy

Significantly, gene therapy for  $\beta$  thalassemia has made substantial progress, but safety is a primary issue, especially due to the use of lentiviral vectors and their intense conditioning regimens for stem cells. One of the most theoretical risks is insertional oncogenesis, which might result if the vectors integrate into the regulation of oncogenes and tumor suppressor genes. This risk was identified in the initial gene therapy trials that are used to  $\gamma$ -retroviral vectors and were associated with the occurrence of leukemia (Cavazzana et al., 2019).

Nevertheless, there are advances in vector design, especially the design of self-inactivating lentiviral vectors, have greatly improved their safety profiles. Clinical results in transfusion dependent  $\beta$  thalassemia TDT patients have demonstrated polyclonal hematopoietic reconstitution without evidence of abnormal dominance of a single modified clone. While clonal dominance in certain instances, such as near the HMGA2 locus, has been described, this has not led to malignant transformation. Lentiviral vectors have also been designed to transduce stem cells in the presence of HIV-1 proteins (Cavazzana et al., 2019). In their  $\beta$  Thalassemia has a molecular basis of HBB gene

mutations, resulting in defective  $\beta$  globin chain synthesis, leading to ineffective erythropoiesis. Patients require blood transfusions and iron chelation therapy for survival. Although HSCT has a curative potential for  $\beta$  thalassemia, the use of this modality is restricted by the availability of suitable donors and the associated risks of transplantation (Cappellini et al., 2020).

Gene therapy, including gene addition via lentiviral vectors and gene editing via CRISPR, Cas9 gene therapy, corrects the underlying genetic defect and has shown success in sustained hemoglobin production with decreased transfusion burden in clinical trials (Frangou et al., 2021). Long term safety evaluation is also important in this regard. In this Cases of myelodysplastic syndrome (MDS) have also been identified in gene therapy trials, but subsequent studies have shown that this could be a result of underlying clonal hematopoiesis, genomic instability, or the effect of conditioning agents such as busulfan rather than gene therapy (Thompson et al., 2018).

#### Insertional oncogenesis risk and clonal dominance

One of the significant safety issues to be faced when developing a gene therapy for  $\beta$  thalassemia is the possibility of the occurrence of insertional oncogenesis, as well as the development of clonal dominance. This issue arises because the most advanced form of  $\beta$  thalassemia therapy makes use of the possibility of genetically modifying the autologous HSPCs, utilizing the lentiviral vectors. This insertion of the vectors occurs semi randomly within the host genome, and there exists a possibility that the process can dysregulate the nearby oncogenes, thereby increasing the possibility of the development of malignancy (Cavazzana et al., 2019).

In response to these observations, contemporary lentiviral vectors used in  $\beta$  thalassemia clinical trials have been designed to contain self-inactivating long terminal repeats. These changes significantly reduce the expression of endogenous gene expression through enhancer activity and reduce the genotoxic risk of the vectors. Studies of integration site use in  $\beta$  thalassemia trials using self-inactivating lentiviral vectors have uniformly shown polyclonal reconstitution of the hematopoietic compartment, without evidence of

insertions near known oncogenes (Lidonnici et al., 2018).

Although isolated cases of clonal dominance have been reported, the most notable of which was the integration of vectors near the HMGA2 locus, resulting in the outgrowth of a single clone, such cases did not progress to malignancies, and such findings suggest that, although clonal outgrowth of dominant clones can occur, it does not necessarily result in leukemic transformation. In summary, the use of lentiviral vectors has a safer profile of integration compared to the  $\gamma$ -retroviral system, where the vectors preferentially integrate into the transcription unit rather than the promoter region, reducing the potential for the activation of oncogenes (Cavazzana et al., 2017).

Clonal dominance refers to the dominance of one genetically engineered hematopoietic stem cell clone compared to the others. This occurs when the integration of the vector inadvertently provides a proliferative advantage to the clone. In one of the early trials of gene therapy for  $\beta$  thalassemia, clonal dominance was observed. This was caused by the integration of the lentiviral vector near the HMGA2 locus. However, despite the dominance of the clone, no signs of leukemia development were observed. In fact, the dominance of the clone was found to decrease with time, indicating that the clonal dominance was self-limiting (Cavazzana et al., 2010).

#### Vector integration site analysis

Vector integration site analysis is a molecular approach for the precise identification of the site where the lentiviral vectors get integrated into the host genome. Using the power of high throughput sequencing technologies, ISA enables the identification of the site of vector integration into the genome and the proximity to protooncogenes, tumor suppressor genes, and other regulatory regions. Vector Integration site analysis is a significant tool for the safety assessment of gene therapy products. The possible adverse effects caused by the integration of the vectors into the genome may manifest at a later stage. For this reason, the regulatory authorities, such as the Food and Drug Administration, recommend the surveillance of the patients for a longer period after the treatment. For example, the patients may be required to undergo the Vector Integration Site Analysis for a period of 15 years after the treatment. This is because the permanent changes

made at the genomic level during the treatment may manifest at a later stage and cause the possible adverse effects (Cavazzana et al., 2010).

#### Toxicity management in gene therapy for thalassemia

The management of toxicities is one of the major aspects that have to be considered in gene therapy for  $\beta$  thalassemia, and this includes the toxicities associated with the conditioning regimen and gene modification itself. Acute toxicities such as cytopenias, mucositis, and increased susceptibility to infections are often encountered after the administration of a conditioning regimen that includes drugs such as busulfan. These toxicities are usually managed with supportive care that includes the administration of broad-spectrum antibiotics and granulocyte colony stimulating factor and blood products to reduce the risk of infections and bleeding. Hepatic veno-occlusive disease is a major complication that occurs after the administration of alkylator-containing regimens and is usually diagnosed with liver function tests. This complication is usually managed with drugs such as defibrotide and has been shown to improve patient outcomes and reduce mortality from the disease treatment (Eskens et al., 2021). Engraftment potential, although these regimens are under clinical investigation to the control management for thalassemia diseases (Hsieh et al., 2020).

#### Comparison lentiviral gene addition and emerging gene editing approaches in thalassemia

Recent developments in gene therapy for thalassemia have moved beyond conventional gene addition using lentiviral vectors to include techniques of gene editing, which involve the CRISPR, Cas9 system for gene disruption and base editing. The conventional method of gene therapy for  $\beta$  thalassemia involves the introduction of a functional  $\beta$  globin gene into stem cells using a lentiviral vector. This method has been successful in demonstrating stem cell engraftment and efficacy. For instance, in clinical trials, over 80% of treated patients have been able to stop regular blood transfusions after sustained expression of haemoglobin (Thompson et al., 2018).

Nevertheless, these lentiviral systems retain a certain risk that is associated with the semirandom vector integration and require myeloablative conditioning. Recent gene editing technologies

have been developed to overcome these drawbacks and correct disease-causing mutations in the endogenous  $\beta$  globin gene locus or reactivate lethal haemoglobin expression through targeted disruption of regulators such as BCL11A. This method minimizes the risk of genotoxicity associated with vector integration (Angelos et al., 2021).

Moreover, pre-clinical and early clinical data suggest that CRISPR, Cas9 edited HSCs can achieve effective rates of editing, engraft well, and decrease transfusion requirements, which can potentially decrease the risk of insertion-related oncogenesis compared to lentiviral methods (Frangou et al., 2021). The recent discoveries in gene editing techniques have led to a wide range of therapeutic possibilities for  $\beta$  thalassemia. Gene editing techniques such as CRISPR, Cas9 mediated disruption of BCL11A and base editing have shown great promise in reactivating the lethal haemoglobin and correcting the genetic mutations. BCL11A is a key transcriptional repressor of  $\gamma$ -globin disruption of BCL11A erythroid specific enhancer using CRISPR, Cas9 increases levels of HbF, which helps to normalize the  $\alpha$ ,  $\beta$  ratio (Frangou et al., 2021).

Base editing, which is a relatively more recent strategy, allows for nucleotide substitution without the creation of DSBs, thus minimizing the risk of unwanted insertions, deletions, and or rearrangements. Preclinical trials of base editing to correct specific  $\beta$  thalassemia mutations have revealed efficient editing with significantly lower off target effects compared to conventional CRISPR/Cas9 nucleases (Levy et al., 2023).

#### **Advantage and disadvantage of emerging gene editing approaches versus lentiviral therapy**

New gene editing technologies like CRISPR, Cas9 mediated disruption of BCL11A and base editing provide promising advantages over conventional lentiviral gene addition therapies for  $\beta$  thalassemia. Unlike lentiviral vectors, which add  $\beta$  globin gene cassettes to the genome in a semi-random manner and carry a small risk of insertional oncogenesis, gene editing technologies precisely target the endogenous  $\beta$  globin locus (Anzalone et al., 2020). Clinical trials of CRISPR, Cas9 edited hematopoietic stem cells have shown strong induction of lethal haemoglobin and reductions in transfusion requirements, with preliminary results

indicating efficacy to be at least, if not better than, lentiviral therapy (Esrick et al., 2021).

Despite these advantages, the techniques have limitations, including the challenges of delivering the edited genes to the hematopoietic stem cells, controlling off target effects, and ensuring the long-term durability of the edited stem cells, which are being investigated (Anzalone et al., 2020). In contrast, the use of lentiviral therapy has a proven track record, and long-term studies have shown that the vectors persist stably, leading to a positive outcome for patients who are transfusion dependent. It requires a process of myeloablation, however, and ongoing surveillance for the possibility of genotoxicity associated with the vectors (Thompson et al., 2018).

#### **Current Limitations**

Gene therapy has transformed the manner in which thalassemia is treated where lifelong supportive therapy is now replaced with the possibility of a functional cure. Although, the clinical outcomes are encouraging, the high price remains the most significant factor as to why these therapies are not more commonly applied. These complex operations increase the cost of healthcare significantly and treatment cost very high (Thompson et al., 2021; Hu et al., 2024).

Therapies that are commercially accepted such as betibeglogene autotemcel have demonstrated the ability to render many patients' transfusion-independent. Nevertheless, the overall cost of the treatment may reach hundreds of thousands to millions of US dollars per individual due to the necessity to manufacture, hospitalize, and provide various forms of clinical follow-up (Locatelli and Thompson, 2024; Malay et al., 2024).

Production of gene therapy is patient-specific and this implies that there is no occurrence of economies of scale that normally reduce the price of drugs. Economic burden is particularly deplorable in those regions where the  $\beta$ -thalassemia is widespread like South Asia, Middle East, and Africa. Healthcare systems of many low and middle-income countries (LMICs) operate on a limited budget that is typically consumed with the basic services such as the iron chelation therapy and transfusion programs. Due to this reason, the solution has not yet been provided due to the unavailability of insurance coverage, reimbursement policies, and government funding mechanisms (Weatherall, 2012; Malik et al., 2019).

This disparity indicates that the disparity in the world is increasing where the scientific advancements are taking place rapidly, yet the patients cannot access it due to their geographical location or the amount of money they possess. Secondly, the initial cost of gene therapy is so great that healthcare policymakers cannot be certain whether it would be cost-effective in the long term, although there is evidence that it may be considered cost saving in the long term as it will eliminate the necessity to conduct chronic transfusions. Economic evaluation models continue to evolve, and various countries pay them in very different ways, which complicates the implementation further (Malay et al., 2024).

Gene therapy requires highly specialized facility to be utilized in a clinical program, such as hematopoietic stem cell transplantation (HSCT) programs. The process of gene therapy is not the same as standard drug administration procedure as it entails a lot of complex procedures including stem cell mobilization, collection of apheresis, genetic modification, conditioning therapy, reinfusion, and long-term monitoring of the molecules. Some of the significant components of the infrastructure include: Vector and cell-processing labs, which is Good Manufacturing Practice (GMP) certified place to harvest and freeze hematopoietic stem cell to provide myeloablative or low-intensity conditioning regimens advanced molecular diagnostic labs to monitor long-term treatment outcomes. The equipment, biosafety systems, adherence to regulations, and well-trained personnel in various disciplines are very expensive to establish and maintain such facilities in operation. The healthcare infrastructure required to carry out transplantation is not available in many LMICs, and thus the only tertiary or quaternary centers in the world can provide gene therapy (Malik et al., 2019; Hu et al., 2024).

Effective implementation needs physical infrastructures, but also coordinated clinical skills by hematologists, transplant physicians, molecular biologists, pharmacologists and specialized nursing staff. Workers are not sufficient in locations with a high burden, contributing to the further difficulty in the delivery of advanced cellular therapies (Locatelli & Thompson, 2024).

In developed countries, gene therapy is only offered in specialized institutes due to safety and regulatory issues. The other issue with the infrastructure is the long-term patient follow-up.

Since gene therapy is a permanent alteration of genes, regulatory agencies must watch gene therapy for many years to determine whether it is effective and whether it has any adverse side effects such as insertional mutagenesis or clonal explosion. It implies that effective systems of patient monitoring are required, which are not usually present in resource-sparse locations (Cavazzana-Calvo et al., 2012).

The inability to make things is a huge issue that renders making gene therapy against thalassemia more difficult. Most ex vivo techniques currently employ the use of lentiviral vectors to transfer functional copies of b-globin genes into hematopoietic stem cells isolated in patients. To prepare these vectors, it requires a considerable amount of time, effort, and compliance with the GMO standards, which are required to ensure that the product is safe and consistent (Cavazzana-Calvo et al., 2012; Ribeil et al., 2019).

Some of the quality control measures in the manufacturing of the vectors include the verification of viral titers, response replication ability, the assurance of sterility, and confirmation of transgene expression. The batch-to-batch variability may alter the efficacy of transduction, and this may impact clinical outcomes. Failure in the production process would either cause delays in the treatment schedule or render the gathered stem cells useless, and this would be a significant logistical issue (Thompson et al., 2021).

The other issue of concern is the preservation of the stem cells during manipulation and expansion whilst also achieving the stable and long-term expression of the genes. Excessive manipulation may damage the engraftment chances whereas inadequate gene transfer may render the treatment less efficient (Hu et al., 2024). Transduction also requires a large number of high-quality viral vectors, the production capacity of the world is also still low. The cost of operation and the regulatory control combined with the necessity to have specialized manufacturing facilities make scaling up the production process to treat large populations of patients even more challenging. Other alternatives to viral vectors-based delivery systems (non-viral) and automated manufacturing platforms are also under consideration as potential alternatives to the currently used clinical practice, although these new technologies remain largely experimental (Malay et al., 2024).

Significant contradiction in the management of thalassemia around the world is that countries that have the highest disease burden accrue the least benefit in the research of gene therapy. In Europe and North America, clinical programs are increasingly becoming common, yet in the low- and middle-income countries (LMICs), they remain an uncommon occurrence due to money, infrastructure, and rule-related issues that are not independent. No long-term financing or reimbursement models, are some of the largest problems. The qualified medical personnel are not sufficient. Regulatory frameworks on the advanced therapies that are not completely developed. The absence of any long-term monitoring and drug safety systems. These issues render most patients unable to receive gene therapy, whether they participate in a medical trial or travel to a foreign country to get medical services (Weatherall, 2012; Malik et al., 2019; Hu et al., 2024).

Prevention programs such as carrier screening, premarital counseling, and prenatal diagnosis have become a popular method of intervention by public health strategies in low- and middle-income countries (LMICs) because they are a relatively cheap means of benefiting a large population at once. This is making the number of new cases of disease drop, however, it does not help those already diseased who require treatment. Unless there is coordination of global policy, transfers of technology, and cost-saving measures, it is only the people who can afford gene therapy. We have to mend these gaps to make scientific findings fair in world health (Malik et al., 2019; Hu et al., 2024).

### **Ethical, socioeconomic, and global health considerations**

The development of gene therapy as a potentially curative therapy of  $\beta$ -thalassemia is a significant breakthrough in the field of genomic medicine, but its clinical efficacy has also increased the ethical issue of equal access to healthcare, as all the patients are now concentrated in Europe and North America, where well-developed healthcare infrastructure and reimbursement are accessible (Thompson et al., 2021; Hu et al., 2024).

This disease burden and lack of treatment is indicative of a severe health inequality in the world. Among the main causes of injustice is the exceptionally high costs of gene therapy. Ex vivo gene-modification technology involves the bespoke production of autologous hematopoietic stem cells,

Good Manufacturing Practice (GMP) laboratories, conditioning chemotherapy, and long-term clinical outcomes, all of which are tremendously costly (Locatelli and Thompson, 2024). Economic studies show that the total cost of therapies can exceed several million US dollars per patient making it affordable to only those healthcare systems that could afford expensive interventions of precision medicine (Malay et al., 2024). Ethically, such a state of affairs is problematic in terms of distributive justice that is concerned with equitable distribution of healthcare resources among the populations.

According to Weatherall (2012), inherited hemoglobin diseases are unequally distributed across the economic backgrounds of the global community, so the unfair access of people to curative treatments is especially harmful. Moreover, the differences in national reimbursement policy also led to the disproportionate access of patients in even high-income nations and proves that the affordability issue is widespread instead of local (Malay et al., 2024).

### **Genomic integration ethical concerns**

$\beta$ -thalassemia has been treated with gene therapy that involves permanent genetic modification via lentiviral gene transfer, or genome editing, including the CRISPR-Cas9. Despite the demonstrated effectiveness of these strategies, their primary limitation lies in their failure to consider the particular culture of a specific nation or area where the project aims to be developed. All these strategies have proven, but their main weakness is that they do not take into account the specific culture of a particular country or region where the project is to be implemented. Promising clinical effects, they are irreversible, which adds complicated ethical factors associated with long-term safety and biologic uncertainty. Viral vectors introduce therapeutic genes into host genome and this creates a hypothetical danger of insertional mutagenesis in which integration close to oncogenes may disrupt normal cellular regulation and may ultimately play a role in malignant transformation (Cavazzana-Calvo et al., 2012).

Engineering of vectors has greatly enhanced safety profiles, but long-term surveillance is still required due to the fact that GM-HSCs engineered will stay longer until the patient dies (Ribeil et al., 2019). The technologies of genome editing pose other

ethical dilemmas. Despite the fact that CRISPR-based methods are designed to ensure a high level of accuracy in genetic defects correction, concerns exist about off-target mutations and unintended changes in the genome (Hu et al., 2024).

The fact that these genetic changes are permanent makes them accept an unknown long-term risk in addition to possible therapeutic benefits, making it difficult to evaluate the ethics. The problem of informed consent is particularly problematic in the case of pediatric patients with thalassemia, as they constitute a significant part of the patients who are candidates of gene therapy. The decision as to what should be done to the child needs to be taken by parents or guardians of the child whose long-term implications are not known quite yet. Ethical principles insist that the patients and the families should be provided with unambiguous information on the probable risks, the lifespan of therapeutic effects and the reproductive or intergenerational unknown effects. Therefore, a tight regulatory control, long term monitoring schemes and extensive patient counseling are all part of a morally responsible application of gene-based therapies. The development of gene therapy as a potentially curative therapy of  $\beta$ -thalassemia is a significant breakthrough in the field of genomic medicine, but its clinical efficacy has also increased the ethical issue of equal access to healthcare, as all the patients are now concentrated in Europe and North America, where well-developed healthcare infrastructure and reimbursement are accessible (Thompson et al., 2021; Hu et al., 2024).

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According to Weatherall (2012), inherited hemoglobin diseases are unequally distributed across the economic backgrounds of the global community, so the unfair access of people to curative treatments is especially harmful. In the absence of international financing models, tiered pricing mechanisms, or technology-transfer programs, gene therapy will turn into an intervention that is available to privileged groups of people, rather than a global treatment of a widespread genetic disease (Locatelli and Thompson, 2024).

Moreover, the differences in national reimbursement policy also led to the disproportionate access of patients in even high-income nations and proves that the affordability issue is widespread instead of local to combat equity, it is thus necessary to launch concerted international health policy that will harmonize scientific innovation and ethical accountability (Malay et al., 2024).

Thalassemia has distinctive socioeconomic issues in countries with a high prevalence of the condition, which hinders the application of gene therapy. Various healthcare systems in South and Southeast Asia, Middle East, and Africa are just learning to live on limited budgets and at the same time dealing with high volumes of transfusion-dependent patients (Weatherall, 2012). Such areas are thus faced with a two-fold challenge of high prevalence and inadequate healthcare facilities. Preventive measures like carrier screening, premarital counseling and prenatal diagnosis have traditionally been viewed as priority programs in the prevention of health in these settings as these offer cost-effective benefits to the population (Weatherall, 2012).

Although gene therapy is a potential solution that would allow cure at the individual level, the potential risks of spending a lot of resources on costly treatments are that the funds would be redirected to other critical services which would save more lives such as blood transfusion safety programs and iron chelation therapy. Ethical dilemmas are also created where the policy makers have to create a balance between investing in innovative biotechnology and ensuring that access to basic health services remains equal.

According to Thompson et al. (2021), sustainable implementation needs to be integrated and not replace existing models of care. Equally, as Hu et al. (2024) observe, a lack of regulatory

infrastructure and trained professionals in the resource-limited setting is also a factor working against the safe adoption of the advanced cellular therapies. A more realistic and ethically balanced way to high-burden regions is to implement a phased implementation strategy, which integrates prevention programs, reinforced healthcare infrastructure, and a gradual introduction of gene therapy of such areas. Global cooperation, employee education, and international investment efforts will be needed to lessen the disparities and make sure that the gains in the field of genomic medicine will be used by the populations that bear the brunt of thalassemia (Cavazzana-Calvo et al., 2012).

The present  $\beta$ -thalassemia gene therapy regimens rely heavily on myeloablative chemotherapy which is primarily busulfan based conditioning to allow the growth of the genetically modified hematopoietic stem cells (HSCs) in bone marrow. These treatments are good, but they are associated with many adverse effects, including infertility, increased susceptibility to infection, organ toxicity, and increased hospitalization (Thompson et al., 2021).

Treatment plans in the future will strive to substitute the conventional chemotherapy with less severe or non-myeloablative conditioning therapies. Scientists are developing antibody conditioning approaches that can be used to address surface markers on the hematopoietic stem cells. These procedures will cause diseased stem cells to be eliminated selectively leaving healthy tissues unaltered (Hu et al., 2024).

These specific strategies can significantly reduce treatment morbidity and increase access to pediatric, geriatric, and medically vulnerable patients. Reduced-toxicity conditioning is also expected to result in lowering the overall cost of treatment and enhance the quality of life of the patients. It is one of the steps on the way to broadening the application of gene therapy in clinical practice (Locatelli and Thompson, 2024; Trends in Molecular Medicine, 2024).

### Gene therapy

The shifts in gene therapy involve going towards an in vivo gene delivery model that involves direct administration of therapeutic genes or gene editing vectors to the target organism, rather than manufacturing the therapeutic gene product in ex vivo culture. The conventional ex vivo techniques

require complex processes of acquiring cells, handling them in the laboratory, and returning them to the body, which complicates them to obtain in most medical systems. In vivo gene therapy is designed to simplify the treatment procedures by delivering the vectors to the organism hematopoietic stem cells. Such a method would eliminate the necessity of special production, simplify processes, and reduces the treatment expenses by several times. Even though it remains largely experimental, initial preclinical findings indicate that they might ease the process of treatment access by individuals across the globe, particularly where the infrastructure is insufficient and there is a lack of advanced cell-processing personnel (Frontiers in Hematology, 2024; Locatelli and Thompson, 2024).

Hybridizing gene editing technologies and classic gene addition techniques is a giant leap in the next-generation treatments. CRISPR/Cas9, transcription activator-like effector nucleases (TALENs) and zinc finger nucleases are other precision genome-editing platforms that enable you to modify specific genetic components that are responsible in causing disease. The reactivation of fetal hemoglobin  $\text{HbF}$  through destabilizing regulatory elements, such as BCL11A enhancer, that suppresses  $\text{HbF}$  following birth is one of the most salient therapeutic approaches. The issues with  $\beta$ -globin synthesis are compensated by increased production of  $\text{HbF}$ , and the disease is not as severe (Ribeil et al., 2019; Hu et al., 2024).

Combining gene editing and lentiviral gene addition may help to increase therapeutic activity and reduce the number of vectors needed, which leads to increased outcomes observed in long-term safety. However, a circumvention of off-target editing and improvement of editing efficiency are essential areas of research that too should be presented before wide clinical implementation (Blood Advances, 2024; Trends in Molecular Medicine, 2024).

The future of  $\beta$ -thalassemia gene therapy is gradually raising more in the spirit of the concept of precision medicine, which emphasizes the necessity of treatment based on the specific biological and clinical characteristics of each patient. Due to genotypic variations, severity of diseases, transfusion burden and tolerance of treatment, individualized treatment plans are required in order to achieve optimal outcomes. Individual vectors, individualized conditioning

regimens, and individualized risk assessments have the potential to substantially improve the treatment outcome without causing undesirable side effects (Thompson et al., 2021). Individualized strategies also assist physicians to identify the appropriate level of treatment intensity and safety with respect to patients with more than one condition or prone to toxicity. Despite all these, the accessibility of personalized gene therapy to all people in a fair manner remains a major issue. The innovations will be significant to address the issues of healthcare infrastructure to eliminate global health inequalities as new treatment options will emerge, and to make these treatments more affordable (Locatelli and Thompson, 2024).

### Future prospective and Conclusion

The primary objective of next-generation gene therapy is to ensure the safety of vectors and at the same time be able to offer long-term therapeutic benefits. The early approaches to gene therapy raised concerns relating to insertional mutagenesis caused by the haphazard genomic site of written viral vectors that may have the ability to activate oncogenes or disrupt important host genes. Consequently, the current literature is focused on the creation of less dangerous delivery systems that could allow controlled and lineage-selective expression of genes. The self-inactivating (SIN) lentiviral vectors represent a remarkable advance in this field because they cause the elimination of promoter activity in long terminal repeats and, therefore, the reduction of genotoxic risk and maintenance of stable transgene expression (Cavazzana-Calvo et al., 2012). Other additions include addition of the chromatin insulator sequences and regulatory elements which also improve regulated expression of b-globin and lessen positional effects following the incorporation (Hu et al., 2024).

New non-viral delivery systems such as lipid nanoparticles and engineered extracellular vesicles are also being researched on to ensure viral vectors generate fewer immune responses and side effects. The systems aim to support short-term or directed gene transfer and avoid long-lasting disruption of the genome though massive clinical validation efforts are currently underway (Blood Advances, 2024; Locatelli and Thompson, 2024).

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